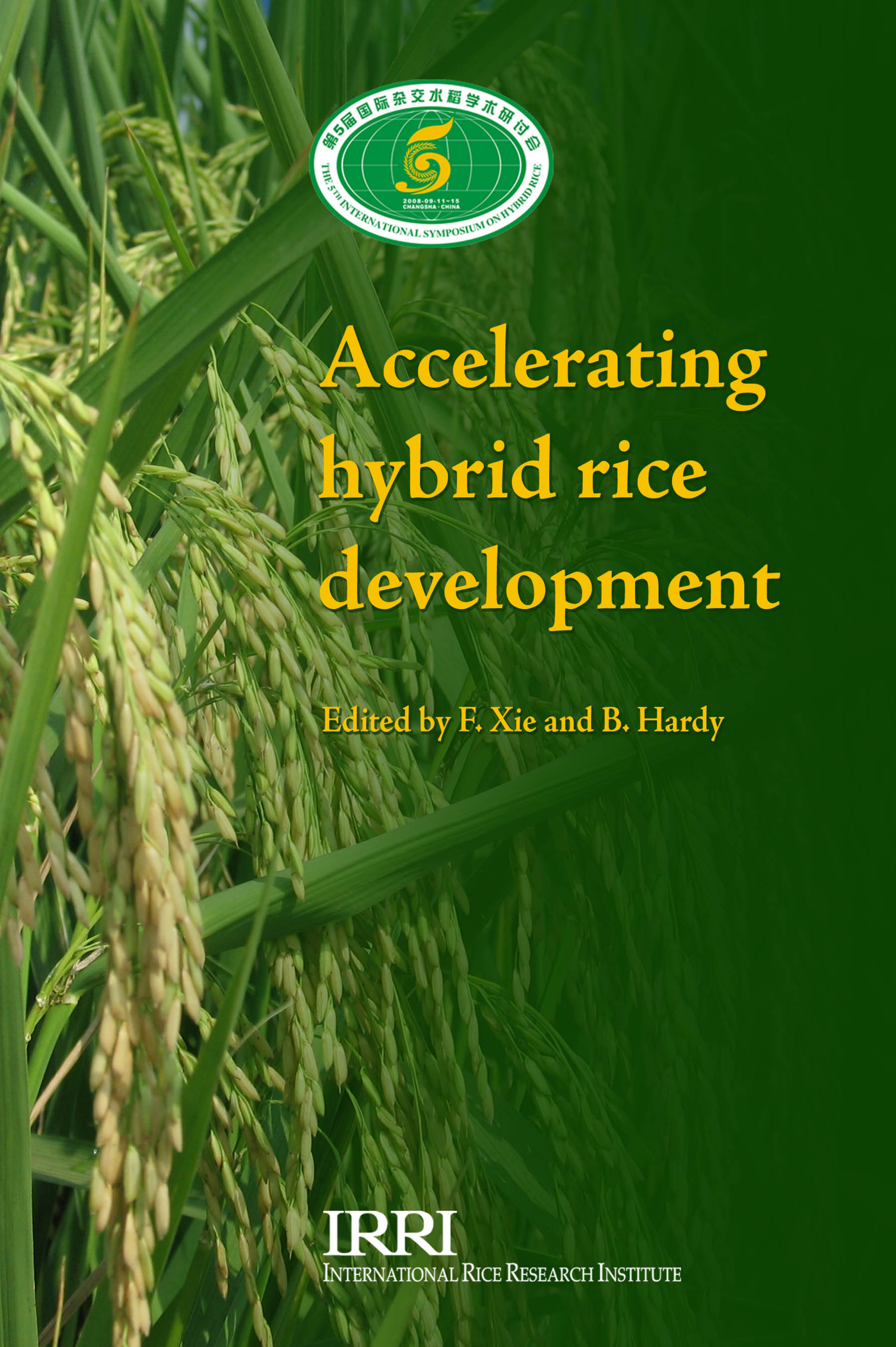

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Accelerating hybrid rice development

Edited by F. Xie and B. Hardy

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Mailing address: IRRI, DAPO Box 7777, Metro Manila, Philippines
Phone: +63 (2) 580-5600
Fax: +63 (2) 580-5699
Email: irri@cgiar.org
Web: www.irri.org.
Rice Knowledge Bank: www.knowledgebank.irri.org
Courier address: Suite 1009, Security Bank Center
6776 Ayala Avenue, Makati City, Philippines
Tel. +63 (2) 891-1236, 891-1174, 891-1258, 891-1303

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Contents

Foreword

Hybrid rice is a proven and successful technology for rice production, having contributed significantly toward improving food security, raising rice productivity and farmers' income, and providing more employment opportunities over the past three decades. Remarkable progress continues as hybrid rice technology makes its way across Asia and to other countries. Since the 4th International Hybrid Rice Symposium held in Hanoi, Vietnam, in 2002, the area grown to hybrid rice worldwide increased to an estimated 19.8 million hectares in 2007, including about 2.8 million hectares outside China. All major rice-producing countries in the world have been investing in applying hybrid rice technology, and, in recent years, the seed industry has also been involved in hybrid rice research and development. The advance of new technologies, such as marker-assisted selection, has provided new approaches to enable scientists to develop hybrid rice varieties with increased yield potential, improved grain quality, and multiple resistance to or tolerance of various biological and environmental stresses. However, with the remaining challenge of increasing food demand with fewer resources, and new challenges from climate change, it is even more important for hybrid rice to receive high priority for increasing rice productivity. Many problems constrain the further development of hybrid rice in the areas of higher yield heterosis, seed production, grain quality, seed cost, field management, and public-private partnership.

The 5th International Symposium on Hybrid Rice was held at Changsha, Hunan, China, on 11-15 September 2008. It brought together leading researchers and industry experts to review and discuss current knowledge and progress on hybrid rice development, seed production, molecular applications, crop and resource management, and economics, as well as to discuss future research strategies to enhance and sustain hybrid rice technology. The first International Hybrid Rice Symposium was held in the same city in 1986, when only 8.5 million hectares of hybrid rice were growing and only in China. A total of 430 hybrid rice scientists from 21 countries and two international organizations (IRRI and FAO) participated in the 2008 symposium.

I am grateful to our co-sponsors, the People's Government of Hunan, the China National Hybrid Rice Research and Development Center, and China National Rice Research Institute, for their contributions to the technical program. Financial support from the People's Government of Hunan (China), Pioneer Hi-Bred International

(USA), RiceTec Inc. (USA), Syngenta Asia Pacific Pte. Ltd. (Singapore), and Sime Darby Plantation (Malaysia) for the symposium and publication of the proceedings is gratefully acknowledged. We hope that this publication will become a valuable source of information for hybrid rice researchers, seed production and technology transfer agencies, and graduate students in plant breeding and seed technology.

Robert S. Zeigler
Director General
International Rice Research Institute

Hybrid rice and food production

Progress in breeding super hybrid rice

Yuan Longping

To meet the food requirement for Chinese people in the 21st century, China's Ministry of Agriculture set up a super rice breeding program in 1996. The yield targets for a single season crop of rice hybrids were

Phase I (1996-2000): 10.5 t ha^{-1}
Phase II (2001-05): 12 t ha^{-1}

Several pioneer super hybrid rice varieties were developed by 2000 that met the yield target of phase I. More than 20 demonstration locations with 6.7 or 67 ha each had an average yield above 10.5 t ha^{-1} in 2000. These pioneer super rice hybrids in large-scale commercial production (1.2–2.0 million ha) have yielded 8.4 t ha^{-1} in recent years.

Good progress has been made in developing phase II super rice hybrids. One promising two-line indica/japonica combination, P88s/0293, yielded more than 12 t ha^{-1} at five demonstration locations with around 7 ha each in 2003 and at 12 locations in 2004. This second-generation super hybrid rice was released for commercial production in 2006 and it yielded around 9 t ha^{-1} on a large scale. Based on these achievements, a phase III super hybrid rice breeding program was proposed, in which the yield target is 13.5 t ha^{-1} and can be fulfilled by 2010. The technological approaches for breeding super hybrid rice are (1) morphological improvement, (2) using intersubspecific heterosis, and (3) using biotechnology. Details on these technical issues are discussed in this paper.

Rice is China's main food crop. It feeds about 60% of China's population and still has great yield potential, especially in hybrid rice. To meet the food requirement for all Chinese people in the 21st century, China's Ministry of Agriculture set up a super rice breeding program in 1996. The yield targets for hybrid rice are listed in Table 1.

With morphological improvement plus the use of intersubspecific (indica/japonica) heterosis, several pioneer two-line super hybrid rice varieties were developed and met the phase I yield standard of single-cropping rice by 2000. More than 20 demonstration locations had 6.7 ha or 67 ha each, where average yield was more than 10.5 t ha^{-1} in 2000. One combination, P64S/9311, was released for commercial production in 2001. Since then, the area under this hybrid has expanded very fast and

Table 1. Yield targets for super rice in China.

Phase	Hybrid rice[a]			% increase
	First cropping	Second cropping	Single cropping	
1996 level	7.50	7.50	8.25	0
Phase I (1996-2000)	9.75	9.75	10.50	More than 20%
Phase II (2001-05)	11.25	11.25	12.00	More than 40%

[a] In t ha^{-1} at two locations with 6.7 ha each in two consecutive years.

it reached 2 million ha in recent years. Its average yield is 8.4 t ha^{-1} while that of nationwide rice is 6.3 t ha^{-1}. Another combination, P64S/E32, reached a record yield of 17.1 t ha^{-1} in an experimental plot (720 m^2) in 1999.

After the success in developing phase I super hybrid rice, efforts have focused on breeding phase II super hybrids and good results have been obtained. For example, a two-line hybrid, P88S/0293, yielded more than 12 t ha^{-1} at four 6.7-ha locations in 2003 and 12 6.7-ha or 67-ha locations in 2004, which indicates that the yield target of phase II super hybrid rice was achieved one year ahead of the plan. These phase II super hybrids have been released for commercial production since 2006. In 2007, the planting area under these super hybrids was more than 200,000 ha and their average yield was around 9 t ha^{-1}.

Based on this progress, a phase III super hybrid rice breeding program was set up in 2006. The yield target is 13.5 t ha^{-1} for single-cropping rice and is to be fulfilled by 2010. So far, some promising hybrids have been developed, which might attain the goal on schedule. For example, two-line hybrid 58S/3128 yielded about 13 t ha^{-1} at a 7-ha demonstration location in 2007 and it is estimated that the yield of this hybrid could surpass 13.5 t ha^{-1} this year at the same location because cultivation has improved. Another example is an excellent R line called giant panicle restorer (GP-1). It has 400 spikelets per panicle on average, and especially its hybrids, T98A/GP-1 and P88S/GP-1, have around 500 spikelets per panicle and the biggest panicle has more than 1,000 spikelets. Spikelet number per m^2 is 75,000. This huge sink means that super high yield potential could be tapped.

Technical approaches

Crop improvement practices have indicated, up to now, that there are only two effective ways to increase the yield potential of crops through plant breeding: morphological improvement and the use of heterosis. However, the potential is very limited when using morphological improvement alone and heterosis breeding will produce undesirable results if it is not combined with morphological improvement. Any other breeding approaches and methods, including high technology such as genetic engineering, must be incorporated into good morphological characters and strong heterosis; otherwise, there will be no actual contributions to a yield increase. On the other hand, the further development of plant breeding for a super yield target must rely on progress in biotechnology.

Morphological improvement

A good plant type is the foundation for super high yield. Since Dr. Donald proposed the concept of ideotype in 1968, many rice breeders pay great attention to this idea and propose various models for super high-yielding rice. Among them, a well-known one is the "new plant type" proposed by Dr. Khush at the International Rice Research Institute. Its main features are (1) large panicles, with 250 spikelets per panicle; (2) fewer tillers, 3–4 productive tillers per plant; and (3) a short and sturdy culm. Whether these models can realize super high yield or not has yet to be proved.

Based on our studies, especially inspired by the striking characteristics of the high-yielding combination P64S/E32, which has achieved a record yield of 17.1 t ha^{-1}, we have found that a super high-yielding rice variety has the following morphological features:

1. Tall erect-leaf canopy

   The upper three leaf blades should be long, erect, narrow, V-shaped, and thick. Long and erect leaves have a larger leaf area, can accept light on both sides, and will not shade each other. Therefore, light is used more efficiently and air ventilation is also better within such a canopy. Narrow leaves occupy a relatively small space and thus allow a higher effective leaf area index. A V-shape makes the leaf blade stiffer so that it is not prone to be droopy. Thick leaves have a higher photosynthetic function and are not easily senescent. These morphological features signify a large source of assimilates that are essential to super high yield.
2. Lower panicle position

   The tip of the panicle is only 60–70 cm above the ground during the ripening stage. Because the plant's center of gravity is quite low, this architecture enables the plant to be highly resistant to lodging. Lodging resistance is also one of the essential characters required for breeding a super high-yielding rice variety.
3. Bigger panicle size

   Grain weight per panicle is around 6 g and the number of panicles is about 250 m^{-2}. Theoretically, yield potential is 15 t ha^{-1} in this case.

Grain yield = biomass × harvest index. Nowadays, the harvest index (HI) is very high (above 0.5). A further raising of the rice yield ceiling should rely on increasing biomass because further improvement of the HI is limited. From the viewpoint of morphology, to increase plant height is an effective and feasible way to increase biomass. However, this approach will cause lodging. To solve this problem, many breeders are trying to make the stem thicker and sturdier, but this approach usually results in a decrease in HI. Therefore, it is difficult to obtain super high yield in this

Table 2. Yield potential of an indica/japonica hybrid.

Combination	Plant height (cm)	Number of spikelets per panicle	Number of spikelets per plant	Seed-setting rate (%)	Actual yield (kg ha^{-1})
Chengte232 (japonica) × 26Zhaizao (indica)	120	269.4	1,779.4	54.0	8,250
Weiyou35 (indica/indica)	89	102.6	800.3	92.9	8,625
Increase (%)	34.8	162.8	122.4	– 41.9	– 4.3

way. The plant model of a taller canopy can combine the advantages of a higher biomass, higher HI, and higher resistance to lodging.

Raising the level of heterosis

According to our studies, heterosis in rice has the following general trend: indica/japonica >indica/javanica>japonica/javanica>indica/indica>japonica/japonica. Indica/japonica hybrids possess a very large sink and rich source, whose yield potential is 30% higher than that of intervarietal indica hybrids being used commercially. Therefore, efforts have focused on using indica/japonica heterosis to develop super hybrid rice. However, many problems exist in indica/japonica hybrids, especially their very low seed set, which must be solved to practically use their heterosis (Table 2). Making use of the wide compatibility (WC) gene (S_{-5}^{n}) and adopting the following fundamental principles, several intersubspecific hybrid varieties with stronger heterosis and normal seed set have been successfully developed.

1. Plant height—higher in the dwarf
   The plant height of an intersubspecific hybrid is normally higher than that of its parents. This is not a problem if the parental lines are selected to have allelic semidwarf genes. On the other hand, it is required that plant height should be higher on the condition that lodging does not occur, so that biomass could be increased for a sufficient source capacity to form a high-yielding basis.
2. Genetic relationship—closer in distance
   Because of too great a genetic difference, typical intersubspecific hybrids result in physiological barriers and unfavorable characters. Presently, javanica, intermediate indica, or japonica rice cultivars should be selected as parental lines instead of typical indica or typical japonica cultivars. It has been proven that heterosis is best exploited in crosses between the U.S. rice WCVs and indica or japonica rice.
3. Heredity—both dominance and overdominance
   Not only should attention be paid to the dominant effects of favorable characters but also stress should be given to maintaining a comparably large genetic heterogeneity in hybrids to make full use of overdominance and additive effects.

4. Panicle size—medium-large panicle type
   One should select combinations of medium-large panicle type with around 200 spikelets per panicle and some 3 million panicles per hectare. It is advisable not to pursue large or super-large panicle-type combinations so that a harmonized source-sink relation resulting in a high seed-setting percentage and good grain filling can be achieved. In terms of an increase in the number of spikelets per panicle, it is essential to augment panicle length and the number of primary branches, rather than seek a high spikelet density, which is disadvantageous to grain filling.
5. Photosynthesis—high ratio of grain weight to leaf area
   High photosynthetic efficiency and a high ratio of grain weight to leaf area indicate a more effective transformation from vegetative heterosis to reproductive heterosis in intersubspecific crosses. Hybrid combinations can be screened through testing. Thus, the selection accuracy and effects can be greatly improved by combining empirical morphological selection with qualitative and quantitative physiological selection, which makes the selecting technique more scientific.
6. Grain filling—plumpness from the plump parents
   Based on surveys and experience, the grain filling of F_1 hybrids is closely correlated with that of their parents. Therefore, to choose varieties or lines with good or excellent grain plumpness as parents is one of the most effective ways to solve the problem of poor grain plumpness often encountered in intersubspecific rice hybrids. In addition, choosing varieties or lines with a heavy unit weight is also an effective way.
7. Grain quality—fine quality from javanica
   One should select long grain and fine-quality javanica-indica intermediate-type cultivars as parents and cross them with indica parental lines so that the grain quality of the hybrids will be fine and retain the characteristics of indica type. Select javanica-type or javanica-japonica intermediate-type cultivars of short grain and fine quality as parents and cross them with japonicas, whose hybrid grains are of fine quality and inclined to japonica type.
8. Ecological adaptability
   In indica rice-growing regions, the main area develops indica-javanica hybrids with consideration of indica-japonica ones; in japonica rice-growing areas, the main area is japonica-javanica hybrids with consideration of indica-japonica ones.

Prospects

By reaching the target of a phase III super hybrid rice program, we can increase yield by 3 t ha^{-1} and produce 30 million t more rice per year when it is commercialized on up to 10 million ha. Thus, another 100 million Chinese people can be fed.

Experiments have shown that super hybrid rice can also greatly enhance rice yield outside China. If 50% of world rice fields were covered by high-yielding hybrid rice and at least 2 t ha^{-1} more rice could be produced, this increased rice production could feed 400–500 million people. Therefore, accelerating the development of super hybrid rice worldwide will play a key role for food security and world peace.

Notes

Author's address: China National Hybrid Rice R&D Center, Changsha, Hunan, China.

Ensuring food security in the 21st century with hybrid rice: issues and challenges

Nguu Van Nguyen

"Rice Is Life" was the theme for the implementation of the International Year of Rice 2004. Rice is a staple food that provides energy, protein, and vitamins for about half of the world population. From 1961 to 1999, global rice production had always increased at a higher growth rate than the population. Consequently, more rice had become available for consumption and people's food security was better ensured during the second half of the 20th century. Entering the 21st century, global rice production started with a substantial decrease, which was caused by a reduction of about 9.3 million hectares in the global rice harvested area from 1999 to 2002. This decrease in global rice production resulted in less rice available for consumption, which is a major cause of the current surge in rice prices and the ensuing global food crisis. The world population still continues to grow and it is projected to increase to about 9.3 billion in 2050, from about 6.5 billion in 2006. Consequently, it is estimated that popular rice demand in 2050 will be about 781 million tons of paddy, or about 146 million tons more than global rice production in 2006. Future global rice production could be increased by efforts to increase rice production area or increase yield or a combination of both. Although future expansion of rice area may require large and costly investments, a substantial yield increase could be obtained with the wide adoption of hybrid rice.

The lesson learned from China in 1975-90 indicated that more rice could be produced even on less land with the wide adoption of hybrid rice. Efforts led by FAO, its member countries, and partner institutions since 1990 have confirmed the yield advantage of hybrid rice in areas and countries outside of China. However, the area under commercial hybrid rice production still occupies only a small percentage of total rice area. This implies great potential for the adoption of hybrid rice to substantially increase global rice production, thus ensuring the food security of people in the 21st century. Analyses on the status of hybrid rice development and adoption as well as the conditions of rice production in general, however, indicate that successful expansion of the commercial production of hybrid rice in the future will require full understanding and appropriate solutions for the following main issues and challenges: increasing the yield of F_1 seed production, improving the economic return from hybrid rice

production, minimizing environmental and resource degradation, responding to climate change, understanding the recent stagnation of yield growth in China, and developing new systems for managing hybrid rice crops. The formulation of appropriate strategies and guidelines for national and international actions will be essential for obtaining the support and commitment of policymakers to the wide adoption of hybrid rice, thus ensuring food security in the 21st century.

"Rice Is Life" was the theme for the implementation of the International Year of Rice 2004. Rice is a staple food that provides energy, protein, and vitamins for about half of the world population. The implementation of the International Year of Rice 2004 reconfirmed that rice will continue to be a global food crop and billions of people around the world will continue to depend on rice for their energy and protein each day. The growth rate of global rice production in the second half of the 20th century was always higher than that of the population. As a result, more rice was available for consumption, and thus food security of the population improved during the 20th century.

Entering the 21st century, global rice production started with a substantial decrease, which was caused by a reduction of about 9.3 million hectares in the global rice harvested area from 1999 to 2002. This decrease in global rice production resulted in less rice available for consumption, which was a major cause of a surge in rice prices and the ensuing global food crisis. This again confirmed the conclusion made during the International Year of Rice that rice plays an important role in providing food security to the world population. The world population continues to increase, although with a declining growth rate. Global demand for rice is therefore expected to further increase along with population growth in the 21st century.

More rice production could be obtained by expanding rice area, but the expansion of rice area in the future will be difficult due to limited water resources and climate change. Also, large and costly investments may be required to expand rice production area. The lesson learned from the development and adoption of hybrid rice in China shows that more rice could be produced even on less land with hybrid rice. This paper discusses the relationship between global rice production and food security in the 20th century, rice demand in 21st century development, the development and use of hybrid rice up till now, and the issues and challenges of ensuring food security of the population with hybrid rice in the 21st century.

Rice production and food security

For thousands of years, the ability to produce a surplus of rice had enabled the development of communities, whereas the failure of a rice crop had caused widespread famine, death, and political instability in many Asian countries. Rice has become the staple food for the people in Gambia, Guinea, Guinea-Bissau, Liberia, Madagascar, Senegal, and Sierra-Leone in Africa. Today, in Latin America, the people in the Dominican Republic, Guyana, Suriname, and others also depend on rice for their daily energy and protein. In 2004, the amount of energy (kcal) per capita supplied from rice

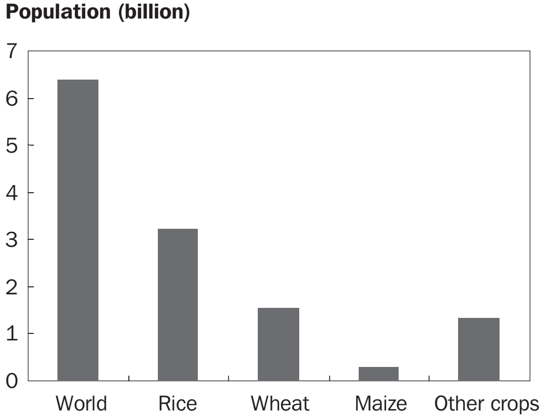

Fig. 1. World population in 2004 based on staple food crops (calculated based on data in FAOSTAT on 22 November 2007); a staple food crop is a crop that provides 700 kcal per capita per day or more (1 kcal = 4.184 kJ).

Table 1. Consumption and production of rice, wheat, and maize in 1997-99.

Crop	Consumption (million tons)			Production (million tons)[a]	Food/ production[b] (%)
	Food[a]	Feed[a]	All uses[a]		
Paddy rice	514.5	17.8	578.5	587.4	87.6
Wheat	421	93.1	582.5	596.9	70.5
Coarse grains	238.9	551.7	891.6	899.9	26.5

[a]From FAO (2002). [b]Calculated values.

was about 700 kcal (1 kcal = 4.184 kJ) per day or more for about 3.23 billion people. In the same year, wheat provided about 700 kcal per capita per day or more to about 1.55 billion people, maize provided about 700 kcal per capita per day or more to 288 million people, and other food crops provided other food kcal to the remaining 1.32 billion people (Fig. 1).

About 90% of global rice production yearly has been consumed as food compared with 70% in the case of wheat and only about 25% in the case of coarse grains, including maize (Table 1). Therefore, the quantity of rice that is available for consumption in a given year depends greatly on rice production in that year.

Rice production and food security in the 20th century

From 1961 to 1999, global rice production had always increased at a higher rate than the population. In 1961, global rice production was about 215.6 million tons and it

Table 2. Rice harvested area, yield and production, population, and rice availability for consumption in 1961 and from 1999 to 2005.

Year	Rice harvested area (million ha)[a]	Rice yield (kg per hectare)[a]	Rice production (million tons)[a]	Population (billion persons)[a]	Rice availability (kg per person)[b]
1961	115.3	1,869	215.6	3.10	69.5
1999	156.8	3,894	610.6	5.99	101.8
2000	154.1	3,884	598.4	6.07	98.5
2001	151.8	3,937	597.3	6.14	97.1
2002	147.6	3,854	568.3	6.22	91.2
2003	148.1	3,943	585.7	6.30	92.9
2004	150.1	4,043	610.9	6.37	95.7
2005	154.4	4,088	629.3	6.45	97.4
2006	154.3	4,112	634.6	6.58	96.4

[a]From FAOSTAT. [b]Calculated.

increased to 610.6 million tons in 1999. The world population was 3.10 billion in 1961 and it was 5.99 billion in 1999 (Table 2). Consequently, rice availability for consumption (or per capita rice production) increased from about 69.5 kilograms per person in 1961 to about 101.8 kg per person in 1999 (Table 2). Therefore, people's food security improved in the 20th century.

Rice production and food security in the 21st century

Global rice harvested area started to decline substantially in 2000, which caused a substantial decrease in global rice production, and this trend continued up to 2002. Global harvested area in 2002 was about 9.3 million hectares less than the 156.8 hectares harvested in 1999. Consequently, global rice production in 2002 was only 568.3 million tons, about 42.3 million tons less than in 1999, although rice yield remained more or less the same (Table 2).

Both global rice harvested area and rice yield have increased again since 2003. Consequently, global rice production in 2006 was 634.6 million tons or 24 million tons more than in 1999. However, per capita rice availability for consumption in 2006 was still less than that in 1999: 96.4 kg per person compared with 101.8 kg per person due to population growth (Table 2). The reduction in per capita rice availability for consumption since 2000 undoubtedly was a major factor causing a surge in rice prices.

World population continues to grow, although with a declining growth rate (Fig. 2). Bruinsma (2003) estimates that the world population will be about 8.3 billion in 2030 and 9.3 billion in 2050. A continued increase in the world's population eventually will have major implications for rice demand and food security in the 21st century. At

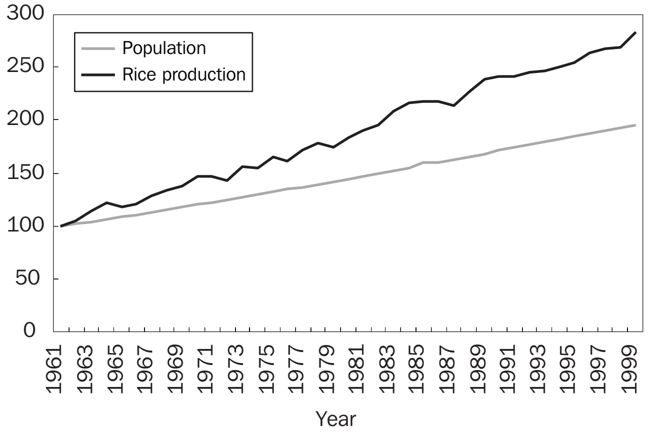

Fig. 2. Relative growth of the world population and that of world rice production (1961 = 100), 1961 to 1999.

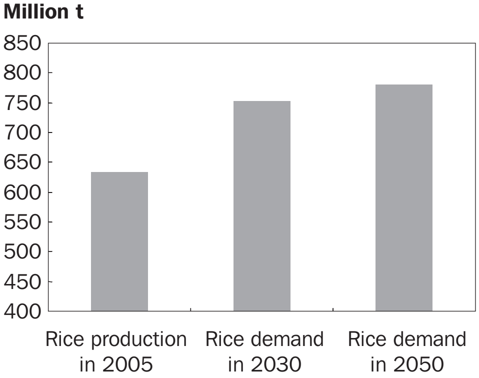

Fig. 3. Global rice production in 2005 and projected rice demand in 2030 and 2050.

the global level, FAO (2006) projected that global rice demand would be about 753 million tons of paddy in 2030 and about 781 million tons of paddy in 2050 (Fig. 3). The gaps between global rice production in 2006 and the projected rice demand in 2030 and in 2050 are considerably large. Producing more rice to fill these gaps will be essential for ensuring food security and poverty reduction in the 21st century.

Ensuring food security with hybrid rice—historical perspectives

The lesson learned from the development and adoption of hybrid rice in China shows that more rice could be produced even on less land with hybrid rice. The Chinese experience could be applied to ensure people's food security in the 21st century.

Table 3. Rice harvested area, yield, and production in China, 1961 to 1990. Commercial production of hybrid rice started in 1976.

Year	Harvested area (million ha)	Yield (kg ha^{-1})	Production (million t)
1961	27.0	2,078	56.2
1965	30.5	2,967	90.7
1970	33.1	3,416	113.1
1975	36.4	3,528	128.7
1980	34.4	4,144	142.8
1985	32.6	5,250	171.3
1990	33.5	5,717	191.6

Source: FAOSTAT.

Hybrid rice and food security in China, from 1961 to 1990

In China, the wide adoption of semidwarf high-yielding varieties together with the development of irrigation and input distribution systems as well as the training of farmers increased national rice yield from about 2.07 t ha^{-1} in 1961 to about 3.52 t ha^{-1} in 1975. Chinese national rice production in 1961 was about 56.2 million tons. To ensure the food security of its population, China had expanded substantially its national harvested area, from 27.0 million ha in 1961 to 36.4 million ha in 1975. Chinese national rice production in 1975 was about 128.7 million tons of paddy rice (Table 3). Therefore, after 14 years (1961-75), Chinese rice production increased by 72.5 million tons, but the rice harvested area increased by 9.4 million ha (Table 3).

A team of Chinese scientists under the leadership of Professor Yuan Longping discovered a wild rice plant with abortive pollen in 1970. In 1974, this team developed cytoplasmic male sterile (CMS) lines (A lines), their corresponding maintainer lines (B lines) and fertility-restoring lines (R lines), and they also developed the first set of 3-line hybrid rice varieties, including Wei-you 2, Wei-you 3, Wei-you 6, Shan-you 2, Shan-you 3, Shan-you 6, Nan-you 2, Nan-you3, Nan-you 6, Si-you 2, Si-you 3, and Si-you 6.

When planted in field trials in Hunan in 1975, these hybrid rice combinations or varieties produced at best 20% higher yield than high-yielding varieties (Yuan 1999). Commercial hybrid rice production in China began in 1976 and the area planted to hybrid rice in China reached about 5% of national harvested area in 1980. It then increased rapidly to reach almost 50% of the national harvested area in 1990 (Xizhi and Mao 1994). In 1990, hybrid rice was planted on about 15 million ha (Yuan 2003).

China's rice production in 1990 was 191 million tons, harvested from 33 million ha, with a national yield of 5.71 t ha^{-1} in 1990 (Table 3). After 15 years of adopting hybrid rice, Chinese rice production increased by 62.9 million tons, whereas rice

Table 4. FAO projects on hybrid rice from 1992 to 2003.

Project	Country	Period	Budget (US$)
FAO/TCP/BGD/6613	Bangladesh	1997-99	201,000
FAO/TCP/EGY/8923	Egypt	1999-2003	248,000
UNDP/IND/91/008 (1st phase)	India	1991-96	4,030,000
UNDP/IND/98/140 (2nd phase)	India	2000-02	2,250,000
FAO/TCP/INS/8921	Indonesia	2000-01	257,000
FAO/TCP/MYA/6612	Myanmar	1997-99	221,000
FAO/TCP/PHI/8821	Philippines	1998-2000	275,000
FAO/TCP/VIE/6614	Vietnam	1996-98	296,000
FAO/TCP/VIE/2251	Vietnam	1992-93	259,000

harvested area decreased by about 3 million ha (Table 3). Professor Yuan Longping was awarded the World Food Prize in 2004.

FAO program on hybrid rice for food security and livelihood improvement

The successful exploitation of hybrid rice in China has encouraged other rice-producing countries to carry out hybrid rice studies. In 1979, the International Rice Research Institute also established its hybrid rice program. However, in 1990, there was no significant commercial hybrid rice outside China. The 19th Session of the International Rice Commission held in Goiânia, Brazil, in 1990 recommended that FAO and member countries promote the development and use of hybrid rice for food security and livelihood improvement.

Between 1992 and 2003, FAO formulated and implemented nine projects on hybrid rice in Bangladesh, Egypt, India, Indonesia, Myanmar, the Philippines, and Vietnam (Table 4). The main objectives of these FAO-supported projects were to (1) build up national capacity in hybrid rice breeding, F_1 seed production, and commercial production; (2) improve the facilities for supporting hybrid rice research; and (3) assist in formulating a program for the sustainable adoption of hybrid rice, at least in the medium term (4 to 5 years).

In addition to those field projects, FAO, together with the International Rice Research Institute, the Chinese Hybrid Rice Research and Development Centers, and national agricultural research and extension systems, established the International Task Force on Hybrid Rice (INTAFOHR) in 1996. Under the framework of INTAFOHR, the Asian Development Bank provided funds for implementing activities of the project "Sustaining Food Security in Asia through the Development of Hybrid Rice Technology," from 1998 to 2006.

Results of FAO projects from 1992 to 2003 confirmed the yield advantage of hybrid rice in countries outside China, at least 15% above the yield of high-yielding varieties (Tran and Nguyen 1998). The first large-scale commercial production of hybrid rice outside China took place when Vietnam planted more than 10,000 ha of hybrid rice in 1992. The commercial production of hybrid rice then gradually expanded in Vietnam and other Asian countries outside of China. In 2004, hybrid rice was commercially cultivated on about 1.5 million ha in seven Asian countries outside China: Bangladesh, India, Indonesia, Myanmar, the Philippines, Sri Lanka, and Vietnam (Nguyen 2006a). In Egypt, hybrid rice was commercially cultivated on about 10,000 ha in 2007 (Dr. T. Badawi, former president of the Egyptian Agricultural Research Center, personal communication in May 2008).

Recently, as requested, FAO approved in 2007 funding support for activities on hybrid rice development and use in Indonesia (TCP/INS/3102) and in Sri Lanka (TCP/SRL/3102). Initial reports of the TCP/INS/3102 indicate that the area of commercial hybrid rice production in Indonesia increased from only about 10,000 ha in 2006 to nearly 80,000 ha in 2008.

Ensuring food security in the 21st century with hybrid rice

Future global rice production could be increased by efforts to increase production area or increase yield or a combination of both. Although future expansion of rice area may require large and costly investments, a substantial yield increase could be obtained with the wide adoption of hybrid rice. The area of commercial hybrid rice production in countries outside of China still occupies only a very small percentage of the total rice harvested area, indicating the large potential for hybrid rice to contribute to ensuring food security in the 21st century (Nguyen 2006b). On the other hand, the slow adoption of hybrid rice in countries outside of China indicates that there are issues and challenges to its adoption. Similarly, the stagnation of Chinese national rice yield in the recent past is a major concern. Major issues and challenges that need to be considered for the successful expansion of the commercial production of hybrid rice in the medium-term future follow.

Increasing the yield of F_1 seed production

In developed countries, farmers traditionally use newly produced seeds from companies to plant, but, in developing countries, the majority of farmers who produce high-yielding rice varieties still use seeds from the previous crop to plant. In commercial hybrid rice production, however, farmers are required to use new F_1 seeds every season. The low yield of F_1 seed production, and thus the availability of F_1 seeds at reasonable prices, had been cited as a major constraint to the wide adoption of hybrid rice in countries outside China (Nguyen 2000, Tran 2002, Virmani 2003). However, substantial improvement has been made in this area, for example, the yield of F_1 seed production in China is now above 2.5 t ha^{-1}, whereas, in Vietnam, yield of local F_1 seed production was about 1.7 t ha^{-1} in 2004 (Nguyen 2004) and in India it was about 2 t ha^{-1} in 2004 (Mishra 2004). These results suggest potential for increasing the yield of F_1 seed production in the future.

Improvement of the economic return from hybrid rice production

The slow adoption of hybrid rice in countries outside China, in spite of its higher yield, suggests that the economic return of hybrid rice production may not be superior to that of the production of other rice varieties. This could be partially due to the high cost of seed in hybrid rice production, especially in areas where direct seeding is used for crop establishment. The low economic return of hybrid rice production may also be due to the lower prices of hybrid rice in open markets where grain quality has an important effect in determining the price of rice.

Direct seeding is widely practiced in Sri Lanka and in the Mekong Delta of Vietnam. Because of the high labor cost in rural areas and pressure from crop intensification, farmers in Asia are increasingly replacing transplanting with direct seeding (Pulver and Nguyen 1999). Techniques for effectively reducing the seed rate used in direct seeding and/or alternative methods of crop establishment will be needed for the wide adoption of hybrid rice cultivation in the future. The rice program in Sri Lanka has been testing and modifying the seedling broadcasting method to reduce the cost of rice crop establishment (Abeysiriwardena et al 2007). On the other hand, an improvement in the grain quality of hybrid rice, while maintaining its yield advantage, would increase the net economic return of hybrid rice production, thus promoting a wide adoption of hybrid rice.

Minimizing environmental and resource degradation

In Asia, the increase in urbanization and industrialization has made water less available for agricultural production in general and rice production in particular (Barker et al 1999). In addition, the intensification of rice production has caused considerable damage to agricultural resources and environmental pollution. The application of only nitrogen, phosphorus, and potassium fertilizers in rice production over the years has led to deficiencies of zinc, sulfur, and other micronutrients in rice soils (Tran and Ton That 1994). With higher yield, hybrid rice could accelerate the depletion of soil fertility. Moreover, the repeated application of pesticides in rice production in the past 40 years has resulted in the development of new pest biotypes. In addition, residues of pesticides that were applied in rice production have polluted the environment and water sources, which are essential for the survival of humans, animals, and other organisms living in and around rice fields. Minimizing these undesirable effects will be essential for the sustainable intensification of hybrid rice production in the future.

Responding to climate change

The increase in temperature, changes in rainfall and its distribution pattern, and rising sea water under climate change may have negative effects on rice production in general and hybrid rice production in particular. In tropical-climate areas, an increase in temperature reduces rice yield (Peng et al 2004). Exposing rice plants at anthesis to 1 or 2 hours of high temperature could produce a large percentage of sterile grains (Yoshida 1978).

Changes in rainfall and its distribution may cause more frequent and intensive drought and flood, which are major factors that limit rice yield. Rising sea water could

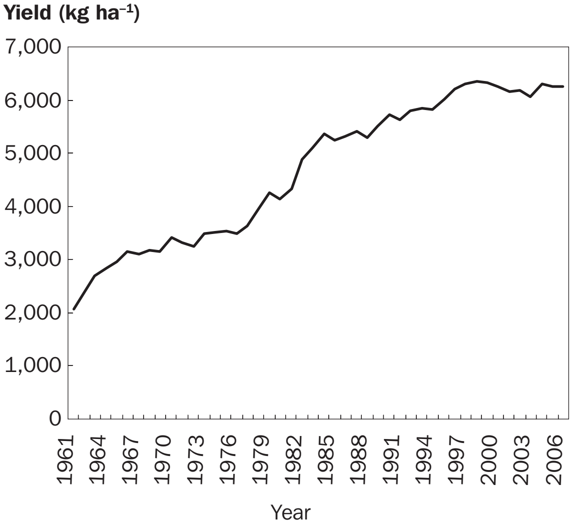

Fig. 4. Chinese national rice yield, from 1961 to 2006.

enlarge the area that is influenced by tidal waves and salinity from sea water in low-lying areas of river deltas and flood plains. Most rice varieties are severely injured in submerged soil culture at an electrical conductivity (EC) of 8–10 mmho per cm at 25 °C (Ponnamperuma and Bandyopadhya 1980). Therefore, the development of hybrid rice varieties that are more tolerant of high temperature, drought and flood, and salinity will be needed for the successful adoption of hybrid rice under climate change.

Understanding the recent stagnation of yield growth in China

Figure 4 shows the evolution of rice yield in China since 1961. Rice yield increased rapidly from about 3.63 t ha^{-1} in 1977 to about 6.33 t ha^{-1} in 1997; since then, it has been stagnant. There may be several reasons for this phenomenon. One possible reason for the stagnation of rice yield in China since 1997 is that the yield potential of hybrid rice has not been improved. However, Yuan (2004) reported that new generations of 2-line hybrids and super hybrid rice varieties with significant yield advantages over 3-line hybrid rice varieties have been developed and released for cultivation. Nevertheless, an in-depth investigation would be beneficial for identifying a way forward in order to promote the adoption of hybrid rice for food security in China and in other countries in the 21st century.

Development of new systems for managing hybrid rice crops

Concern about environmental and resource conservation and the necessity of further improvement in the economic return of hybrid rice production imply that the sustainable expansion of hybrid rice adoption would require new crop management systems. In this regard, the rice integrated crop management (Rice-ICM) systems that FAO, in collaboration with national research systems, has developed and tested since 2000 could be adopted for commercial hybrid rice production in the future. Rice-ICM systems are based on the understanding that (Tran and Nguyen 2006)

- The agroecological and socioeconomic conditions of rice production vary substantially from one region/area to another.
- Production limitations are closely linked: Stronger seedlings from high-quality seeds will not benefit yield if the crop is inadequately fertilized; likewise, the rice crop cannot respond to fertilizer application if weed infestation is intense and water supply is inadequate.
- The rice crop has distinct developmental phases and, at every phase, rice crops should attain a certain amount of growth in order to be able to produce a (certain) yield. Defective growth during a developmental phase could be partially remedied only with crop management in the next developmental phases.

Rice-ICM systems integrate all improved crop management technologies and apply them in a holistic way during the rice cropping season, from land preparation to harvest. Rice-ICM systems provide indicators of rice crop growth at different growth phases for farmers to refer to when evaluating their practices. Farmers are encouraged and helped in observing, measuring, and recording rice growth and development at critical growth phases, as well as yield and yield components at harvest, and in interpreting growth and development of the rice crop during the season using the recorded data.

Results of Rice-ICM systems application in Brazil, Indonesia, the Philippines, Thailand, and Venezuela demonstrated that the yield of existing irrigated varieties increased by 1 t ha^{-1} or more (Kueneman 2006 and Figure 5). The results of pilot tests on the development and transfer of Rice-ICM systems confirm the observations made on a rapid yield increase in Japan due to improved crop and resource management from the mid-1950s to the mid-1970s (Horie et al 2005) and a substantial yield increase in Australia with the adoption of a Rice-ICM system, called RiceCheck, since 1986 (Clampett et al 2001).

The economic analysis of data from the adoption of a Rice-ICM system in Indonesia showed that the profit of rice production generally increased with the application of the system (Abdulrachman et al 2005, Table 5). Similar observations were made in India (Balasubramanian et al 2005) and Vietnam (Pham et al 2005), showing lower production costs and higher profits from rice production with improved crop management.

Strategy and guidelines for the sustainable adoption of hybrid rice

The successful development and adoption of hybrid rice in China was made possible thanks to the consistent support and commitment of policymakers. The Chinese government provided 8 million yuan in 1976 (US$4 million at that time) to support the production of F_1 seed on 4,000 ha on Hainan Island and this helped to accelerate the adoption of hybrid rice (Yuan 2002). Also, generous funds were provided to support the development of new hybrid rice varieties, the improvement of F_1 seed production,

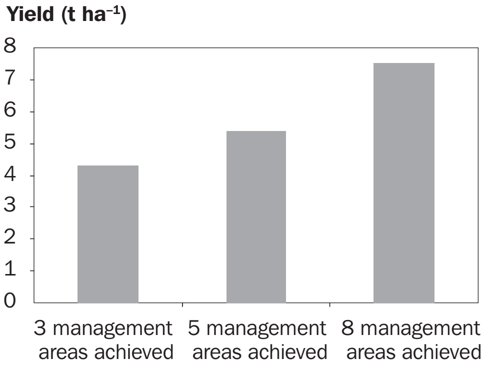

Fig. 5. Grain yield responses to farmers' skill in adoption of the PalayCheck system in the Philippines (adapted from Cruz et al 2005). The PalayCheck system has eight management areas: (1) variety and seed selection, (2) land preparation, (3) crop establishment, (4) nutrient management, (5) water management, (6) pest and disease management, (7) weed management, and (8) harvest. In each management area, indicators were provided for farmers to refer to during the evaluation of their practices.

Table 5. Change in profit from the application of a Rice-ICM system in 10 districts in Indonesia during 2002-03.

District	Change in profit (1,000 Indonesian rupees; US$1 = 8,000 rupees)	
	2002-03 wet season	2003 dry season
Deli Serdang	1,635.5	2,294.8
Solok	1,035.9	970.5
Pinrang	1,933.4	1,797.2
OKU	309	1,065
Bojonegoro	868	934
Sambas	729.8	640.8
Blitar	1,008	1,212.5
Padang Pariaman	724	743.5
Bima	1,321.3	534.6
Lampung Tengah	1,422.5	1,261.6

Source: Abdulrachman et al (2005).

and the strengthening of human resources and expertise in hybrid rice.

Similarly in Vietnam, after successful field tests on hybrid rice in 1991, the Vietnamese government provided funding support and subsidies for local F_1 seed production, and low-interest loans and tax exemptions to seed companies, and established the National Hybrid Rice Research Center in 1994. The cost of F_1 seed used in the commercial production of hybrid rice in Vietnam was subsidized at 30% in favorable areas, at 50% in difficult areas, and at 100% in mountainous areas (Nguyen 2002). In 2005, about 800,000 hectares were under commercial hybrid rice production in Vietnam (Le 2006).

The support and commitment of policymakers would be greatly enhanced by the formulation of strategies and guidelines for an effective and sustainable adoption of hybrid rice. The conditions and the environment of rice production internationally, regionally, and nationally have evolved rapidly and substantially. Also, the level of hybrid rice development and use varies greatly from one country to another. Therefore, the formulation of strategies and guidelines for an effective and sustainable increased adoption of hybrid rice will need the expertise and participation of all stakeholders. Expert consultations and neutral forums would provide an appropriate environment for the formulation of strategies and guidelines for the adoption of hybrid rice internationally and regionally. The International Rice Commission organized its Regular Sessions and Expert Consultations to provide a forum for the formulation of strategies and guidelines for major initiatives to promote sustainable intensification in the past. The 21st Session of the Commission will be held in 2010.

Concluding remarks

"Rice Is Life" was the theme for the implementation of the International Year of Rice 2004. Rice is a staple food that provides energy, protein, and vitamins for about half of the world population. The recent food crisis due to a surge in rice prices confirmed the important role of rice in providing food security to the world population. Although the world population is still growing, land and water resources for rice production are limited. A wide adoption of hybrid rice is a viable technological option for ensuring food security in the 21st century. FAO and its member countries as well as other international and regional institutions have made considerable efforts and provided resources to promote the development and use of hybrid rice for food security and livelihood improvement in the past. However, there is still a large potential for a further expansion of hybrid rice production.

A successful expansion of the commercial production of hybrid rice in the medium-term future, however, will require solutions for the following issues and challenges: increasing the yield of F_1 seed production, improving the economic return from hybrid rice production, minimizing environmental and resource degradation, responding to climate change, understanding the recent stagnation of yield growth in China, and developing new systems for managing hybrid rice crops. The formulation of appropriate strategies and guidelines for national and international actions will be essential for obtaining the support and commitment of policymakers to the wide adoption of hybrid rice, thus ensuring food security in the 21st century.

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Notes

Author's address: Executive Secretary, International Rice Commission, Food and Agriculture Organization of the United Nations, Vialle delle Terme di Caracalla, 00153 Rome, Italy.

A breeding strategy for hybrid rice in China

Cheng Shi-hua, Cao Li-yong, Zhuang Jie-yun, Wu Wei-ming,
Yang Shi-hua, and Zhan Xiao-deng

The commercial use of hybrid rice has made a great contribution to rice production in China. It was estimated that the planting of hybrid rice on a total area of 370 million ha from 1976 to 2005 increased rice production by nearly 450 million tons. The rapid development of hybrid rice in China has been attributed to innovations in breeding technology. Since the first WA-CMS plant of rice was found in 1973, diverse breeding approaches have been adopted, including the exploitation and use of various resources with cytoplasmic or genic male sterility, the development of male sterile lines with a high outcrossing rate, improvement of pest resistance and grain quality for parental lines, and combinations of the ideal plant type with interspecific heterosis in the F_1 hybrid. Other innovative breeding techniques, such as root system selection, molecular marker-aided selection, and wide hybridization, are also integrated into the hybrid rice breeding program in China.

Keywords: hybrid rice, male sterility, marker-aided selection

The commercial use of F_1 hybrid rice has made a great contribution to China's rice production. It was estimated that hybrid rice increased rice production by nearly 450 million tons on 370 million ha from 1976 to 2005. The rapid development of hybrid rice in China has been attributed to innovations in breeding technology. Since the first wild-abortive cytoplasmic male sterile (WA-CMS) plant of rice was found in 1973, diverse breeding approaches have been adopted, including the exploitation and use of various cytoplasmic or genic male sterility resources, the development of male sterile lines with a high outcrossing rate, the improvement of parental traits on pest resistance and grain quality, and combinations of an ideal plant type with intersubspecific heterosis in the F_1 hybrid. Other innovative breeding techniques, such as root system selection, molecular marker-aided selection (MAS), and wide hybridization, are also incorporated into the hybrid rice breeding program in China.

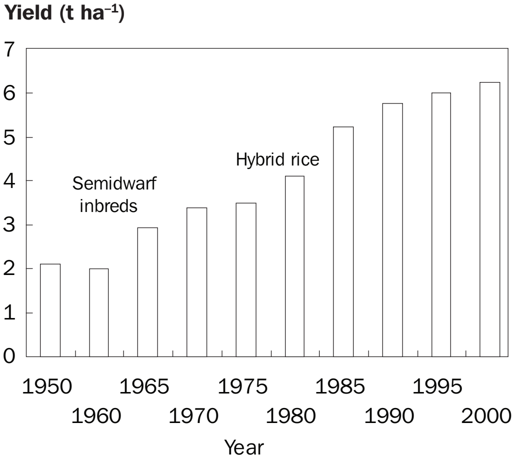

Fig. 1. Yield enhancement in rice through genetic improvement in China.

Hybrid rice development in China

Rice is the staple food of more than 60% of the Chinese population. Since 1949, China has made a great effort to increase its rice yield by exploiting the genetic resources of rice. With the use of semidwarf resources of the *sd1* gene and the wide use of modern semidwarf varieties, China increased its rice yield from 2.0 t ha^{-1} in the 1960s to 3.5 t ha^{-1} in the 1970s. Afterward, the finding of a wild-abortive cytoplasmic male sterile (WA-CMS) plant of rice on Hainan Island in 1973 resulted in tremendous success of three-line hybrid rice breeding in China. Hybrid rice that has a yield advantage of 10–20% over conventional varieties has been grown commercially since 1976, which helped China surpass 6.0 t ha^{-1} in rice yield (Fig. 1).

The successful use of hybrid rice has kept China's leading place in rice production in the world. Official statistics show that China now has about a 70% advantage in rice yield compared with the global average. Hybrid rice has made a great contribution to self-sufficiency in rice for the Chinese.

Nowadays, hybrid rice has been planted throughout the rice-growing regions in China as well as in some other countries in the world. In China, the area under hybrid rice increased from 0.14 million ha in 1976 to about 17 million ha in recent years, more than 60% of the total rice-planting area in China (Table 1). In some regions, such as Jiangxi and Sichuan provinces, more than 90% of the rice varieties grown belong to hybrid rice. Considering the overwhelming majority of new hybrid rice evaluated in the national trial of the southern Chinese rice-growing region (Table 2), we can anticipate that the area under hybrid rice in China will reach 70% (about 20 million ha) of the total rice area in 2010. It is also expected that hybrid rice will be grown on 30–35 million ha in 2020 in countries outside China.

Table 1. Annual planting area under hybrid rice in China in 1976-2006.[a]

Year	Planting area (million ha)	% of total rice area
1976	0.14	0.4
1978	4.34	12.6
1982	5.62	17.0
1986	9.00	27.9
1990	13.62	41.2
1997	17.73	55.8
1999	16.55	52.9
2002	18.30	64.9
2004	18.61	65.6
2006	19.19	65.5
2010	–	>70?

[a]Data for 1976-97 are provided by Dr. Zhu Defeng of CNRRI, and the others are collected from the Year Statistics of the Ministry of Agriculture, China.

Table 2. Proportion of new hybrid rice tested in the national trial of the southern China rice-growing region from 1998 to 2005.

Type[a]	Total no. of new varieties tested	No. of hybrids			Proportion of hybrids (%)		
		1998-2005	1998	2005	1998-2005	1998	2005
Early indica	87	57	6	18	65.5	37.5	81.8
Mid indica	277	270	19	110	97.5	90.5	100.0
Late indica	182	171	18	45	94.0	94.4	100.0
Late japonica	50	24	2	8	48.0	20.0	72.7
Huanan early indica	83	62	7	18	74.7	77.8	81.8
Huanan late indica	44	41	5	11	93.2	71.4	100.0
Total	723	625	57	210	86.4	69.1	95.0

[a]Huanan indicates the southernmost part of China, including Guangdong, Guangxi, and Hainan provinces.

Table 3. Different CMS resources used in the hybrid rice breeding program.

CMS type	MS model	Origin of CMS	Representative CMS lines/hybrids
WA-CMS	Sporophyte sterility	Natural wild rice abortive plant in Hainan	Zhenshan 97A/Shanyou 63
G-CSM	Sporophyte sterility	Gambiaka from West Africa	Gang 46A/Gangyou 22
D-CMS	Sporophyte sterility	Indica rice Dissi D52/37	D62 A/Dyou 68
ID-CSM	Sporophyte sterility	Indonesia 6 from Indonesia	II-32 A/IIyou 838
DA-CSM	Sporophyte sterility	Dwarf wild rice in Jiangxi	Xieqingzao A/Xieyou 46
K-CSM	Sporophyte sterility	Japonica rice K52	K17 A/Kyou 402
HL-CSM	Gametophyte sterility	Red-awned wild rice	Yuetai A/Honglianyou 6
BT-CSM	Gametophyte sterility	Chinsurah BoroII/Taichong 65	XiulinA/Xiuyou 57
DT-CSM	Gametophyte sterility	Japonica rice Taibei 8	DianyiA/Diaza 25

The rapid development of hybrid rice in China has been mainly attributed to innovations in breeding technology. Since the first WA-CMS plant of rice was found in 1973, diverse breeding approaches, including the integration of traditional methods and novel technologies, have been adopted.

Breeding strategy of hybrid rice in China

Exploitation and use of various male sterility resources

To date, CMS has been found to be the most effective system for developing rice hybrids in China and outside China. WA-CMS was the first male sterile type to be used in hybrid rice breeding. To minimize the potential damage from rice diseases caused by a unique CMS genetic background, other CMS resources have been exploited and used in the breeding of new hybrids. Other than WA-CMS, seven kinds of CMS resources are being used in the hybrid rice breeding program in China (Table 3). Although the proportion of CMS-WA-type hybrids dropped from 69% in 1996 to 46.7% in 2003 (Fig. 2), it remains the dominant type in three-line hybrid rice production. Among the other types of CMS, ID-CMS has been used widely in recent years because of its good flowering habits and grain quality. Its proportion has increased quickly from less than 10% in 1996 to more than 20% in recent years.

Although the CMS system has been successful in exploiting heterosis in rice, it is labor-intensive as it involves three lines (CMS, maintainer, and restorer). Its use is also restricted to available maintainers and restorers, which are not abundant. Shi (1981) first reported that Nongken 58S, a male sterile mutant from japonica variety Nongken 58, was a photoperiod-sensitive genic male sterile (PGMS) line. The development of environmentally induced genic male sterile (EGMS) rice, including PGMS,

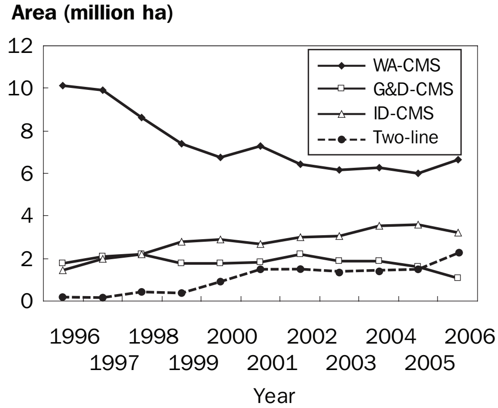

Fig. 2. Annual change in planting area of two-line hybrids and major types of CMS hybrids.

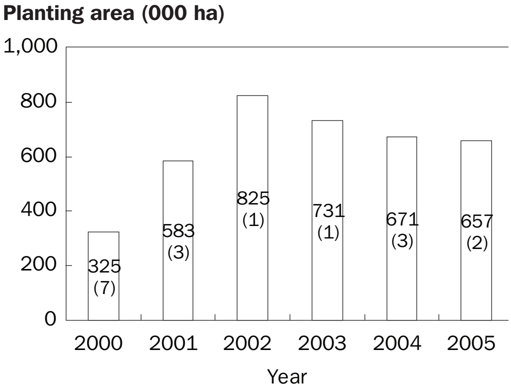

Fig. 3. Change in planting area of Liangyoupei 9, a pioneer two-line hybrid (numbers in parentheses refer to the rank of planting area).

thermosensitive genic male sterilily (TGMS), and photo-thermosensitive genic male sterility (P-TGMS), has laid the foundation for using a two-line system for producing hybrid rice seeds (Cheng et al 1996). Nongken 58S has been a main donor for developing new EGMS. Peiai 64S, an indica P-TGMS line derived from Nongken 58S, has been widely used in the two-line hybrid rice breeding program. In 2006, the planting area of the two-line hybrids occupied 15% of the total rice area, replacing the third place of G&D-type hybrids (Fig. 2). Liangyoupei 9, a pioneer two-line hybrid developed from Peiai 64S × 9311, moved into first place in planting area in 2002-03 (Fig. 3), replacing Shanyou 63, a WA-CMS-type hybrid. The production of two-line hybrids in China is promising as long as the unstable fertility problem in EGMS can be solved.

Table 4. Some traits concerning outcrossing capacity in Zhong 9A and Zhenshan 97A.

Outcrossing traits	Zhong 9A	Zhenshan 97A
Exserted stigma (%)	82.3	39.6
Outcrossing seed setting (%)	75.6	35.7
Seed yield (t ha^{-1})	3–4.5	2.2–3

Development of male sterile lines with high grain quality and outcrossing capacity

The first generation of CMS lines generally has a high general combining ability (GCA) with restorer lines, but they have unfavorable grain quality and usual outcrossing. To improve the grain quality of hybrid rice, it is necessary to improve the grain quality of both the maternal and paternal lines. To enhance the efficiency of hybrid seed production, it is necessary to increase the yield of hybrid seed by improving the outcrossing capacity of CMS lines. With great efforts, Chinese scientists have developed a large number of high-quality CMS or EGMS lines with improved outcrossing capacity, which has accelerated overall improvement in the grain quality of newly bred hybrids (Yang et al 2006). For instance, Zhong 9A, an ID-CMS line developed by CNRRI, has been used widely because it possesses excellent outcrossing capacity (Table 4) and high grain quality (Cheng et al 2007).

Combination of ideal plant type with intersubspecific heterosis

According to pedigree analysis, the majority of inbred varieties and hybrids of rice grown in China have few ancestors. For semidwarf indica rice in southern China, most of the *sd* genes can be traced to Aizizhan and Aijiaonante. Moreover, IR varieties are the major donors of the *Rf* gene in restorer lines of three-line hybrid rice, and Japanese varieties compose the major genetic background of japonica rice in northern China. Results of RFLP variations confirmed the narrowing genetic base in indica cultivars and their parental lines (Zhuang et al 1997). This places a potential limit on further exploiting intraspecific heterosis.

The use of heterosis between the two subspecies of rice, indica and japonica, has long been considered a promising approach to further enhance the heterosis of rice. However, F_1 semisterility has generally been encountered in these crosses of rice, making it meaningless for direct use in hybrid rice breeding. In addition, distant crosses do not always increase F_1 yield, and this is particularly true when the parental lines belong to different subspecies. It is necessary to innovate with breeding techniques to use intersubspecific heterosis.

We constructed a set of doubled-haploid (DH) populations derived from an intersubspecific cross. All lines were detected with 28 indica-japonica differential RFLP markers, and the indica-japonica differential index was calculated for each line. Using the DH lines as the male parent, and maintainer lines Xieqingzao B showing typical indica characteristics and 064B having an intermediate differential index inclinable

Table 5. Some super hybrids derived from hybridization of indica (*i*), japonica (*j*), or intermediate-type (*m*) rice.

Variety	Pedigree	Pedigree of parental lines
Xieyou 9308	Xieqingzao A/R9308	R9308: C57 (*j*)//No. 300 (*j*)/IR26(*i*)
Liangyoupei 9	Peiai64S/9311	Peiai 64S: Nongken 58S(j)/Peiai 64(i)//Peiai 64) (i)
Iiyou 602	II-32A/Luhui 602	Luhui 602: 02428(*j*)/Gui 630(*i*)//IR24(i)
Iiyou 7954	II-32A/Zhehui 7954	Zhehui 7954: R9516/M105 R9516: Peiai 64S/Teqing M105: Milyang 46(*i*)/Lunhui 422(*m*)
Guodao 1	Zhong9A/R8006	R8006: IRBB60//T2070/Duoxi1
Guodao 3	Zhong8A/R8006	T2070: WL1312(*j*)/Lunhui 422(*m*)//Minghui
Guodao 6	Nei 2A/R8006	63(*i*)
Liaoyou 5218	Liao 5216A/ C418	C418: Lunhui 422(m)/Milyang 23(i)

to indica as the female parents, two sets of F_1 hybrids were produced, respectively. Results indicated that F_1 yield was related to the indica-japonica differential index of the parents. The medium-type lines, including indica-inclined or japonica-inclined lines, were generally advantageous for a higher F_1 yield and had more harmonious plant type (Cheng et al 2007). In recent years, medium-type parents have been widely used in the super hybrid rice breeding program in China. In the areas of southern China that grow indica rice, japonica components have been integrated into an indica background in the rice breeding program, and the reverse in the regions of northeastern China growing japonica rice. By this means, a series of indica-inclined or japonica-inclined restorer lines has been developed and employed as parental lines to develop super hybrids (Table 5).

Consideration of the root system as a selection criterion for super rice

The root system is a vital part of the rice plant. It plays a very important role in absorbing nutrients and water from the environment, and in anchoring rice plants to the ground firmly. It is related to both drought tolerance and lodging resistance. It is the foundation of rice productivity. In recent years, some root parameters have been considered in the super rice breeding program.

Our research indicated that roots with a diameter of less than 0.2 mm were very important for rice growth. Although these roots account for only less than 8% of the total root volume, they play a very important role in water and nutrient absorption because they occupy about 75% of the total root length and more than 50% of the

Table 6. Comparison of yield and root traits among four rice varieties.

Variety[a]	1,000-grain weight (g)	No. of productive panicles m^{-2}	No. of spikelets m^{-2}	Seed setting (%)	Yield (kg ha^{-1})	Percentage of dry root weight (%)		Root depth index
						0–20 cm	<20 cm	
Xieyou 9308	25.31	278.7	4.1×10^4	86.2	8,325.0	74.5	25.5	14.2
Shanyou 63	28.89	293.1	3.5×10^4	77.2	7,276.5	77.9	22.2	13.0
Xiushui 11	29.12	340.2	2.9×10^4	86.2	6,844.5	80.6	19.4	12.4
Teqing	22.35	246.4	3.1×10^4	78.2	5,517.0	95.5	4.5	8.5

[a] Shanyou 63, indica hybrid variety; Xiushui 11, japonica inbred variety; Teqing, indica inbred variety.

total root surface area. To increase the functional efficiency of the root system, the total amount of roots should be controlled and the mean length and number of root branches should be increased. The volume of the roots 20 cm below the soil surface should occupy 20–40% of the total root system. Another important root parameter that should receive attention is nonrooting nodes. The suitable number of nonrooting nodes per productive tiller is 5–8. Xieyou 9308, a super hybrid derived from an intersubspecific cross, had a high yield because of its high proportion of roots 20 cm below the soil surface and high root depth index (Table 6).

Marker-aided selection for restorer lines of hybrid rice

As a result of the development of rice genomics research since the late 1980s, molecular marker-aided selection (MAS) has been widely used in rice breeding programs. In our hybrid rice breeding program, MAS has been used successfully to develop restorer lines with the bacterial blight (BB) resistance gene.

Backcrossing was done using L1 as the recurrent parent and IRBB60 as the donor parent. L1 is a restorer line selected from Duoxi 1/R2070, and it has good combining ability but is susceptible to BB. IRBB60 introduced from the International Rice Research Institute (IRRI) is a restorer line resistant to BB carrying the dominant resistance genes *Xa4* and *Xa21* and the recessive resistance genes *xa5* and *xa13* (Huang et al 1997). The offspring of the backcrosses were detected with DNA marker pTA248, which is tightly linked to *Xa21* to select resistant lines, whereas restoring ability was tested by conventional testcrosses. Four BB strains were inoculated artificially to verify the resistance of candidate lines.

On the basis of phenotypic selection in each generation, the individuals carrying *Xa21/xa21* heterozygous were selected. By means of four successive backcrosses and selection, 43 individuals from BC_4F_1 carrying *Xa21* were selected. After selfing, the individuals carrying *Xa21/Xa21* homozygous were screened based on the DNA marker. Two elite restorer lines (R8006 and R1176) with resistance to BB and excellent agronomic traits were selected (Cao et al 2003).

The restorer R8006 has shown great potential in the hybrid rice breeding program. In total, five hybrids, Guodao 1, Guodao 3, Guodao 6, IIyou8006, and Tianyou 6, have been developed by crossing R8006 with various CMS lines. All five hybrids have been commercially released and they showed high resistance to diseases, good quality, and high yield potential in national and provincial trials for adaptability and yield. Among them, Guodao 1, Guodao 3, and Guodao 6 have been nominated as super rice varieties.

Future outlook on hybrid rice research

China has been a leading country in hybrid rice breeding. To further increase the yield of rice through heterosis breeding technology, some key issues for future research should be considered.

Root system vigor at various growth stages, particularly during the grain-filling period, should be comprehensively considered in a hybrid rice breeding program. However, it is very difficult to study roots using the traditional method in the field. We have used a new planting method using a float-bed on water instead of the traditional field method, which greatly decreases damage to the roots. We have screened the root parameters for parents on a large scale using the float-bed planting method. A primary study indicates that controlling root number, improving root depth, and developing efficient root activity per unit of root biomass will be needed for high-yielding rice in a future breeding program (Wu and Cheng 2005).

Although hybrid rice has yielded more than 10% more than popular inbred rice varieties, little is known about the genetic mechanism of the formation of heterosis in rice. The availability of high-density genetic linkage maps now makes it possible to identify and study the effects of the individual loci underlying quantitatively inherited traits (QTLs). Many scientists work to elucidate the genetic basis of heterosis, but progress is slow. Rice breeders still select F_1 hybrids on the basis of large-scale crossing. Recently, we tried to pyramid different yield QTLs and major genes for disease resistance into restorer lines by MAS. The new restorer lines have shown a high combining ability with different CMS lines (Cheng et al 2004).

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Notes

Authors' address: China National Rice Research Institute (CNRRI), Hangzhou, China; email: shcheng@mail.hz.zj.cn.

Toward the development of a Green Super Rice using genomics-based strategies

Qifa Zhang

From a global viewpoint, several challenges need to be met to sustain rice production: (1) increasingly severe occurrence of insects and diseases and indiscriminate pesticide applications, (2) high pressure for yield increase and overuse of fertilizers, (3) water shortage and increasingly frequent occurrence of drought, and (4) extensive cultivation on marginal lands. A combination of approaches based on recent advances in genomics research has been formulated to address these challenges, with the long-term goal of developing rice cultivars referred to as Green Super Rice, which should possess multiple resistance to insects and diseases, high nutrient efficiency, and drought resistance. This approach can greatly reduce the consumption of pesticides, chemical fertilizers, and water. Breeding efforts have focused on identifying germplasm and discovering genes for resistance to diseases, insects, and stresses such as drought and genes that can enhance N- and P-use efficiency, grain quality, and yield. Genes for almost all traits have now been isolated from a global perspective and are being gradually incorporated into the genetic backgrounds of elite cultivars by molecular marker-assisted selection or transformation. It is anticipated that such strategies could eventually lead to the development of a Green Super Rice.

In the last half century, rice yield has had two big leaps, primarily the result of genetic improvement—increasing harvest index by reducing plant height and making use of the semidwarf gene and heterosis by producing hybrids. To further increase yield potential, several major national and international programs initiated in the last decade have made significant progress. The goal is to develop "super rice" or "super hybrid rice" that can break the yield ceiling.

However, several challenges have to be met to increase rice production in a sustainable manner. The first is the increasingly severe occurrence of insects and diseases in almost all rice-producing areas, causing huge yield losses. Three diseases—bacterial blight, fungal blast, and sheath blight—are considered to be the most devastating diseases in most rice-growing regions. Similarly, three groups of insects—stem borers, leaffolders, and planthoppers (mostly brown planthopper, or BPH)—are regarded as the most damaging pests. For a long time, disease and insect control has relied heavily on indiscriminate application of chemical pesticides. Chemical control is not very effective and poses many hazards. Such an intensive use of chemicals gives rise to serious environmental pollution, putting the health of rice producers and rice consumers at risk. It kills natural enemies, resulting in pest outbreaks. Heavy crop loss is a frequent occurrence in many rice-producing areas.

The second challenge is related to fertilizer application. There has been a dramatic increase worldwide in fertilizer applications in the last 40 years, and this has particularly been the case in certain regions of the world. For example, in 2002, China used approximately 30% of N and P fertilizers produced worldwide, although its arable land accounts only for less than 10% of the world's total. Peng et al (2002) compared China with other major rice-producing countries in terms of N fertilizer efficiency. They found that N application per unit rice area in China was 75% higher than in other countries, but that N-use efficiency was much lower. Overfertilization not only greatly reduced economic returns from fertilizer use, placing a heavy economic burden on farmers, but also resulted in widespread water eutrophication. Moreover, overapplication of N fertilizer often reduced rice grain yield as plants grown under excess N conditions became more susceptible to lodging and pest damage. Overapplication of N fertilizer also partly accounts for the poor eating and cooking quality of the rice grains produced. Thus, developing crops that do not heavily depend on fertilizers is essential for agricultural sustainability.

The third major challenge stems from the global water shortage. Again, taking China as an example, it is estimated that total water usage in this country is about 557 billion m^3 per year. Agriculture uses 392 billion m^3, accounting for 70% of the country's total water consumption, of which approximately 70% is used for rice production alone (Tang et al 2000). Drought stress is still identified as the single most important constraint to rice production in many rice-producing areas in China (Lin and Shen 1996). Various factors—variation in annual rainfall patterns, uneven distribution of rainfall in the rice-growing season, and inadequate rainfall in many areas—contributed to this situation.

There is also a tremendous need to improve rice grain quality. Historically, many breeding programs focused on yield potential alone as the primary target, particularly in China. Consequently, many popular high-yielding cultivars and hybrids have relatively poor quality. With the rise in standards of living, improvement in cooking and eating quality and grain appearance has become a priority. Additionally, more than half of the world's population, mostly the poor in developing countries, suffer from the devastating consequences of micronutrient malnutrition. In countries where rice is the major staple food, there is a need to improve the nutritional quality of rice grains to enhance micronutrient intake.

Thus, as the main staple, rice has to be produced in such a way as to meet increasing requirements, both in quantity and quality, and in harmony with the environment. Ensuring sustainability means a gradual reduction in the use of pesticides, fertilizers, and water, while still achieving a continuous yield increase and quality improvement. The goal is to produce Green Super Rice (GSR) with the following characteristics:

adequate resistance to major diseases and insects, high efficiency in nutrient uptake and use, resistance to drought and other abiotic stresses, good quality, and increased yield potential. This chapter aims to provide a perspective of the strategies and useful resources that can be used and to describe the progress made so far in the development of GSR.

Strategies for developing GSR

Rice is rich in germplasm resources. The International Rice Gene Bank holds more than 105,000 types of Asian and African cultivated rice and around 5,000 ecotypes of wild relatives (www.irri.org/GRC/GRChome/Home.htm). In addition, many major rice-producing countries have established their own national germplasm banks. Collectively, these germplasm collections contain genes that can be used to address a broad range of research objectives.

With the completion of the rice genome sequencing project, there have been rapid developments in functional genomics resources, including large mutant libraries by T-DNA insertion, transposon tagging, and chemical mutagenesis (Hirochika et al 2003). Whole-genome microarray techniques have been developed and applied to obtain profiling expression of all the genes in the entire life cycle of rice growth and development. Full-length cDNAs for both indica and japonica rice have been constructed, with a total of more than 40,000 full-length cDNA clones becoming available (The Rice Full-Length cDNA Consortium 2003, Liu et al 2007).

Such germplasm and genomics resources have provided an unprecedented opportunity for rice genetic improvement. To develop GSR, a combination of strategies has been formulated by integrating germplasm, genomics resources, and molecular technology and breeding with insect and disease resistance, N- and P-nutrient-use efficiency, drought resistance, quality, and yield as target traits (Fig. 1). The approaches used for identifying genes and germplasm for the defined traits include screening of germplasm collections, mapping and identification of genes, screening of mutant libraries, microarray analysis of differentially regulated genes, and functional testing of candidate genes by transgenic analysis. The genes would then be incorporated into breeding lines, either by transformation or molecular marker-assisted selection (MAS), and accumulation of the desired genes would result in progressive improvement of rice cultivars, eventually leading to GSR.

Progress in gene identification and development of GSR

Resistance to stem borers and leaffolders

In the last two decades, considerable research effort has been invested in introducing insecticidal crystal protein genes from *Bacillus thuringiensis* (*Bt*) into crops, including rice. Although the use of these *Bt* crops has benefited growers and the environment by greatly reducing the use of chemical insecticides (James 2007), there is increasing concern that widespread adoption of *Bt* crops may lead to the development of resistance to insecticidal genes in pest populations. To manage resistance, high-dose/refuge and

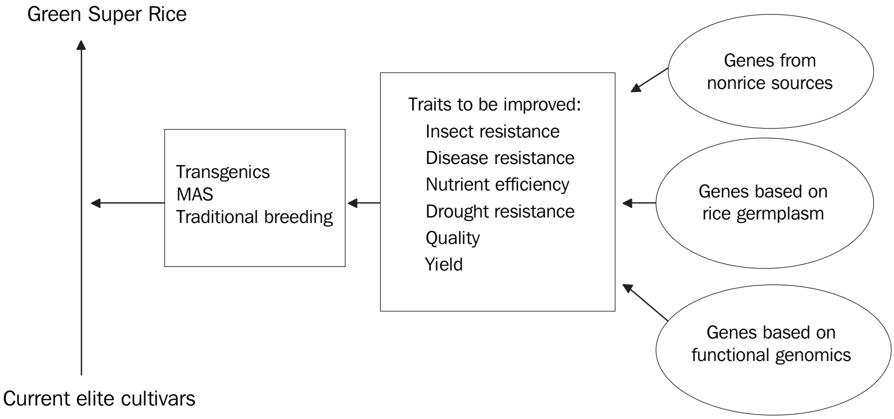

Fig. 1. Schematic representation of combinations of genes and approaches for the development of Green Super Rice (adapted from Zhang 2007).

gene stacking have been proposed as two effective strategies to prevent or delay the occurrence of pest resistance to *Bt* toxins (Frutos et al 1999, Ferre and Van Rie 2002, Shelton et al 2002). Nonetheless, the high-dose/refuge strategy does not seem to be applicable in most rice-producing countries as the majority of the rice growers are small-scale farmers, which makes it difficult to designate certain areas of nontransgenic crops for refuge. The gene-stacking strategy suggests that plants containing two or more dissimilar *Bt* toxins have the potential to delay resistance more effectively than those producing only a single toxin, as the insects have to develop resistance to two or more insecticides in order to survive.

At present, the most commonly used *Bt* genes in transgenic crops, including rice, are *Cry1Ab*, *Cry1Ac*, and a fusion gene of *Cry1Ac/Cry1Ab*, *Cry1Ab/c*. However, binding tests of midgut brush border membrane vesicles showed that *Cry1Aa*, *Cry1Ab*, and *Cry1Ac* toxins share a common binding site (Escriche et al 1997, Ballester et al 1999, Karim and Dean 2000). Thus, a mutant that is able to overcome one of the *Cry1A* genes is also likely to be resistant to other *Cry1A* genes as well. Therefore, combinations of *Cry1A* genes with other groups of *Bt* genes should be explored to prevent or delay the emergence of pest resistance. Based on the assay of δ-endotoxin binding to brush border membrane vesicles of rice stem borers, it was proposed that *Cry1A* genes could be combined with *Cry1C*, *Cry2A,* or *Cry9C* for more durable resistance in transgenic plants (Alcantara et al 2004).

A *Cry1Ab/c* fusion gene was transformed into Minghui 63, the restorer line for a number of elite rice hybrids widely cultivated in China (Tu et al 2000). Results from large-scale field tests in several provinces of China showed that this line and its hybrid with Zhenshan 97A, the male sterile line of the most popular hybrid Shanyou 63, were highly resistant to leaffolders and stem borers under field conditions. There is thus great potential to significantly reduce insecticides, labor, and related costs, while at the same time increase the yield and protect the health of farmers and consumers

(Huang et al 2005). This line and the hybrid have now completed the production demonstration stage following the Chinese government's regulatory procedures.

To address the issue of resistance management, transgenic rice lines were developed using Minghui 63 as the recipient to individually harbor the codon-optimized *Cry1C* and *Cry2A* genes (Tang et al 2006, Chen et al 2005). Transgenic plants with a single-copy transgene were crossed with Zhenshan 97A. Field tests showed that both transgenic lines and their hybrids with Zhenshan 97A exhibited strong resistance to natural infestation of leaffolders and stem borers. Together with the lines individually harboring *Cry1Ab/c*, *Cry1Ab*, and *Cry1Ac* in the Minghui 63 background, the transgenic lines with the new *Bt* genes have provided the critical materials and the flexibility for developing rice lines with various combinations of multiple resistances by gene stacking.

Resistance to brown planthoppers

Brown planthoppers (BPH) are often regarded as the most destructive pest of rice in many countries. Nineteen BPH resistance genes have been identified from cultivated and wild rice species (Zhang 2007). Molecular mapping of these genes has facilitated MAS of BPH resistance genes.

Sharma et al (2004) performed a molecular marker-assisted pyramiding of two BPH resistance genes, *Bph1* and *Bph2*, into a japonica line. BPH bioassays showed that the resistance of the pyramided line was equivalent to that of the *Bph1*-single introgression line, which showed higher resistance than the *Bph2*-single introgression line.

Making use of a molecular marker linkage map, Huang et al (2001) identified two genes—*Qbh1* and *Qbh2* (renamed *Bph14 and Bph15*)—for BPH resistance from B5, a highly resistant line that derives its resistance genes from wild rice *Oryza officinalis*. Both genes had large effects on BPH resistance and the two loci acted essentially independent of each other in conferring the resistance. These two genes were subsequently incorporated by MAS into a number of important parental lines in hybrid rice breeding programs in China. Lines containing either or both of the genes showed enhanced resistance to BPH with artificial infestation (He and Qi, unpublished).

Although the large number of genes identified so far has provided a rich source for developing BPH-resistant cultivars, knowledge about insect biotypes in various rice-producing areas is still lacking. Thus, a detailed characterization of the resistance genes against insect populations in the field is essential for efficient deployment of these genes.

Identifying genes for disease resistance and developing disease-resistant rice

Dozens of genes that confer resistance to bacterial blight and fungal blast have been identified, many of them having been cloned in recent years (Zhang 2007). In addition, a number of genes with strong resistance have been fine-mapped. This development has provided markers either based on DNA sequences of the genes (frequently referred to as functional markers) or closely linked to the genes for pyramiding by MAS.

For targeted improvement of an elite rice hybrid, MAS was conducted to introgress *Xa21* into Minghui 63 (Chen et al 2000). The improved hybrid has been used in commercial rice production in China. MAS has also been successfully applied to pyramid four genes for bacterial blight resistance (*Xa4*, *xa5*, *xa13*, and *Xa21*) in various combinations (Huang et al 1997). The resulting lines showed enhanced resistance in terms of both resistance spectra and disease level, demonstrating the usefulness of marker-assisted pyramiding in developing cultivars with multiple resistance.

It is important to understand the pathogenicity spectrum of the pathogen populations in order to have targeted development in breeding for disease resistance. Chen et al (2001) assayed pathotypes of 792 single-spore isolates of *Pyricularia grisea* using samples collected from 13 major rice-growing provinces of central and southern China. These isolates were tested by inoculation with 13 host differentials consisting of six indica and seven japonica near-isogenic lines (NILs), collectively carrying most of the genes for blast resistance previously identified. The results showed a large difference in the frequencies of the isolates producing compatible reactions on the NILs. For example, a very small proportion (10% or less) of the isolates could cause compatible reactions on NILs carrying *Pi1* or *Pi2*, but a large proportion (41.5%) could overcome the resistance of the NIL carrying *Pi3*. Moreover, a combination of *Pi1* and *Pi2* would be susceptible to only 2% of the isolates and adding *Pi4* would make this proportion even smaller. The data provided very useful information for formulating strategies for improving blast resistance in rice breeding programs, which have now been implemented.

Although MAS is effective for pyramiding disease resistance genes, the transgenic approach may still have a role in using cloned genes. For example, *Xa26* was isolated from indica cultivar Minghui 63 (Sun et al 2004), in which it confers moderate resistance against bacterial blight at the seedling and adult stages. When this gene was introduced into several japonica cultivars with its native promoter, transgenic plants showed enhanced resistance, featuring a broadened resistance spectrum, and increased the resistance in extended growth stages (Cao et al 2007). It was shown that the enhanced resistance is closely associated with the elevated expression of the gene. Overexpressing *Xa26* with a constitutive strong promoter further enhanced the resistance in both indica and japonica backgrounds, whereas regulating the gene expression by a pathogen-inducible weak promoter impaired resistance. These results suggest that optimizing the expression of resistance genes by the transgenic approach could be combined with MAS to develop resistant cultivars.

With the increasing availability of cloned disease resistance genes, new strategies are now feasible for developing cultivars with multiple resistance. For example, cloning of multiple genes with different resistance spectra into a single construct to transform rice may be employed for developing cultivars with broad-spectrum and durable resistance. Compared with pyramiding of multiple genes using MAS, the transformation of a single construct containing multiple genes not only takes less effort to develop; it also has the advantage of being transferred and transmitted as a single unit to different genetic backgrounds and from one generation to another, given that the efficacy of the genes can be individually tested. This could be expanded to include in the same cassette genes for insect resistance as well as those for other traits, in addition to the disease resistance genes. If properly optimized, this strategy hold great promise for the efficient development of cultivars with multiple resistance.

The difficulty now is the improvement of resistance to sheath blight, a major disease of a number of cereals in addition to rice. So far, no major gene for sheath blight resistance has been identified, although a few QTLs have been reported. Thus, intensive efforts based on advances in genomics research in both host and pathogen systems are needed to develop innovative technology to combat this disease.

Identifying genes for nutrient-use efficiency

Reducing fertilizer application in fertile soils and increasing productivity in poor soils both require improvement of nutrient-use efficiency, including both uptake and use efficiency. Unlike the traits described above, not many genes are currently available to be directly useful for developing nutrient-efficient cultivars. Thus, most of the work is still at the stage of discovering genes and QTLs for nutrient efficiency.

N uptake and assimilation pathways in higher plants have been well documented. They involve a variety of transporters functioning to absorb the nutrients from the soil and a number of enzymes for assimilation and transfer of the absorbed N into amino acids and other compounds. However, little is known regarding how these elements and the processes are regulated, especially under low-N conditions, although efforts have been made to identify QTLs for low-N tolerance (Lian et al 2005) and characterize the genes and processes involved in early response to low-N stress (Lian et al 2006).

The overwhelming majority of soils in rice-producing areas are P-deficient with a high P-fixing capacity (Li 1985). Moreover, it is highly alarming that global P resources will be exhausted before the end of this century (Vance et al 2003). Thus, improving the uptake efficiency of the rice plant in P-fixing soils has been a major research target. Yi et al (2005) identified *OsPTF1,* a P-deficiency responsive transcription factor containing a bHLH domain. Transgenic plants of a low-P sensitive rice variety Nipponbare overexpressing *OsPTF1* showed enhanced P efficiency in both solution and soil cultures. Tillering ability, root and shoot biomass, and P content of the transgenic plants were about 30% higher than those of wild-type plants in P-deficient culture solution. In soil pot and field experiments at low-P levels, tiller number, panicle weight, and P content increased more than 20% in transgenic plants compared with wild-type plants.

Wissuwa and Ae (2001) analyzed P uptake of 30 rice varieties representing a wide diversity of cultivated rice germplasm on normal and P-deficient soils. The analysis revealed very wide variation in low-P tolerance, as measured by P uptake on P-deficient soil relative to that on normal soil, indicating a high potential for using natural variation to improve the P efficiency of rice cultivars. Wissuwa and Ae (2001) further developed NILs for two QTLs—a major one on chromosome 12 and a minor one on chromosome 6—by introgressing the alleles from Kasalath, a P-efficient variety, into Nipponbare, a P-inefficient variety. P uptake of the NIL carrying the Kasalath allele of the QTL on chromosome 12 on a P-deficient upland soil was three to four times that of Nipponbare, whereas the advantage of the NIL carrying the Kasalath allele of the QTL on chromosome 6 was in the 60–90% range.

These genes hold promise for improving P-uptake efficiency of the rice crop, although further study is needed to evaluate their effectiveness in the genetic backgrounds of elite cultivars under diverse field conditions.

Identifying genes for drought resistance and developing drought-resistant rice

The mechanisms of drought resistance include drought escape via a short life cycle or developmental plasticity; drought avoidance (DA) via enhanced water uptake and reduced water loss; and drought tolerance (DT) via osmotic adjustment, antioxidant capacity, and desiccation tolerance. It is thus important to understand the genetic basis of the individual components, especially DA and DT, in order to formulate strategies for developing drought-resistant cultivars. Yue et al (2006) analyzed the genetic bases of DT and DA at the reproductive stage in rice using a recombinant inbred line population from a cross between Zhenshan 97 (irrigated rice) and a drought-resistant upland cultivar IRAT109. A total of 27 QTLs were used for seven traits of relative performance of fitness and yield, 36 QTLs for five root traits under control, and 38 for seven root traits under drought stress conditions, suggesting the complexity of the genetic basis of both DT and DA. Only a small portion of the QTLs for fitness and yield-related traits overlapped with QTLs for root traits, indicating that DT and DA had distinct genetic bases.

MAS has been applied to pyramid QTLs for several root traits (Shen et al 2001, Steele et al 2006), which resulted in positive effects for increasing root length and root mass. However, the effects of the change in root traits on drought resistance at the field level remained to be determined.

Great efforts were also made to identify genes for the development of drought-resistant rice. Garg et al (2002) overexpressed a fusion gene made of two *Escherichia coli* trehalose biosynthetic genes (*otsA* and *otsB*) in rice. Depending on growth conditions, the transgenic rice plants accumulate trehalose at levels three- to tenfold higher than that of the nontransgenic controls. Compared with nontransgenic rice, several independent transgenic lines exhibited sustained plant growth, less photo-oxidative damage, and more favorable mineral balance under salt, drought, and low-temperature stress conditions.

In another study, Hu et al (2006) identified a transcription factor gene, *SNAC1,* as showing elevated expression by drought stress, based on data from a cDNA chip analysis. Overexpression of *SNAC1* in rice significantly enhanced drought resistance in transgenic rice (22–34% higher seed setting than the control) in the field under severe drought stress conditions at the reproductive stage while showing no phenotypic changes or yield penalty. The transgenic rice also showed significantly improved drought resistance and salt tolerance at the vegetative stage.

The challenge ahead is to incorporate these genes into the genetic backgrounds of elite cultivars and hybrids and to evaluate their performance under real field conditions. Gene discovery and innovative strategies based on germplasm screening and functional genomics research are also needed for developing drought-resistant rice cultivars.

Identifying genes for quality improvement

Grain quality of rice consists of several components: cooking quality, eating quality, appearance, milling quality, and nutritional quality. Cooking and eating qualities are mostly determined by amylose content (AC), gelatinization temperature (GT), and gel consistency (GC) of the grain starch. Appearance is mainly specified by grain shape as defined by grain length, grain width, length-width ratio, and translucency or chalkiness of the endosperm.

Molecular marker-based genetic analysis in the last decade has established that each quality trait is mainly conditioned by a major locus. For example, the *Wx* locus on chromosome 6 plays major roles in specifying AC and GC and it has a minor role in GT (Tan et al 1999, Wang et al 2007). The *Alk* locus, tightly linked to *Wx*, has a major effect on GT (He et al 1999, Wang et al 2007). As for quality traits related to appearance, grain length is mostly controlled by the *GS3* locus on chromosome 3 and grain width is largely conditioned by *GS5* on chromosome 5 (Tan et al 2000). A major locus for chalkiness (*Chk5*) was also identified on chromosome 5 (Tan et al 2000). Several genes for these traits have been cloned (Wang et al 1990, Gao et al 2003, Fan et al 2006, Song et al 2007).

The single locus inheritance clearly indicated that MAS can play a major role in quality improvement. Indeed, Zhou et al (2003) were able to simultaneously improve the quality of Zhenshan 97, the female parent of a number of widely used hybrids in China, which has poor quality because of its high AC, low GC, low GT, and chalky endosperm.

The transgenic approach has been successfully applied to enhance micronutrient contents of rice cultivars. Perhaps the most successful example is the development of Golden Rice with an engineered pathway for provitamin A biosynthesis (Ye et al 2000, Paine et al 2005). Currently, there is a coordinated international initiative for biofortification of the rice grain with provitamin A, iron, and zinc. Such effort will provide rice cultivars that can help improve the nutritional status of people in target areas.

Identifying genes for yield traits

For a long time, yield has been generally regarded as a complex trait that is controlled by multiple genes with small effects. However, the development of QTL-based NILs revealed that many QTLs have major effects in homogeneous genetic backgrounds, which has enabled QTL cloning following the map-based cloning approach. Several QTLs for yield components have now been cloned, including ones for number of tillers per plant (Li et al 2003), number of grains per panicle (Ashikari et al 2005, Xue et al 2008), and grain size (Fan et al 2006, Song et al 2007).

The major effects observed between the NILs and the cloning of QTLs have fundamental implications for yield improvement. This suggests that yield, like other traits, can also be improved by individually manipulating component traits using both MAS and transformation. A limiting factor may be the biological activity of rice plants to provide sufficient carbohydrates to achieve the grain yield set by the component traits, so that the gain in one component trait is not compensated for by a loss in other traits. Progress in expressing C_4 photosynthetic enzymes in rice to increase photosynthetic rate has been reported in recent years (Ku et al 2000); this may prove to be a promising line of research. Genetic variation in photosynthesis-related traits in rice germplasm may also be explored.

Prospects

The development of GSR with improved insect and disease resistance, greater N- and P-use efficiency, drought resistance, high grain yield, and superior quality is a critical factor in sustainable rice production. The goals of GSR can be achieved in a two-stage process. In the first stage, which has been partly realized, elite lines carrying single genes of interest are developed and thoroughly evaluated, which by themselves are useful for varietal release. In the second stage, the genes introduced into these lines will be combined in various ways to develop cultivars with the desired traits for GSR. Use of these cultivars will result in increased rice productivity with much reduced inputs, thus ensuring greater sustainability of rice production in particular and agriculture in general.

A big challenge is the assembling process to combine all the favorable alleles into a single cultivar and ensure their proper functioning. In this regard, it may be more advantageous to focus on hybrids than on conventional pureline cultivars, as it may take less effort to have two complementary sets of genes in two parental lines than to stack all the genes in a single genetic background.

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Notes

Author's address: National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan) and National Center of Crop Molecular Breeding, Huazhong Agricultural University, Wuhan 430070, China. Email: qifazh@mail.hzau.edu.cn.

Priorities of IRRI hybrid rice breeding

Fangming Xie

IRRI has been playing a crucial role in researching and developing hybrid rice in the tropics for 30 years, with a large number of hybrid rice varieties and parental lines released and being used in commercial rice production. Hybrid rice technology provides a tool for raising rice yield per unit area and also a great opportunity for agricultural business. For a further sustainable increase in rice production and hybrid rice technology dissemination, priorities for hybrid rice research at IRRI will focus on increasing yield for both hybrid seed and grain production, reducing seed cost, improving grain quality and resistance to stress, and increasing breeding efficiency. Close collaboration and partnership between public research institutes and the private sector are needed for hybrid rice acceleration.

Keywords: hybrid rice, heterosis, seed production, priorities

The International Rice Research Institute (IRRI) has been doing research on hybrid rice heterosis and adaptation of hybrid rice technology in the tropics since 1979 with the rationale of increasing rice production and productivity beyond the level of semi-dwarf high-yielding inbred rice varieties. In collaboration with IRRI, many national agricultural research and extension systems (NARES) in Asian countries have also given priority to hybrid rice development as a tool for meeting the future challenges of food security and environmental protection. After 30 years of development, hybrid rice technology has been extended widely into many countries in large-scale commercialization that has been making a significant impact on rice production and the food supply, as well as social and economic development. Technology research, refinement, product development and release, as well as capacity building and international collaboration at IRRI and in NARES for developing hybrid rice have been playing a crucial role for dissemination of this technology. Moreover, as the technology developed, many private-sector organizations, including multinational and local seed companies, grower associations, nongovernmental organizations, etc., have been involved in technology development as an agribusiness investment, which has been accelerating the extension of hybrid rice commercialization. In 2007, an estimated 2.8

Table 1. Major hybrid-rice growing countries and estimated growing area in 2007 (000 ha) outside of China.

Country	Bangladesh	Brazil	India	Indonesia	Philippines	Vietnam	USA	Total
Area	300	35	1,100	130	341	650	242	2,798

million hectares of commercial rice hybrids were grown in the countries outside of China (Table 1). These major hybrid rice-growing countries are Bangladesh, Brazil, India, Indonesia, the Philippines, Vietnam, and the United States. Other countries, such as Egypt, Malaysia, Pakistan, Thailand, Sri Lanka, and Myanmar, are accelerating research and investment in hybrid rice technology development and product commercialization.

During the past decades, IRRI has developed a large quantity of hybrid rice parents, germplasm, and hybrids, and distributed these products globally to NARES and the private sector. From 2004 to 2007, a total of 2,150 samples of IRRI-bred hybrid rice germplasm and hybrids were shared with NARES and the private sector in 25 countries for evaluation, which accounted for an average of 1.4 samples per day. The countries that used mostly IRRI hybrid rice germplasm were India, the Philippines, and China. These parents and hybrids have been evaluated under different environments for yield and other agronomic traits, stress tolerance, and grain quality. Some elite and heterotic rice hybrids adapted to local environments were released directly by NARES as commercial hybrid rice varieties or as local hybrid rice parents (Virmani 2003). However, the most important application of IRRI-bred hybrid rice germplasm was for use as gene donor resources for developing local germplasm and hybrid products. IR58025A, for instance, an IRRI-bred cytoplasmic male sterile (CMS) line that has been distributed widely, has been used extensively as a female parent for commercial hybrid rice production and as a donor parent in the hybrid rice breeding programs in Asian countries. The obvious advantages of IRRI-bred lines are high yield, adaptation to tropic environments with great genetic diversity, resistance to or tolerance of diseases, pests, and other stresses, and superior grain quality.

Although hybrid rice has been commercialized on a large scale in rice production, Asian countries face some major constraints to further hybrid rice development. After a survey conducted among NARES and careful analysis of the status of hybrid rice research and development in the region, the major constraints were identified and breeding priorities were set for tackling them in the IRRI hybrid rice program. These breeding priorities are to

- Increase and stabilize yields of seed production
- Enhance yield heterosis in both the dry and wet seasons to more than 20%
- Improve hybrid rice grain quality
- Improve resistance to biotic stresses
- Develop hybrids for unfavorable environments
- Improve breeding efficiency by using biotechnology

Table 2. Ranking of constraints to hybrid rice development.[a]

Trait or factor	Bangladesh	Egypt	India	Indonesia	Korea	Myanmar	Philippines	Thailand	Vietnam	Average
Seed yield and cost	5	1	4	2	3	5	4	1	2	3.0
Yield heterosis	1	4	2	6	2	3	2	4	3	3.0
Grain quality	–	–	1	–	–	1	7	–	–	3.0
Disease or insect resistance	3	2	3	1	–	6	3	6	1	3.1
Grain quality	2	5	7	7	5	4	1	5	5	4.6
Germplasm diversity or source	6	3	6	3	4	8	9	2	4	5.0
Government policy (technology dissemination)	9	9	5	5	1	2	6	3	–	5.0
Production extension service	4	8	8	4	–	7	5	7	6	6.1
Drought tolerance	7	7	–	–	–	–	8	8	8	7.6
Other tolerance (deepwater, cold, heat, salinity, etc.)	8	6	–	–	–	–	10	9	7	8.0

[a] Lower numbers mean more concern as a constraint.

Improving the yield of hybrid seed production to reduce hybrid seed cost

The availability of affordable hybrid rice seeds to farmers is crucial to the success of hybrid rice commercialization since farmers have to use fresh hybrid seeds in each crop season. The low yield of hybrid seed production and high cost of hybrid rice seeds have been the main complaints raised by seed producers and farmers, and were listed as one of the major limitations in Asian countries for hybrid rice extension (Table 2). Currently, the yield of hybrid rice seed production in China is 2.7 t ha^{-1} and it has been reported as high as more than 4 t ha^{-1} in large-scale seed production in many cases. Vietnam has the second highest seed yield in seed production, with an average of 2.0 t ha^{-1}. However, the yields of hybrid rice seed production are still low (generally 0.5 to 1.5 t ha^{-1}) in other Asian countries.

During the 2005 wet season and 2006 dry season, 92 IRRI-bred CMS lines were grown side-by-side in the field with their corresponding maintainer lines. Seed sets produced by natural pollination on the CMS plants were observed as their outcrossing rates. It was found that these CMS lines yielded poorly, with an average of 10.7% seed setting, and most (86%) of these lines observed had only less than 20% seed set, compared with two Chinese commercial CMS lines that were averaging 47% seed set (Fig. 1). IR58025A, the most popular CMS line used in Asian countries, had seed

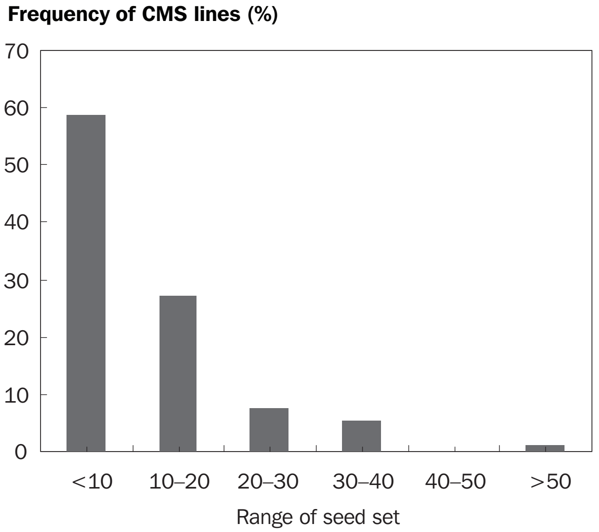

Fig. 1. Outcrossing performance of IRRI CMS lines.

set of only 14.7%. The possible cause of the low outcrossing of IRRI-bred CMS lines was that all CMS B lines have been directly derived from inbred breeding programs without specific improvement for outcrossing. The maintainer lines identified in the testcross process were directly converted into CMS B lines and A lines. Although a lot of maintainer lines were identified and converted into CMS A lines in the past decades, only a few were used in commercial production because of the weaknesses of poor outcrossing, incomplete male sterility, and poor lodging resistance, etc. The improvement of female parents, especially for the trait of high yield in seed production, has become a key target of hybrid rice breeding at IRRI.

Two approaches are generally followed to increase the yield of hybrid rice seed production: genetic improvement of outcrossing traits of parental lines and technical refining of field management in seed production, including location and season selections. Experience obtained from countries with high-yielding seed production, such as China, Vietnam, and the U.S., has showed that the most efficient way to increase the yield of hybrid rice seed production and reduce the seed cost was to improve the traits related to outcrossing in female parents. IRRI can do the best, as an international research organization, for the improvement of parental outcrossing because of its large and diverse germplasm pool and breeding lines developed from various breeding programs, as well as strong breeding and research capacity. Since 2005, a large quantity of rice germplasm and breeding lines, including those lines derived from wild rice crosses, has been screened for high-outcrossing traits, such as floret opening time and angle, stigma size, and exsertion. Many new breeding crosses were specifically made with the target of integrating high-outcrossing traits into new CMS B lines. Progenies from these crosses have been selected and advanced to develop new CMS B and A

Table 3. Yield growth rate of inbred and hybrid rice in IRRI Advanced Yield Trials (AYT).

Entry	Growth ($\text{kg ha}^{-1}\ \text{y}^{-1}$)	Yearly growth rate (%)	R^2
Dry season			
Average inbred	27.9	0.47	0.1042
Average hybrid	91.1	1.50	0.4019
Top 20% yielding inbred	32.1	0.47	0.1143
Top 20% yielding hybrid	66.0	0.89	0.2462
Wet season			
Average inbred	80.7	2.05	0.4547
Average hybrid	93.7	2.13	0.5071
Top 20% yielding inbred	57.5	1.17	0.3139
Top 20% yielding hybrid	77.0	1.53	0.4751
Yearly			
Average inbred	64.3	1.35	0.4608
Average hybrid	99.8	1.93	0.7000
Top 20% yielding inbred	52.7	0.93	0.3405
Top 20% yielding hybrid	71.8	1.17	0.5359

Data source: AYT at IRRI, 1985-2007.

lines and these newly developed lines have demonstrated high yield potential for seed production in small-scale experiments.

Enhancing yield heterosis in both the dry and wet season to more than 20%

Over the years, IRRI has developed thousands of parental lines and experimental hybrids. Selected elite hybrids, about 20 entries per year, were evaluated together with advanced inbred breeding lines in Advanced Yield Trials (AYT) from the 1985 wet season to 2002 wet season. However, as more parents and experimental hybrids were developed, hybrid rice AYT were separated from the inbred AYT from the 2003 dry season, with an average of 100 entries per year and with hybrids derived from the two-line system.

Data collected from AYT showed that the yield potential of IRRI breeding products, including inbred varieties and hybrids, has been sustainably increasing over the years (Table 3, Figs. 2 and 3). The average yield of experimental hybrids increased from 4.5 t ha^{-1} in 1986 to 6.5 t ha^{-1} in 2007, with yearly growth of 1.36% in the dry season and 2.19% in the wet season. For heterotic hybrids (the top 20% yielding hybrids), the yearly growth rates were 0.85% in the dry season and 1.55% in the wet

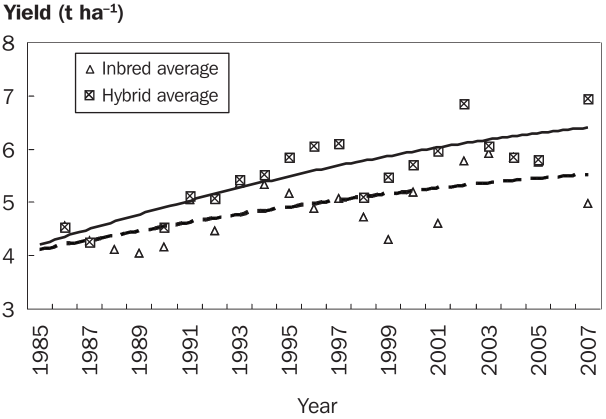

Fig. 2. Yield performance of IRRI inbred and hybrid rice in Advanced Yield Trials (AYT) (average inbred vs. average hybrid; data source: IRRI AYT, 1985-2007).

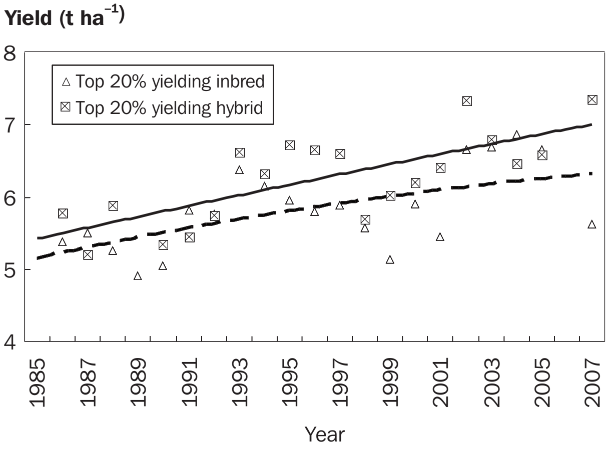

Fig. 3. Yield performance of IRRI inbred and hybrid rice in Advanced Yield Trials (AYT) (top 20% yielding inbred vs. top 20% yielding hybrid; data source: IRRI AYT, 1985-2007).

season. Annual yield gains were greater in the wet season than in the dry season for both inbreds and hybrids, and for both average entries and high-yielding hybrids. The yearly yield growth rates for both inbred and hybrid rice products generally agreed with that estimated by other IRRI scientists for released IRRI inbred varieties, that is, about 1% per year (Peng et al 2000). Even though commercial hybrids generally have 8–9 t ha^{-1} of yield potential, with more than a 15% yield advantage over inbred check varieties, a major concern is that most elite hybrids evaluated in AYT did not have the expected yield of a minimum 15% yield heterosis over the inbred check varieties. The hybrids showed an average of only 658 kg ha^{-1} yield advantage over inbred checks, with a 13.3% yield advantage, and for those hybrids yielding in the top 20%, they outyielded the top 20% of inbred varieties by only 435 kg ha^{-1} with a 7.5% yield advantage from 1985 to 2007. The possible cause of low yield heterosis from the experimental hybrids was possibly the same as that for poor outcrossing, that all parents from hybrid rice programs were directly derived from inbred breeding programs without further improvement specifically for hybrid rice breeding. Inbred breeding has a breeding strategy and methodology different from those of hybrid rice breeding because genetic diversity among the parents is a fundamental prerequisite for breeding hybrid crops. If all hybrid rice parental lines were derived directly from inbred breeding without consideration of heterogeneity among the parents, it was quite possible to develop parents closely associated by pedigree and lines with narrow genetic diversity, which would turn the significant advantage of numerous breeding lines created by inbred breeding programs into a weakness for hybrid rice breeding.

Heterotic groups have been identified and used to breed superior commercial hybrid crops. Among the approaches to study heterotic groups, DNA-based molecular marker technology has been one exploited widely in recent years, including for developing hybrid rice. With a historical collection of 247 IRRI-bred hybrid rice parents, 21 simple sequence repeat (SSR) markers were used to study parental structure and genetic diversity. The results showed that most of these hybrid parents could obviously be classified into four main germplasm groups with some subgroups within each group (Fig. 4). Group 1 included all CMS B lines and group 2 had mostly B lines, but with a few R lines. Group 3 and group 4 clearly all belonged to CMS R lines. This study was only primarily analyzed with a limited number of small SSR markers. Further studies are needed to clarify the relationship between the genetic diversity and pedigree among these parents and the relationship between marker-based genetic diversity and agronomic traits. As knowledge increases on parental population structure and the relationship between genetic diversity and agronomic traits, well-planned breeding strategies will be applied to develop diverse and heterotic parents from different heterotic groups in tropical rice germplasm. Primary studies already showed that hybrids derived from an indica × new plant type (NPT) could have higher yield potential than hybrids derived from indica × indica (Virk et al 2003). More studies will be conducted to identify the contributors of heterosis from different heterotic pools combined with the technology of two-line hybrid rice that has the advantage of using more diverse rice germplasm.

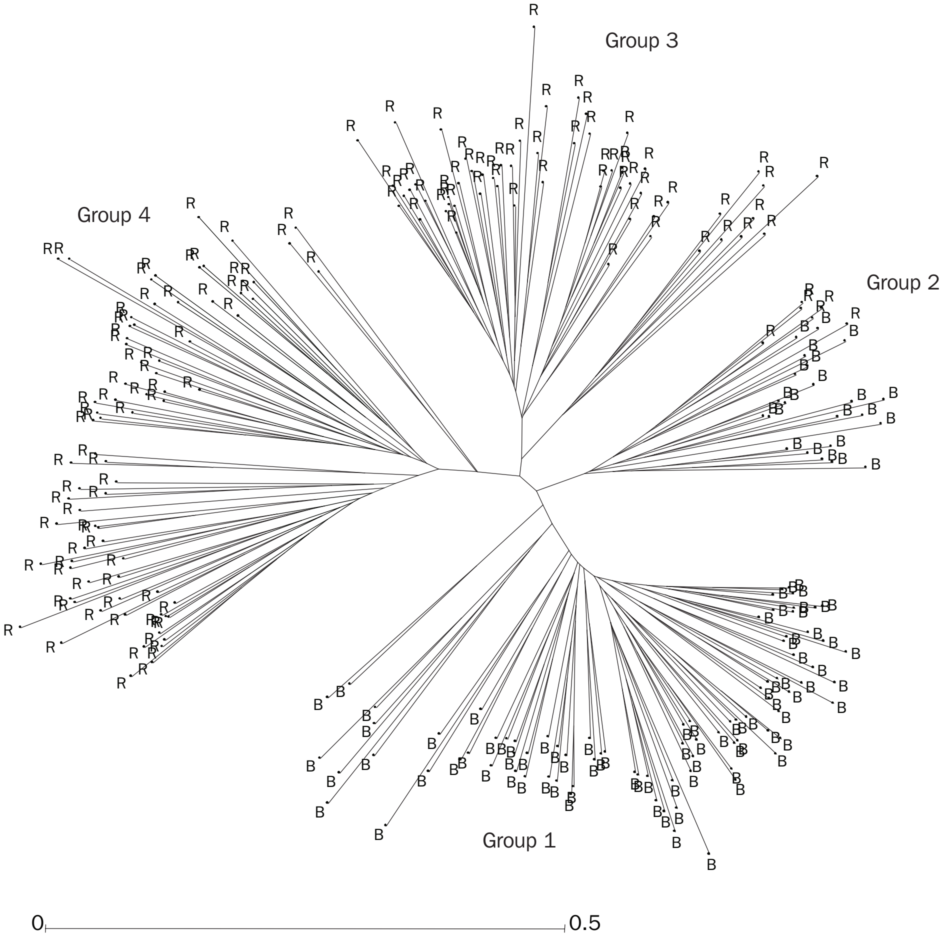

Fig. 4. Dendrogram of 247 IRRI hybrid rice parents based on DARwin 5 using simple matching dissimilarity and unweighted neighbor joining from 21 simple sequence repeat markers.

Improving hybrid rice grain quality

In comparison with inbred rice varieties, two major concerns were related to grain quality of the first generation of commercial hybrid rice, that is, the low milling yield of head rice and high chalkiness. Other quality traits, such as grain size and taste, are mostly locally preferred, and could be manipulated by the breeding process without much technical difficulty. By using grain quality data collected from the National Cooperative Testing (NCT) of the Philippines from 1997 to 2007, we compared the two traits of head-rice milling yield and chalkiness between commercial hybrids (Mestizo 1, Mestizo 3, and Mestizo 7) and inbred check varieties (PSB Rc 18, PSB Rc 28, and PSB Rc 82). It was found that the averages of head-rice milling yield and

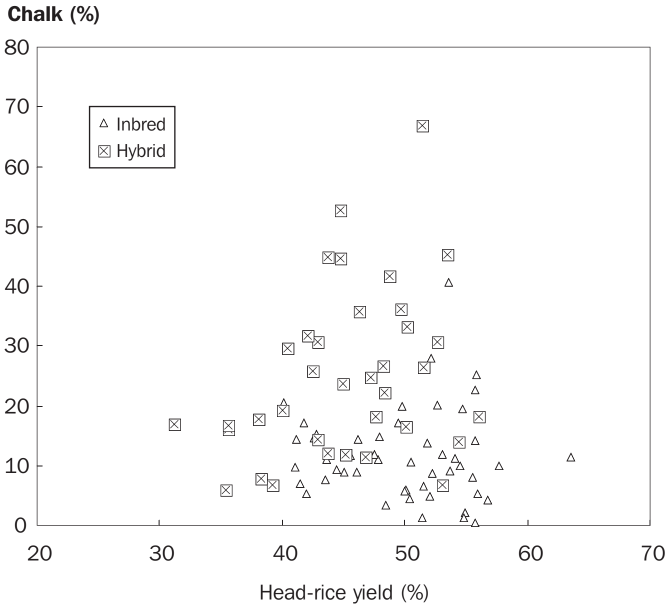

Fig. 5. Head-rice yield and chalk between inbred rice varieties and rice hybrids.

chalkiness of the hybrids were 45.2% and 25.1%, respectively, although for inbred varieties they were 50.2% and 11.3%, meaning that the commercial hybrids had 5% lower head-rice milling yield but 14% higher chalkiness than the inbred varieties in the Philippines (Fig. 5). Head-rice recovery and grain chalkiness are two genetically related traits and they are influenced much by environment (G × E interaction). The disadvantage of hybrid rice for these two traits is possibly due to its heterotic characteristics of larger and more panicles than inbred varieties. However, commercial hybrid rice with desirable and market-acceptable grain quality could be developed through critical parental screening and multienvironment evaluation with more understanding of the trait relationship between the parent and hybrid, and between the hybrid and the environment. An earlier study showed that hybridity per se does not induce poor grain quality for hybrid rice and the key to breeding rice hybrids with desirable rice quality is the selection of parents (Khush et al 1988). The success of hybrid rice commercialization in the United States comes from the strict quality requirements implemented that have provided the best example for the possibility of improving hybrid rice quality. The Chinese experience also showed that grain quality of hybrid rice can be improved to meet market requirements. The earlier complaint of poor grain quality against the first generation of hybrid rice in China has disappeared in the past decade because of the new released rice hybrids with much improved quality, especially for two-line hybrid rice.

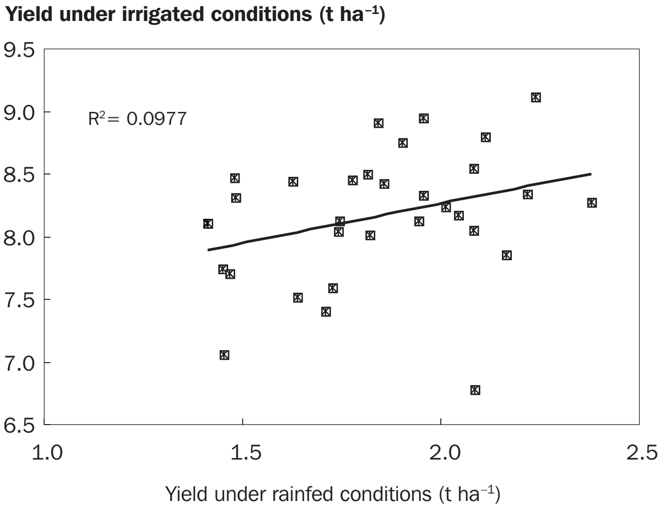

Fig. 6. Hybrid rice yield under irrigated and rainfed conditions (2007 dry season).

Improving resistance to biotic stresses

Resistance to biotic stresses is one of most important subjects for hybrid rice in the tropics. Although no evidence showed a significant effect on resistance to/tolerance of biotic and abiotic stress with hybrids derived from wild abortive (WA) cytoplasm (Faiz 2000), in the past few years, bacterial blight, blast, false smut, and kernel smut have been reported in many Asian countries for serious damage to hybrid rice grain and seed production, especially the new epidemics of kernel smut, false smut, and brown planthopper. There is a lack of knowledge whether these threats are specifically associated with hybrid rice germplasm. It is critical to identify, incorporate, and pyramid multiresistance to parental lines through traditional breeding and DNA-based biotechnology.

Developing hybrids for unfavorable environments

IRRI has allocated significant resources to increasing rice production in stress environments (IRRI 2006). Experimental data collected from primary studies have showed the yield superiority of hybrid rice under unfavorable environments, for example, rainfed drought-prone lowlands and problem soils (Virmani 2003). During the past few years, more studies have been conducted for evaluating hybrid rice under drought-prone areas at IRRI. Hybrid rice developed for the irrigated environment has been critically evaluated under drought environments. Data indicated that some hybrids yielded very well, with high yield heterosis under both irrigated and drought conditions (Figs. 6 and 7). However, it is interesting to note that the yield correlation between the two environments is low, that is, a high-yielding hybrid under an irrigated environment

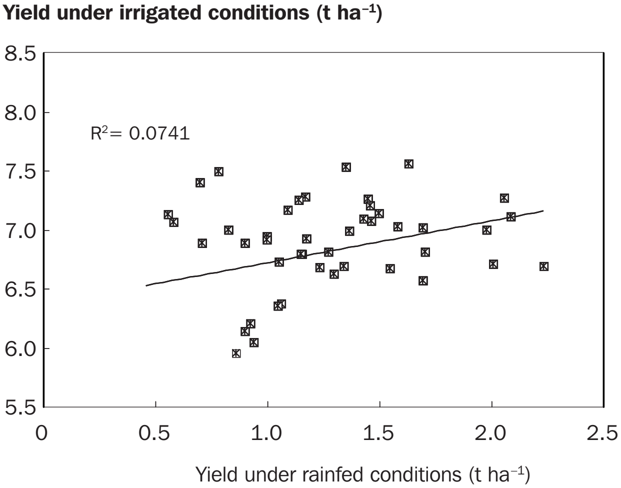

Fig. 7. Hybrid rice yield under irrigated and rainfed conditions (2008 dry season).

is not a guarantee of high yield under a drought-stressed environment. On the other hand, some hybrids are more specifically adapted to drought stress, that is, a hybrid yielding average or low under an irrigated environment may have acceptable yield under drought conditions, with remarkable yield heterosis. Developing hybrid rice for a drought-stressed environment requires scientists to confront some thoughtful questions before deploying appropriate breeding strategies and germplasm: Is the drought tolerance of the hybrid truly controlled genetically or is it because of hybrid vigor? How does it or does it need to integrate the quantitative trait locus (QTL) conferring drought tolerance into hybrid rice parents? How do these drought-tolerance QTLs function in a heterozygous (in one parent) and homozygous background (in both parents), and what are their effects on heterosis? What will be the economic impact of hybrid rice growing in rainfed areas, which are generally associated with severe and extensive poverty? As is always true in heterosis breeding, there are no easy answers, but it has never been more vital than to look for them.

Improving breeding efficiency (biotech)

More recently, DNA-based molecular technology has been developed as an effective and essential tool in improving crop varieties and breeding lines. Various DNA markers have been routinely used in hybrid rice research and they have provided a remarkable means to study genetic diversity, to identify heterotic groups, and to be used in marker-assisted selection (MAS). Some molecular markers, such as those related to fertility restoration, thermo-genic male sterility (TGMS), resistance to major diseases, as well as other agronomic and quality traits, have been available for use in hybrid rice breeding. We will continue the effort to employ the new molecular-based tools, combined with traditional breeding methods, in hybrid rice research and product development, especially for studies of heterotic grouping, heterosis enhancement, and MAS to improve breeding efficiency.

A new platform for developing hybrid rice

Hybrid rice technology has been proven to be an efficient way to increase rice production in many countries. However, it involves many aspects of product development and evaluation, seed production, distribution, and marketing. Successful on-farm deployment of hybrid rice will rely primarily on the establishment of effective collaboration between public research institutions and the private sector in research, seed production, and technology dissemination. Traditionally, public research institutes have advantages in science research, breeding, innovation, product testing, assessment, training, and capacity building as well as close connection with policymakers. However, the private sector usually has more experience in commercial seed production on a large scale, seed production systems and facilities, and marketing. It is essential to establish new modalities to combine the complementary advantages of both the public and private sector to mutual benefit, and accelerate the dissemination of the technology, as well as strengthen the partnership among IRRI, NARES, and the private sector. In 2008, IRRI organized and established a Hybrid Rice Research and Development Consortium (HRDC) as a new platform to link the hybrid rice research programs at IRRI, in NARES, and in the private sector to accelerate hybrid rice technology. In the first year of the consortium, 38 members from the public and private sector joined. The objectives of the HRDC are (1) to provide research support in developing parental lines and hybrids through strengthening hybrid rice development programs at IRRI and in NARES, and enhancing the product pipeline and ensuring hybrid rice breeding priorities; (2) to provide better information on the performance of hybrids and develop best management practices through multilocation hybrid performance trials in collaboration with IRRI's partners and local research on hybrid rice seed production and best management practices in key hybrid rice mega-environments in Asia; and (3) to support information sharing, public awareness, and capacity building. The first activity the HRDC organized in 2008 was a multilocation hybrid rice yield trial in eight locations, which provided a forum for the public and private sector to evaluate and demonstrate their products in various environments.

With clear objectives and appropriate breeding strategies as well as a strong supporting mechanism, hybrid rice technology will be further refined, and more and better rice hybrids will be employed to make a large impact on rice production.

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DuPont's global strategy for increasing agricultural productivity

Dennis F. Byron

Global demand for most of the world's food crops—including rice—is growing rapidly. To achieve food security, each hectare of land needs to produce more. DuPont's seed business, Pioneer, is committed to improving the productivity of farmers around the globe. Pioneer has a long and successful history of developing improved crops through plant breeding, with years of product research and development resulting in an extensive germplasm collection. Pioneer applies the latest molecular genetics technologies, and uses biotechnology tools to deliver products with traits that will help farmers increase yields, protect crops, safeguard the environment, and produce grains that improve the quality of the foods we eat. Pioneer has a global network of scientists that work together to develop traits, technologies, and germplasm that ultimately result in products with improved productivity and quality that the world is demanding. Examples of improved traits, enabling technologies, and products are presented. Special emphasis is given to Pioneer's hybrid rice research program.

Global demand for most of the world's food crops is growing rapidly. The need to increase the productivity of each hectare of land is critical for achieving food security. Over the past ten years, the growth of the world's population has been greater than 13%, growth in global income has exceeded 35%, growth in meat consumption has increased more than 20%, world maize consumption has grown more than 30%, and rice consumption has increased more than 10%. These factors, combined with irregular weather patterns, high energy costs, reduced investment in agricultural research for some crops, and little growth in arable crop land, resulted in dramatic grain price increases for most commodity crops.

Farmers face a tremendous challenge. Between now and 2050, we will have to double the world's food, feed, fiber, and fuel production on less land than is currently in production. In addition, we must accomplish this in a sustainable fashion. Agriculture is competing and will continue to compete for land against urbanization, salinization, and other factors. Why do we need all this increased production? The world population is expected to grow to nine billion in 2050, compared with six billion in 2000. We are seeing growing demand for protein: meat from cattle, pigs, and chickens, which depend on feed grains. Emphasis is also increasing on food safety and nutritional value. As economies improve, so will diets. All of these factors combine to further increase demand for grain crops.

China faces one of the greatest challenges of all. China must feed 20% of the global population with 9% of the world's arable land. The U.S., in contrast, has only 5% of the world's population and approximately 13% of the globe's arable land. China will need to use all of the production tools available to produce food for its people. Molecular plant breeding and biotechnology are among the key tools for sustainably feeding and fueling the world—tools that producers and consumers around the world will increasingly depend on.

Until the 1990s, crop improvement relied primarily on improvements in crop management practices and plant breeding. It is interesting to compare yield improvements for maize, rice, and soybeans over the past 50 years. Although rice in China and maize in the U.S. started out at about the same yield levels in the 1950s, the rate of growth for maize hybrids has been much greater than the rate for the improved rice semidwarf varieties of the Green Revolution. China developed and quickly adopted hybrid rice in the 1980s and 1990s and there was a significant shift in productivity that reduced the gap. However, China has not significantly expanded hybrid rice areas in recent years and average yields have somewhat leveled. India has experienced positive growth in rice yields but at a much lower rate. Hybrid adoption is still not high enough in this country to influence overall yields. At Pioneer Hi-Bred and other organizations, we think that achieving productivity gains in rice that rival those in maize is not only possible but necessary to improve food security, especially for Asians, whose major staple food is rice.

A combination of technologies will be required to increase productivity to meet the growing demand for rice. Technologies such as molecular breeding to develop higher yielding hybrids, improved hybrid seed production systems, traits that increase yield and protect yield, and improved efficiencies for water and nutrient use through both management practices (tillage, planting techniques, fertilization and watering practices, postharvest storage) and biotechnology will be needed.

DuPont and its seed subsidiary Pioneer are committed to improving the productivity of farmers around the globe, including rice farmers. Pioneer uses an integrated system consisting of molecular breeding, trait development, phenotypic evaluation, and supporting bio-information systems to develop improved hybrids and varieties. The foundation of our integrated crop development system is our plant breeding program. This system is most advanced in our maize breeding projects, but we think the same principles will apply to our hybrid rice breeding projects. In maize, we are increasingly using molecular breeding techniques that include pedigree, phenotypic, and genetic characterization of our germplasm. We have the ability to detect marker trait associations as a routine part of our breeding programs based on pedigree and marker information. The ability to phenotype traits through managed environments is the most important part of identifying marker trait associations, and the key to our success. Given good genetic information, we can predict the performance of individuals, populations, and hybrids. This allows us to evaluate a very large sample of genotypes in the early stages and then quickly sort them, with better products coming out of the funnel in the end.

Complementing the plant breeding effort is a large biotech trait development program that focuses on crop protection and agronomic traits. Protecting maize from insects is a major undertaking in most maize-growing regions. Pioneer partnered with Dow AgroSciences to develop the Herculex® Bts that confer resistance to a wide range of lepidopteran insects. Bt traits have allowed maize hybrids to more fully reach their genetic potential by protecting the hybrids from these insects and the resulting yield losses from reduced water and nutrient uptake and diseases. Pioneer maize hybrids with Herculex® Bt have reduced or eliminated the need for insecticides and have been proven to be convenient and safe. Most of the insect-resistance traits have resulted in much more consistent and reliable yields for our customers.

Yield reduction caused by weed competition is also a major factor throughout the world. Pioneer has developed a multiple mode of action herbicide resistance for use in maize and soybeans called Optimum® GAT®. This trait allows for the application of glyphosate plus a broad range of sulfonylurea herbicides for protecting maize and soybean yields from yield-robbing weeds. One of the key benefits of this technology is that herbicides can be applied well into the crops' growing seasons.

The availability and cost of water and fertilizer are becoming more challenging in most parts of the world. Pioneer is devoting considerable resources toward developing maize hybrids that are more effective in their use of both. We have developed several gene leads that are showing yield increases when maize is grown under drought conditions. The genes allow the maize plants to continue growing and developing yield when grown under water-stress situations. Similarly, we have several gene leads for nitrogen-use efficiency that allow maize hybrids to yield 10–25% more under reduced nitrogen environments. Pioneer has industry-leading programs for the development of insect resistance, herbicide resistance, and agronomic traits, as well as strategic alliances with external organizations to bring these value-added traits to market in our core crops, including hybrid rice.

Rice is facing a somewhat similar situation as maize as demand for rice and rice products is increasing. Ninety percent of the world's rice is grown and consumed in Asia. Seven hundred million Asians currently rely on rice for more than 60% of their daily calories. Population growth is outpacing rice yield growth and there is a clear need to improve rice productivity. As the International Rice Research Institute and other organizations have stated, there is a need to accelerate research on the development of new rice varieties and hybrids. Many organizations, including Pioneer, are increasing investment in the development of rice hybrids to meet this challenge.

Pioneer's hybrid rice breeding program currently operates in India, Indonesia, and the Philippines. Hyderabad is the main center for our rice research work, where we have a large breeding station and a biotechnology center. In addition, we have recently established breeding stations in Indonesia and the Philippines. These research projects also receive support from our extensive research and data management programs in the U.S. The hybrid rice breeding program has objectives that are similar to those of our maize breeding programs. The top priority is developing products with high yield.

We must access and develop diverse germplasm to form a strong germplasm base for our breeders' programs. We also need to gain a better understanding of this germplasm and how to better use it. Pioneer's maize programs have evolved into well-defined heterotic groups and the breeders are improving their understanding of the genetics underlying these heterotic pools through marker associations. For hybrid rice, we are poised to learn from maize's experiences and we can apply the same principles to increase heterosis and yield.

To increase the rate of genetic gain in maize and other crops, Pioneer breeders are using molecular markers to increase the number of genotypes entering the breeding funnel, and subsequently improving the quality of experimental inbreds and hybrids coming out of the funnel. We have an extensive network of researchers involved in our marker-assisted breeding programs. This work requires coordination among breeders, assay specialists, marker lab scientists, and data mangers. We are leveraging processes already in place for maize and soybeans and are applying them to our hybrid rice programs. We are currently producing millions of data points for maize and soybean programs and are ramping up efforts for rice.

High yield is very important; however, it must also be stable yield. To protect the high yield potential of our rice hybrids, Pioneer is developing traits for insect, disease, and herbicide resistance. In addition, we are developing agronomic traits to improve performance under both high management conditions and stress. This work includes new options for developing male sterile inbreds and higher yielding hybrids. If we can evolve hybrid rice similarly to maize, in which both female and male gene pools are broad, we can increase the rate of genetic gain for yield and other traits. One of Pioneer's advantages is that we can leverage traits from our internal maize and soybean research programs as well as from our external biotechnology partners. This gives us a large set of trait options to potentially test in rice.

Two highly significant aspects of trait and product development are trait integration and trait testing. Integrating traits into rice germplasm and inbreds, whether they are native traits or transgenes, is a key competency Pioneer aims to achieve. We are borrowing processes already in place in our maize and soybean breeding programs and are building greenhouses, screen houses, and growth chambers to aid rice trait integration. Fast and effective trait integration using molecular markers and screen houses is a must for successful hybrid rice breeding programs. Perhaps even more important is the development of effective assays to screen for efficacious native traits and transgenic events. The development of managed environments, whether it is with growth chambers, with greenhouses, or in the field, takes considerable effort and care. Pioneer has trait-screening experts to help breeders screen their genotypes for effectiveness.

Fortunately for Pioneer, we have strong maize and soybean breeding programs that we can leverage for developing high-performing rice hybrids. We think that by using many of these technologies and processes, we can develop rice hybrids that will yield 20% or more than current hybrids by 2020. The foundation of this effort is broadening our rice germplasm collection and setting up broad male and female heterotic groups. Increasing genetic gain of the breeding programs with molecular markers will further increase yields. Protecting the genetic potential of high-yielding hybrids through crop protection traits will add another 5% or more to rice productivity if these traits are approved for commercialization. Finally, yield improvement traits such as high yield per se, drought tolerance, and nitrogen-use efficiency could add another 5% to hybrid rice yield by 2020. DuPont and Pioneer believe that rice production can be improved significantly over the next 10–12 years if we wisely use the technology tools that are already being applied to other crops. We have a great opportunity ahead of us.

Notes

Author's address: Vice president, Pioneer Hi-Bred International, Inc., Crop production development, 7300 NW 62nd Ave., Johnston, IA 50131, USA; e-mail: dennis.byron@pioneer.com.

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Improvements in breeding methodologies and products

Advances on the molecular mechanism and use of Honglian-CMS in rice

Shaoqing Li, Jun Hu, Yanping, Tan, Wenchao Huang, Xiaojue Peng, and Yingguo Zhu

With increasing population, improvement of rice, the world's most important cereal crop, has attracted more research attention. Hybrid rice based on cytoplasmic male sterility (CMS) increases grain yield by more than 20% relative to improved inbred rice varieties. It remains a valuable approach to grain yield enhancement. Honglian (HL)-CMS is one of the three widely used CMS systems in rice. The area planted to HL-type hybrid rice is expanding in China as it has good quality, high yield, wide adaptability, and improved resistance to diseases. Also, HL-CMS has been the subject of genetic, cytological, and molecular studies on CMS and fertility restoration as a CMS model system in recent years. This paper summarizes the latest progress in research on the mechanism of CMS, including the structure and activity of F_0F_1-ATPase, oxidative stress in mitochondria, growth viability of seedlings, transgene characterization and protein analysis of anther mitochondria of HL-CMS lines, new sterile cytoplasm screening and development of corresponding gametophytic CMS lines based on *orfH79*, coevolution of fertility restorer and CMS in *Oryza* species and others, and the development and popularization of hybrid rice using the HL-CMS system.

Keywords: cytoplasmic male sterility, Honglian, hybrid rice

Rice, one of the most important cereal crops, has been cultivated in 113 countries in the world. The FAO reports that the price of rice has doubled from US$350 per ton to more than $1,000 per ton in the last two years, resulting in social turbulence in some countries. This implies a looming food shortage worldwide. It is predicted that world population will increase from the current 6 billion to 8 billion by 2030 (Hugo 1999). Corresponding to the expansion of the human population in developing countries, rice consumption will increase rapidly. Facing such a great challenge, it is obvious that the only way to solve the food crisis is to improve the yield of cereal crops such as rice, wheat, maize, etc. Rice is also known as a model cereal crop for basic research on crop improvement and it has attracted great interest from scientists around the world.

Historically, significant breakthroughs occurred in grain yield improvement in the 1960s and 1970s. In rice, the use of heterosis through cytoplasmic male sterility

(CMS) developed in the 1970s in China contributed to the increase in grain yield by more than 20% over that of improved inbred rice varieties. It is estimated that hybrid rice has been grown on 334 million hectares and about 300 billion tons more rice have been produced in China since the 1970s (Yuan and Peng 2005). Hybrid rice has been released in more than 20 countries, playing a key role in increasing rice grain yield in developing countries. Considering genetic and cytological features, commercial CMS lines can be categorized into three groups—wild-abortive (WA), Honglian (HL), and Baotai (BT) (Zhu 2000). Of these, the HL-CMS system has been carefully studied as a model CMS system for genetic, cytological, and molecular analysis of male sterility and fertility restoration in recent years. Because the newly developed HL rice hybrids have good quality, high yield, wide adaptability, and improved resistance to diseases, the area planted to hybrid rice has expanded in the Yangtze River valley and in southern China.

The HL-CMS system is also a valuable model for discerning the molecular mechanism of CMS and fertility restoration, cytoplasm-nucleus interaction, and heterosis of hybrid rice (Li et al 2007). A series of alloplasmic CMS lines and near-isogenic lines (NILs) with different *Rf* loci has been developed to elucidate the working mechanism behind cytoplasm-nucleus interaction. Here, we summarize the latest progress in studies about the mechanism of CMS and the development of commercial hybrid rice using the HL-CMS system.

Molecular mechanism of male sterility and fertility restoration in Honglian-CMS rice

A transgene confirms that *orfH79* determines HL-CMS and retards root development in rice

The mitochondrial chimeric gene *orfH79* was suggested to be a candidate for HL-CMS in rice (Yi et al 2002). However, no direct evidence shows whether *orfH79* expression could induce CMS and how the ORFH79 product interferes with pollen development during microsporogenesis. *orfH79* was fused to a mitochondrial transit peptide and transformed into a wild-type rice. The phenotype and some physiological events of transgenic plants were investigated. Localization and the expression pattern of ORFH79 in HL-CMS lines were studied using immunocytochemistry. *orfH79* expression induced gametophytic male sterility and slow growth of the primary and lateral roots in transgenic rice. Results revealed that the ORFH79 product was localized mainly in the mitochondria, disrupting the structure of mitochondria in microspores of the CMS line. The strong expression of *orfH79* concentrated in meristematic tissues (including the root tip, sporogenous cells, the tapetum, and the intercalary meristem of the stem internode) was accompanied by excess accumulation of reactive oxygen species (ROS) and reduced cellular ATP levels. We conclude that overexpression of *orfH79* in the mitochondria results in excess ROS production, causing male sterility and defective development of the roots in HL-CMS rice.

An HL-CMS line displays aberrant F_0 of F_0F_1-ATPase in mitochondria

Honglian-CMS has been shown to be associated with a chimeric gene (*orfH79*) that is co-transcribed with an extra *atp6* in the mitochondria. Recent studies demonstrated that the intact F_0F_1-ATPase in HL-CMS lines was specifically reduced in terms of both protein quantity and enzyme activity, whereas its F_1 sector was not affected (Zhang et al 2007). This implies that the F_0 sector presents a labile linkage with F_1. In the presence of the fertility restorer gene, F_0F_1-ATPase can be recovered. Furthermore, *orfH79* transcripts were preferentially polyadenylated and consequently degraded rapidly in florets of restored hybrid plants, indicating that *atp6-orfH79* is involved in the sterile phenotype. With inhibition of the cytochrome pathway of the electron transfer chain, the biomass of sterile plants grown in the dark was significantly lower than that of fertile lines. However, respiration measurements showed an increase in electron transfer capacity in the sterile plants, suggesting that reduction of biomass in the sterile line was caused by the disruption of F_0F_1-ATPase.

HL-CMS lines undergo severe mitochondria damage caused by oxidative stress during microgenesis

Previous studies on HL-CMS have suggested that pollen abortion of sterile plants resulted from a special programmed cell death (PCD) program, which started at meiosis in the microspores (Li et al 2004). To elucidate the molecular basis of pollen abortion, we compared biochemical and physiological properties such as content of ROS, ATP, NADH, total glutathione, and ascorbate acid; activities of dehydroascorbate reductase, glutathione reductase, ascorbate peroxides, and superoxide dismutase; and the integrity of mitochondrial genome DNA isolated from an HL-CMS line, Yuetai A (YtA), and its maintainer line, Yuetai B (YtB). Our results indicated that the mitochondria of the HL-CMS line suffered from a serious oxidative stress during microspore development. Oxidative stress induced by the abnormal increase in ROS at the meiosis stage resulted in the depletion of ATP and NADH and the degradation of mitochondrial genomic DNA. This suggests that the presence of a redox signal originating in the mitochondria affects the rest of the cell. Therefore, it is possible that the abortion of premature microspores in the HL-CMS line is induced by the chronic oxidative stress in the mitochondria during the early stage of pollen development (Wan et al 2007).

Seedlings of HL-CMS lines exhibit intense susceptibility to H_2O_2 relative to a fertile maintainer line

CMS lines and their corresponding maintainers have an identical nuclear genome but different cytoplasm. This confers a CMS system with good enough quality to investigate the interaction between the nucleus and cytoplasm. To investigate the relationship between mitochondrial mutation and physiological response to environmental stimuli, mitochondrial gene expression and the biochemical character of seedlings under H_2O_2 stress between the HL-CMS line and its maintainer were analyzed. Biochemical and molecular analysis showed that ATP content and mitochondrial membrane potential in HL-CMS line YtA declined significantly compared with those in maintainer line YtB. Parallel to this, ROS content rose swiftly in the CMS line but remained stable in the maintainer line. These biochemical changes were closely accompanied by DNA fragmentation, an indicator of plant PCD index, occurring in the root cells of the CMS lines. Expression analysis revealed that the genes for mtETC subunits and the mtETC complex activity were also down-regulated. These differences in biochemical index indicate that the CMS line is more susceptible to H_2O_2 stress than the maintainer line, which may be the result of co-damage between the toxic ORFH79 peptide and the induced stress from the outside H_2O_2.

Proteomic analysis of anthers in HL-CMS lines

To better understand the molecular mechanism of CMS at the protein level during the development of anthers and microspores, proteomic analysis was performed to investigate the varying patterns of protein synthesis in rice anthers at the tetrad stage among CMS line YtA, maintainer line YtB, and the F_1 hybrid (Honglian You-6). Total proteins of anthers were extracted and separated by two-dimensional gel electrophoresis. About 1,500 protein spots, including 70 differentially expressed spots, were reproducibly detected. Of these, about 49 differentially expressed spots representing 49 different proteins (including putative lipoamide dehydrogenase, pyruvate dehydrogenase E1 beta subunit, ADP-glucose pyrophosphorylase, 14-3-3 protein, etc.) were characterized through mass spectrometry and database searches. The subcellular localization and the function of the identified proteins were predicted using software. The 49 specially expressed proteins were sorted out according to their possible function. These fell mainly into groups related to metabolism, protein biosynthesis, transcription, signal transduction, and so on, all of which are involved in cell activities that are essential to pollen development (Wen et al 2007).

Cloning of a fertility restorer in HL-CMS rice

There are two unallelic *Rf* (restorer of fertility) genes, designated as *Rf5* and *Rf6*, from Milyang 23 and 9311 for HL-CMS rice (Liu et al 2004). To clone these two *Rf* genes, two NIL lines with *Rf5* and *Rf6* loci were developed from HL-CMS line YtA and the corresponding restorer line of Milyang 23 and 9311. Then, two BC_1 populations of YtA//YtB/NIL_{Rf5} and YtA//YtB/NIL_{Rf6} were constructed to map these two *Rf* genes. *Rf5* was mapped to a region at about 60 kb, flanked by markers RM6469 and RM25661, at each side. *Rf6* was mapped to the locus in a region at about 50 kb between markers RM6737 and SBD07. Further, physical maps containing the *Rf* loci were completed after fully screening the clones of 9311 and Milyang 23. A BAC library was formed with an average insert at about 60 kb using special probes tightly linked to *Rf5* and *Rf6*. One candidate BAC clone with *Rf5* and three candidate BAC clones with *Rf6* were screened and sequenced to analyze possible *Rf* genes. Then, the selected BACs were subcloned and transformed into the HL-CMS line of YtA by *Agrobacterium tumefaciens*–mediated transformation. Phenotyping and sequencing confirmed that the *Rf5* in Milyang 23 was identical to PPR791 (i.e., *Rf1*). To further discern the network of the *Rf* gene, a bacterial two-hybrid system was used to identify the protein potentially interacting with *Rf5*. The recombinant bait plasmid pBT-PPR791 was co-transformed with the target plasmid pTRG cDNA library DNA into the reporter stain.

After screening and isolation of positive pTRG clones, the target genes were identified by validation reporter and DNA sequencing. This has provided us with some valuable information to gain a deeper understanding of the working mechanism of *Rf5*. The identification of the transformant plant with *Rf6* is under way.

Exploitation of new gametophytic CMS and *Rf* in *Oryza* species

Development and genetic analysis of a gametophytically alloplasmic CMS line of rice with variant *orfH79* haplotype

Identification and exploitation of new CMS cytoplasm resources in wild relatives of rice always motivate rice breeders to develop alternative new CMS lines. In this report, we used the chimeric *orfH79* gene related to HL-CMS as a molecular marker to screen wild rice with the AA genome, and found eight out of 42 investigated accessions sharing *orfH79*. Sequence analysis revealed a total of nine nucleotide substitutions that led to the change of nine amino acids being detected in the newly identified *orfH79* in wild rice, thus falling into seven haplotypes. To detect the latent relationship between *orfH79* haplotypes and the corresponding fertility restorers, we selected four accessions with different *orfH79* haplotypes as the female parent to hybridize with a Honglian maintainer line, YtB. After eight consecutive recurrent backcrosses, four alloplasmic CMS lines with different *orfH79* haplotypes were developed (Li et al 2008). Microscopic observations revealed that pollen grains of the newly developed CMS lines were all spherical and as clear in 1% I_2-KI solution as those of HL-CMS lines. Test crosses showed that the four newly developed CMS lines displayed various fertility-restoring models, which indicates that each *orfH79* haplotye represents a new CMS type and corresponds to its specific *Rf* allele.

Genetic and allelic analysis of fertility restorer genes for WA-, HL-, and BT-CMS lines in *Oryza* species

It is essential to investigate the status of *Rf* genes for WA-, HL-, and BT-CMS for hybrid rice development, including the genetic mode and allelism of *Rf* genes and the relationship between *Rf* and CMS. The fertility of all test-cross F_1 plants showed that the restorer-maintainer relationship was similar between HL-CMS and BT-CMS, but that between WA-CMS and HL- or BT-CMS differed. Genetic analysis indicated that HL- or BT-CMS is controlled by a single dominant *Rf* gene and WA-CMS is controlled by one or two pairs of dominant *Rf* genes, thus reflecting the characters of the gametophytic and sporophytic restoration CMS type. Allelism analysis revealed at least three *Rf* loci in different accessions with *Rf* genes for each CMS type.

Phylogenetic analysis reveals *Rf* and CMS being co-evolved in *Oryza* species

The characterization of genes associated with CMS and fertility restoration (*Rf*) has been well documented. However, studies on the phylogenetic or evolutionary relationship between nuclear *Rf* and CMS factors in the mitochondria of *Oryza* species have usually been ignored. In our research, 41 accessions from seven *Oryza* species with the AA genome were employed and analyzed for the phylogenetic relationship between CMS factors and *Rf* candidates on chromosome 10. These accessions were categorized into five groups, according to restoring ability for HL- and WA-CMS—i.e., eight accessions were capable of restoring neither the HL-CMS line nor the WA-CMS line (group A); 20 accessions restored both HL- and WA-CMS lines, 11 of which had stronger restoring ability for the WA-CMS line than for the HL-CMS line (group C); and the other nine demonstrated the opposite (stronger restoring ability for the HL-CMS line than for the WA-CMS line [group D]). A phylogenetic tree based on restriction fragment length polymorphism (RFLP) patterns of CMS-associated mitochondrial genes showed that the 41 *Oryza* accessions fell into five distinct groups. Another phylogenetic tree based on PCR profiles of nuclear *Rf* candidates on chromosome 10 was also established, and five groups were distinctively formed. The accessions in each subgroup of the two phylogenetic trees are parallel to each other. Furthermore, according to the distribution of *Rf* genes for HL- and WA-CMS, accessions with a similar fertility restoring pattern were always clustered in the same subgroup of the two phylogenetic trees. Therefore, we conclude that genetic diversity of CMS-associated mitochondrial genes is compatible with that related to nuclear *Rf* genes; there seems to be an existing coevolutionary relationship between CMS and *Rf* in *Oryza* species.

Genetic relationship among elite restorer rice for CMS in China

Three-line hybrid rice has spread all over the world and has shown good yield potential. Usually, the development of three-line hybrid rice relies on a few sterile lines and their corresponding fertility restorer lines, thereby reducing the cost of commercial seed production. This means that production risk would increase with vulnerable DNA polymorphism. To overcome this problem, increasing the diversity of hybrid parents, especially in restorer lines, is always a primary breeding goal.

RAPD and SSR are two common techniques used to analyze variation in crop plants—these are convenient, rapid, and easily operated, and they provide enough information. Here, we selected 35 elite restorer lines for CMS lines in China and we detected their genetic relationship using these two methods.

All 35 restorer lines of hybrid rice were analyzed by 25 SSR primer pairs dispersed on the 12 chromosomes in rice. Sixty-five polymorphic fragments at 25 loci were detected from these selected SSR primer pairs, with each locus possessing 2.6 alleles on average in the restorer lines tested. The polymorphic index content ($PIC = 1 - \Sigma f_i^2\, f_i$, the frequency ratio of I alleles) values ranged from 0.206 to 0.682 and averaged 0.414. The genetic similarity coefficients (Gs) of the 35 rice restorer lines were between 0.67 and 0.98, and all lines tested could be divided into two groups near Gs = 0.710. Results of cluster analysis showed that IR38, Moroberekan, and New Bonnel were clustered in group I; all others were clustered in group II. The latter can be divided further into five subgroups at Gs = 0.80; almost all of them originate from IR series or are derived from Minghui 63.

There are 106 reproducible fragments, of which 93 fragments showed polymorphism. These were detected from 13 RAPD primers with reproducible polymorphism screened in 258 primers; the number of fragments varied between 6 and 14, and each primer possessed 7.1 bands on average. Cluster analysis showed that the Gs of the 35 rice restorer lines was in the 0.38–0.98 range, and all lines tested could be divided into four groups near Gs = 0.57. IR8 was located in group I separately; Teqing, Moroberekan, and New Bonnel clustered in group II; Shenghui 11, IR24, Forbiprotife, C418, E32-1, Mianhui 725, IR54, Guangqing 124, and Milyang 23 clustered in group III; Fuhui 838, IR64, 288, 9311, Wanhui 88, Minghui 53, J413, 71068, Zihui 100, Lihui 6, R527, Ce49, Duoxi-1, MBP98, Minghui 86, 6078, IR38, Ce64, Shenghui 747, IR36, CDR22, and Mianhui 734 clustered in group IV. The Gs of most restorer lines (>83%) was more than 0.80.

From the information, we can say that rice restorer lines are abundant in China, but genetic diversity was small and the genetic background was relatively vulnerable, which will further restrict the application of rice heterosis to a large extent.

Development and planting of HL-type hybrid rice varieties

With the swift spread of HL-hybrid rice, new HL-CMS line selection is now attracting more and more rice breeders. Recently, several newly developed elite CMS lines were used for commercial production. For example, Lu-3A and Luohong 3A have been approved by the Anhui Academy of Agricultural Science and Wuhan University, respectively. From these, a number of new HL-type hybrid combinations, including Yuelou-9, Hongyou-30, Xiannong-404, and Hunzhi-1, have been developed and approved by the national or provisional approval committees in different regions. One such variety is Luoyou-8. Developed by Wuhan University, this new hybrid has good quality, high yield, and wide adaptability; it has become popular in the Yangtze River region. It was developed from the CMS line of Luohong-3A and restorer of R8308, a restorer line selected from the 9311/E32 cross. Luoyou-8 was approved as a commercial variety by the Hubei Crop Variety Approval Committee in 2006, and further approved by the National Crop Variety Approval Committee in 2007. Luoyou-8 is well adapted to areas in southern China and the Yangtze River valley. The area planted to Luoyou-8 has expanded and it is estimated that accumulated planting area will reach 200,000 ha from the time it was commercially released. Luoyou-8 usually has a yield potential of about 11.3 t ha^{-1} under general cultural conditions. It gave a high yield of 12.35 t ha^{-1} in Wuhan, a new record yield in Hubei Province.

In conclusion, we predict that planting area of HL-type hybrid rice will cover 540,000 ha every year; the total will be more than 2 million ha nationally.

Prospects for developing HL-type hybrid rice

As a novel gametophytic CMS type, breeding and exploitation of HL-type hybrid rice will greatly enrich the genetic diversity of hybrid rice. Furthermore, HL-CMS lines, such as Yuetai-A, Luohong-3A, and Luyuan-A, are usually characterized by good grain quality, stable sterility, and high hybrid-seed yield, and they can overcome the yield, quality, and adaptability constraints that exist in current hybrid rice varieties. The development and popularization of HL-type commercial hybrid rice varieties will not increase farmers' income but will strongly ensure food security in China. In addition, HL-type hybrid rice showed good performance in Southeast Asian countries such as the Philippines and Vietnam, with 20–40% higher yield than advanced rice varieties widely planted in these countries.

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Notes

Authors' address: Key Laboratory of MOE for Plant Developmental Biology, College of Life Sciences, Wuhan University, Wuhan, Hubei 430072, China. Email: zhuyg@public.wh.hb.cn.

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Breeding and application of restorer lines for three-line hybrid rice

Hua-An Xie

Hybrid rice breeding is mainly based on the advantages of male sterility and effective restorer lines. The strong restorer lines Minghui 63 and Minghui 86 and other related restorer lines were successfully bred and widely used in the 20th century. The approaches and technologies of restorer line breeding for hybrid rice are summarized in this paper. Breeding methods have been improved with pyramiding of restoring genes with genetic diversity, setting up a protocol for blast resistance, screening restorer lines, and combining characteristics of high yield, high grain quality, blast resistance, wide adaptability, and ideal plant type in different ecosystems. Thus, breeding efficiencies were promised. The new generation of restorer lines was much improved by integrating elite characteristics. Furthermore, the current status of the application of restorer lines for hybrid rice is discussed and ideas on hybrid rice development are proposed.

Keywords: hybrid rice, restorer line, approaches, technologies

Rice is one of the world's most important crops. It is the staple food for more than 50% of the world's people. From the 1950s to 1960s, rice yield increased 20–30% by adapting semidwarf indica rice, leading to a leap in rice production. At the beginning of the 1970s, Chinese rice breeders overcame many difficulties and developed hybrid rice under the leadership of academician Longping Yuan. The yield of rice went up one step more based on the semidwarf plant type breeding and increased by 20% because of the advent of three-line hybrid rice. Two important milestones in the history of modern rice breeding were dwarf breeding and three-line hybrid rice; thus, China has kept a leading position for hybrid rice in the world (Xie et al 2004). From the advent of hybrid rice in the early 1970s, several important milestones were achieved in its development. The representative combinations Nanyou 2 and Shanyou 2 were intermediate rice varieties and late-maturity rice varieties with restorer lines introduced from the International Rice Research Institute (IRRI). Since the early 1980s, Shanyou 63 has been a representative of high-yielding hybrid rice with restorer lines created by hybridization by different rice varieties. Great progress has been made in blast resistance, high yield, and wide adaptability for this hybrid. From the mid-1980s,

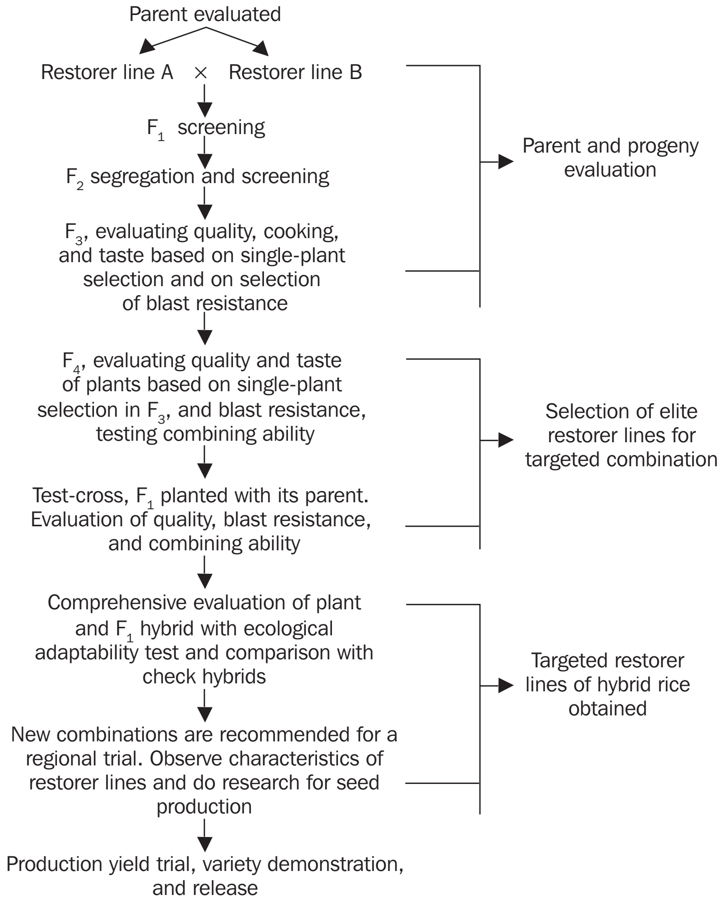

Fig. 1. The technical processes of restorer line breeding for three-line hybrid rice.

early-maturity combinations such as Weiyou 64 and others were extended quickly in China. In the late 1990s, the representative combinations for early-maturity hybrids were Weiyou 77 and Shanyou 77, with characteristics of early maturity in restorer lines. The contradictions for high quality and early maturity were overcome when early-maturity varieties were bred successfully (Xu et al 2004). The breeding of elite restorer lines plays a very important role in developing hybrid rice.

Breeding technology of restorer lines for hybrid rice

Technical processes of restorer lines for hybrid rice

Figure 1 shows the breeding process of restorer lines.

Pyramiding strong restorer genes by hybridization of strong restorer lines

In the breeding of restorer line Minghui 63, a hybridization plan aimed to replace imported varieties as restorer lines. IR30, an IRRI variety that originated at low latitude with restorer genes, and Gui 630 from tropical South America were screened as the parents of restorer line breeding. Restorer genes and other elite genes were pyramided in a line called Minghui 63 effectively (Xie et al 1987, 1994, Xie 1998). Based on our experience, if there is a wide genetic diversity between parents or a wild rice used as germplasm, a multiplex cross or multiple backcross could be used to enhance excellent traits or overcome some poor characteristics. In breeding for Minghui 86, another elite restorer line, we used a multiplex cross of subspecies between indica and japonica [(IR54/Minghui 63//IR60/Gui 630) × GK148 (japonica187/IR30)]. The R line has a strong stem, large panicle, and elite plant type.

Testing and screening for restorer line combining ability

In general, if the segregation is not too wide in progenies, line screening for restoring ability can be started before the F_4 generation. A male sterile line difficult to restore could be used as a tester for screening restorer ability of male parents. F_1 fertility should be observed for R-line restoring ability. When doing retesting, F_1 offspring should be seeded to target the F_1 flowering time in normal and late seasons. Flowering time should be planned at a cooling period for the late-cropping season so as to provide an environment for screening better restorer ability.

Identifying and screening resistance in earlier generations of restorer lines

Because of complicated terrain, various ecological environments, high temperature, and high humidity in Fujian, China, rice blast is a serious disease. Strong blast resistance is very important for a variety. Through practice for several years, we developed an effective breeding process, in which breeding lines of early or intermediate generations were screened in blast hotspot areas in multilocations and years, and lines of late generations were screened with blast (caused by *Magnaporthe grisea*) isolates indoors. These lines were analyzed for their spectrum of blast resistance. Hence, the varieties bred would have strong blast resistance in southern China (Xie 2007). Rice blast resistance of restorer line Minghui 63 was tested with 51 isolates of 12 races in 5 groups of *M. grisea* by inoculation. This line demonstrated that the rate of isolate resistance was 96.1% and the rate of race resistance was 83.3% (Table 1) (Yang and Li 1986). Moreover, a male sterile line, Fuyi A, was bred at the Fujian Rice Research Institute. It had a strong and broad spectrum of blast resistance (screened with 86 isolates of 7 races, including A, B, C, D, E, F, and G races in southern and northern China) with stable sterility. Sixteen combinations, including Fuyou 77 with the female line of Fuyi A, had been used commercially in Fujian, Sichuan, Hubei, and Guangdong provinces. Furthermore, three combinations were certified nationally. A total of 1 million growing hectares for the 16 combinations were accumulated in the Wuling mountain areas of Hunan, Hubei, and Guizhou provinces.

Table 1. Resistance to *Magnaporthe grisea* races for Minghui 63 and Siyou 30.

Race	Number of *Magnaporthe grisea* isolates	Minghui 63		Siyou 30 (check)	
		S	R	S	R
ZB1	1		1	1	
ZB5	3		3	3	
ZB9	6	1	5	5	1
ZB13	5		5	4	1
ZB15	1		1	1	
ZB29	4	1	3	3	1
ZB31	2		2	2	
ZC13	2		2	1	1
ZC15	2		2	2	
ZE1	1		1		1
ZF1	9		9		9
ZG1	15		15	1	14
Total	51		49	23	28
Rate of *Magnaporthe grisea* resistance (%)		96.1		45.1	
Rate of race resistance (%)		83.3		16.7	

Screening high-yield characteristics and developing the new plant type to improve yield potential

It was important to screen restorer lines with the high-yield trait, which was the basis of high vigor for combinations. Furthermore, biological yield was the basis of economic yield, so, a moderate plant type was required, with erect leaves, short basal internodes, 100 cm height, 25 cm length of panicles, moderate seeding density, and leaf color changing quickly at late maturity with yellow seeds, green branches, and yellow stem. Yield potential could be greatly improved by increasing grain weight and plant height.

The application of new restorer lines in hybrid rice

Since 1980, the Sanming Institute of Agricultural Sciences, Fujian Province, had bred 10 Minghui series of restorer lines, including Minghui 63, Minghui 77, and Minghui 86 (Figs. 2 and 3). The release of Minghui 63 changed the situation of only IRRI varieties being used as restorer lines in China. From 1984 to 2005, 33 combinations were released with Minghui 63 as the restorer line, occupying 83 million rice hectares. This

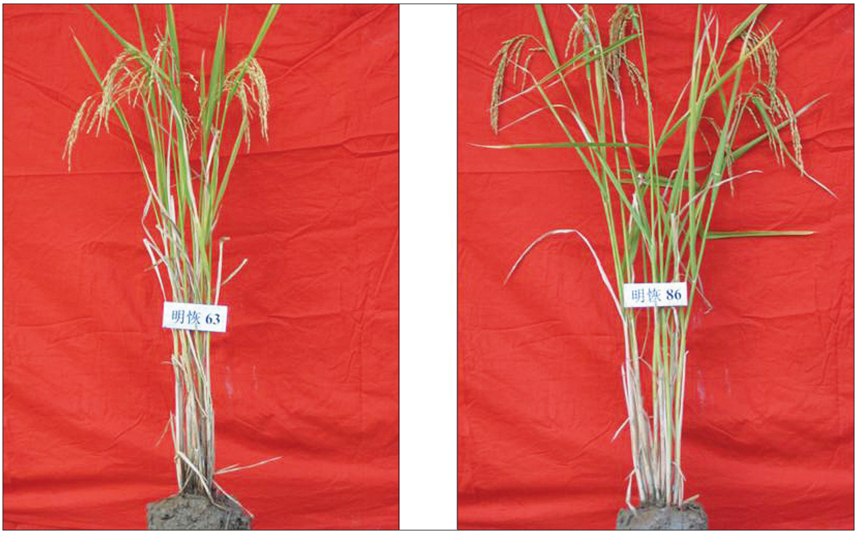

Fig. 2. The plant type of restorer lines Minghui 63 (A) and Minghui 86 (B).

Fig. 3. Spikelet type of partial restorer lines and their combinations.

produced a landmark effect on promoting the development of hybrid rice in China. Furthermore, Minghui 63 was used as an excellent germplasm for developing other new restorer lines. Some 295 new restorer lines were developed with Minghui 63 as a parent. Among the 477 new hybrids certified nationally and provincially during 1984 to 2005, 103 combinations have Minghui 63 in the restorer line pedigree. The total area of hybrid rice with Minghui 63 pedigree was 139.9 million hectares, which was 49.4% of the total hybrid rice area in China from 1984 to 2005.

In the 1990s, the Fujian Rice Research Institute also developed eight restorer lines, including Hang No. 1, Fuhui 148, and others, through space radiation mutagenesis, and indica and japonica hybridization.

Table 2. Area of certified rice hybrids with Minghui 63 as the restorer line from 1984 to 2005 (million ha).

Combination	Area	Combination	Area
Shanyou 63	62.5	Jinyou 63	0.69
Dyou 63	6.4	D you No. 10 (D297 You 63)	0.52
Teyou 63	3.5	Syou 63	0.16
Xueyou 63	3.5	Maxueyou 63	0.14
IIyou 63	3.2	Liangyou 2163	0.07
Gangyou 12 (Gangyou 63)	1.7	Hengliangyou No. 1	0.07
Weiyou 63	0.76	Gangyou 63	0.07
You I 63	0.56		

Combinations with large extended area from new restorer lines

The combination Shanyou 63 had the largest planting area from 1986 to 2001, with a yearly average of 3.79 million hectares, about 28.74% of the total hybrid rice area in China. In 1990, Shanyou 63 was planted on 6.85 million hectares, which was about 43.9% of the total hybrid rice area in China. The total extended area for Shanyou 63 was 62.5 million hectares from 1984 to 2005, with a grain production of 69.5 billion kg and economic value of 62.6 billion yuan (Table 2).

In 1991, the Sanming Institute of Agricultural Sciences developed the combination of Minghui 77, which promoted hybrid rice for the early season in China in the 1990s. The combinations of Weiyou 77 and Shanyou 77 had the largest extended area from 1990 to 1995 in the early season of China. The extended area was 5.1 million hectares in total, resulting in an increase of about 2.30 billion kg of rice grain and an economic benefit of 2.30 billion yuan.

In the 1990s, this institute also bred strong restorer line Minghui 86 derived from a cross of indica and japonica rice. The combination II youming 86, whose restorer line was Minghui 86, was planted in Taoyuan of Yunnan Province, and it yielded 79.8 kg ha^{-1} d^{-1}, which set a new world record of yield in 2001 (Table 3). Moreover, the combination of II youming 86 was planted in Fujian Province in the late season and yielded 6,674 kg ha^{-1}, which was 5.15% higher than control variety Shanyou 63. From 1999 to 2000, II youming 86 yielded 8,387 kg ha^{-1}, 5.67% over control Shanyou 63 in the indica rice trial of the southern region of China. From 1999 to 2001, the combination Shanyouming 86 was planted on a 6.7-hectare field in Youxi County in Fujian Province for three continuous years, and its yield surpassed 12,000 kg ha^{-1}. It was the first super hybrid rice combination with an area validated on 6.7 hectares with a yield of 12,000 kg ha^{-1}.

In the 1990s, the Fujian Rice Research Institute had bred restorer line Hang No. 1 by space radiation mutagenesis. The yield of combination II youhang 1, whose restorer line was Hang No. 1, reached 13,927 kg ha^{-1}. II youming 86, II youhang 1, and Teyouhang 1 were approved as the first super hybrid rice combinations by the

Table 3. Yield components of hybrid rice grown at Taoyuan in Yunnan Province of China.

Combination	Spikelets per ha (million)	Number of spikelets per panicle	Number of filled spikelets per panicle	Seed set (%)	1,000-grain weight (g)	Calculated yield (t ha^{-1})	Actual yield (t ha^{-1})	Year
IIyouming 86	299.7	209.9	201.3	95.9	30.0	12.1	12.0	2001
Teyou 175	349.7	187.4	170.5	91.0	30.5	12.1	11.9	2001
Teyouhang 1	290.6	235.7	223.0	94.2	29.0	12.5	11.9	2003
IIyouhang 1	359.1	191.6	177.2	92.5	29.0	12.3	11.6	2003

Chinese Ministry of Agriculture. The extended areas of II youming 86 and II youhang 1 were 1.1 million hectares and they producd 0.595 billion kg of grain, with a social benefit of 0.81 billion yuan.

Combinations with strong regenerating ability suitable for ratoon rice

Combination Shanyou 63 has not only high yield, good quality, blast resistance, and wide adaptability, but also is suitable for cultivation as ratoon rice. Thus, a new rice cultivating system with a ratoon crop was practiced in southern China. Combination Shanyou 63 was cultivated as a ratoon crop, representing 85% of the total ratoon cropping area in China. The accumulated area reached 4 million hectares in the past 7 years, with a significant social benefit.

To exploit the yield potential of ratoon rice, the Fujian Rice Research Institute has been cooperating with the Agricultural Bureau of Youxi County of Fujian since 1999. Experimental fields with super high-yielding hybrid rice targeted for a ratoon crop were demonstrated in Youxi County, so as to promote and develop cultivation technology for super high-yielding hybrid rice as a ratoon crop, and to screen super high-yielding rice hybrids. In recent years, four super high-yielding hybrids with strong regenerating ability, including II youming 86 and II youhang 1, yielded more than 12,000 kg ha^{-1} in the main crop, and more than 7,500 kg ha^{-1} in the ratoon crop. In 2002, the average yields were 12,711 kg ha^{-1} in the main crop and 7,596 kg ha^{-1} in the ratoon crop in the demonstration fields of super hybrid rice in Mayang Village in Youxi County. The average yield from the two crop seasons set a world record for yield measured on 6.7 hectares.

In 2005, II youhang 1 was planted on 7.1 ha in Mayang Village of Xicheng of Youxi County, Fujian (Fig. 4). The average yield of the ratoon crop was 7,506 kg ha^{-1} and the highest-yielding field yielded 8,775 kg ha^{-1}. In 2005, II youhang 1 was planted on 67 ha in Lingtou Village of Lianhe of Youxi County. The average yield from the ratoon crop was 7,326 kg ha^{-1}, with the highest-yielding field at 8,796 kg ha^{-1}. In 2007, II youhang 2 was planted on 7.3 ha in Mayang Village of Youxi County and the ratoon crop produced an average yield of 8,271 kg ha^{-1} and a record high of 8,883 kg ha^{-1}. All of these examples showed that super high-yielding rice hybrids

Fig. 4. Ratoon rice of II youhang 1.

could be cultivated not only as a main crop but also as a ratoon crop with great yield potential.

Prospects

Combinations with high yield, disease resistance, high quality, and wide adaptability should be improved

In the new period of hybrid rice development, in addition to aiming for high yield, breeders should focus more on high yield with high quality, blast resistance, and wide adaptability. Some of the super high-yielding hybrid rice now has good quality and strong disease resistance, but it still needs to be further improved. To meet different market requirements, the high quality of super hybrid rice should be taken fully into account for various uses, including the consumption of high-quality rice, processing, and feed. With the development of agricultural production, various germplasm accessions with different purposes should be introduced, screened, and developed based on the main physical and chemical characteristics by using enhanced screening technology.

It was very important for breeders to develop blast-resistant varieties with high yield and high quality. In addition, the environmental differences in ecological systems and soil types are diverse in China. Variety adaptability should be improved to meet the needs of different ecological conditions and increase economic efficiency.

Extensively introducing germplasm with blast resistance, enhancing blast resistance screening, and improving biological yield

Enhancing blast resistance is a key to ensuring high and stable yield in super hybrid rice. Varieties bred by Japan and IRRI had difficulty in being used commercially because of blast susceptibility. So, it was necessary to enhance disease and insect resistance and cold tolerance to improve yield and stability in super hybrid rice breeding (Chen 2005). There were four major types of rice diseases—fungal, bacterial, virus, and nematodes. Blast, sheath blight, and bacterial blight are considered to be three major rice diseases. In past decades, resistances to these three diseases have been extensively studied**.** *Xa*21 and other disease-resistance genes have been mapped and cloned. Moreover, the sequence of the rice genome was completed, which provided a great opportunity for using marker-assisted selection (MAS) and other biotechnologies to develop disease-resistant varieties (Qian Qian 2007). IRRI has succeeded in pyramiding rice bacterial blight resistance genes *Xa4*, *xa5*, *xa13*, and *Xa21* into the restorer line IR24 by MAS (Cheng et al 2004). IRRI has also been using molecular markers to screen blast resistance, and applying MAS for germplasm improvement and breeding for multiple resistance through gene pyramiding. The PCR markers developed from the sequences of blast-resistance genes would further speed up rice blast resistance.

Pests and diseases were more serious in southern China because of high temperature and humidity. Introducing new resistant germplasm, enhancing screening in multiple locations/years, and combining with MAS would enhance the breeding of varieties with stronger and a broader spectrum of disease and pest resistance.

Enhancing research and extension of cultivation technologies for super high-yielding rice

Generally speaking, improved varieties need good cultivation methods to achieve high yield potential. Physiological functions of a high-yielding variety should be studied in order to achieve high yield potential. Suitable cultivation measures should be adapted to meet the special requirements of a variety. Different varieties with different ecological adaptability have different cultural requirements. Whether a variety can achieve its high yield potential depends on its genetic characteristics as well as cultivation measures. To achieve super high yield fully, the photo-thermo reaction of a variety must be studied first for suitable planting area. Moreover, different varieties have different requirements for soil conditions, fertilizer, water, density, etc. Scientists must do more research on nutrition, physiology, and photosynthetic performance to provide guidelines for super high-yielding cultivation. At the same time, research on technologies of ratoon cropping for super hybrid rice should be strengthened. Furthermore, more research should be done for the physiological and biochemical traits of a ratoon crop for good cultivation technology. Results from Fujian showed the advantages of saving labor, increasing production efficiency, and obtaining high economic value in a ratoon rice crop. There are 3.3 million ha of single-rice land suitable for ratoon rice cultivation. Developing ratoon rice is a key to ensuring food security in the future, with great significance for total grain production capacity in China.

Combining conventional technology with biotechnology

The sequence of the rice genome was completed and many important functional genes have been fine-mapped and cloned. Rice breeding needs to combine MAS with conventional breeding. The application of modern breeding methods would play a strong role in accelerating super hybrid rice breeding. Transgenic technology has great prospects in breeding for resistance to herbicide, pests, and stress; improving quality; and increasing yield potential. Further development of biotechnology would certainly bring a revolutionary change to super hybrid rice breeding. The application of biotechnology should focus at some point on problems that could not be solved by conventional technology. Modern breeding technology should be combined with conventional breeding methods to breed new varieties of super high-yielding rice more quickly and efficiently.

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Author's address: Fuzhou Subcenter, National Rice Improvement Center of China, Fujian Academy of Agricultural Sciences, Fuzhou 350018, Fujian Province, China.

Developing new-generation restorer lines for high yield heterosis

I.A. dela Cruz, L.S. Baldedara, S.M.F. Ablaza, C.D. del Rosario, T.F. Padolina, R.T. Cruz, E.D. Redoña, M.C. Casimero, L.S. Sebastian, and J.C. de Leon

New high-yielding restorer lines were developed from five hybrid rice varieties introduced to PhilRice through a collaborative undertaking with one of the leading public research and development institutions in China. The selection of lines highly adapted to local conditions from the initial hybrid varieties (IHVs) started in the 2004 dry season at the PhilRice-Central Experiment Station (CES) using the pedigree method. In the 2006 dry season, in an unreplicated observational nursery at CES, these inbreds yielded from 7.9 to 11.2 t ha^{-1}. The inbred and hybrid check varieties in this test yielded from 6.6 to 8.9 t ha^{-1}. A highly promising hybrid that we bred using one of these lines reached the national cooperative test (NCT) for hybrid rice in the 2008 dry season. Good heterobeltiosis was further observed in our experimental hybrids based on these restorer lines in the 2008 dry-season testcross nursery. These restorer lines are characterized by intermediate plant height, medium to very high tillering, late and slow leaf senescence, medium-early to medium maturity, medium to long panicles, and medium to long and slender grains. They represent a new generation of restorer lines that could be exploited to obtain higher yields in our breeding program.

Readily understood examples of plant breeding as an impact science include plant domestication, Green Revolution crops, disease and insect resistance, wide environmental adaptability, heterosis, and increased productivity. In the future, plant breeding is projected to impact the attainment of competitive agriculture in a global economy, a healthy and well-nourished populace, harmony between agriculture and the environment, a safe and secure food system, and even adaptation to climate change, to mention a few possibilities (Stuber and Hancock 1008, Wassmann 2008).

A technology-driven attainment of food self-sufficiency and security is the goal and policy of both the Philippine government and China. Building on immense cultural and economic commonalities, goodwill, and friendship, the Philippine Rice Research Institute (PhilRice) ventured into bilateral agreements with leading Chinese rice research and development (R&D) institutions and agricultural universities starting in 1988. Through formal and informal collaborative arrangements with about 13 institutions in China in particular (until 2005), several Chinese scientists sent to the country have also obtained degree and nondegree training from PhilRice and from Philippine institutions of higher learning (Redoña et al 2005).

Collaborations with R&D institutions in China and in other national agricultural research and extension systems (NARES) as well as support from other organizations such as the International Rice Research Institute (IRRI), Food and Agriculture Organization (FAO), the Asian Development Bank (ADB), and the private sector were instrumental in building our national capacity in tropical hybrid rice technology development, use commercialization, and benefit generation. During the past 22 years, PhilRice, as the national agency mandated to develop and promote new technologies for increasing rice production and attaining rice self-sufficiency, has actively sought the establishment of linkages with Chinese R&D institutions. Forging technical and scientific cooperation is an approach that can expedite the spread of viable technologies (Redoña et al 2005).

Materials and methods

A memorandum of understanding (MOU) on technical cooperation on rice research and development was signed between the Fujian Academy of Agricultural Sciences (FAAS) and PhilRice on 20 September 2001. Areas given emphasis in this MOU were hybrid rice technology, inbred rice technology, rice biotechnology, rice-based farming systems, sustainable resource management, effective technology promotion strategies, training of technicians, and the exchange of scientists and scholars (Redoña et al 2005).

In the 2003 wet season (i.e., June to November cropping), a regular adaptation trial (AT) was conducted at the PhilRice-Central Experiment Station (CES) and it included five hybrids form FAAS. These five hybrids were Rong You 1, Rong You 2, Rong You 3, Rong You 4, and Rong You Hang 1. In the AT implemented, these hybrids were compared with local hybrid varieties PSB Rc72H, NSIC Rc114H, and NSIC Rc116H, two high-yielding inbreds, PSB Rc28 and PSB Rc18, and IR64 as the check cultivar for quality. The hybrid Rong You 3 yielded 5.6 t ha^{-1} in 118 days, which was the highest in the trial. Rong You 4 ranked third, Rong You Hang 1 ranked fourth, and Rong You 1 ranked sixth. Rong You 2, which matured in 117 days, yielded 3.7 t ha^{-1}, the lowest in the trial (dela Cruz 2003).

In the succeeding 2004 dry season (DS) (i.e., December to May cropping) at CES, selection of new high-yielding pure lines with ability to restore fertility began. A modified pedigree nursery of F_2 segregating populations was established and this included F_2 seeds form the five FAAS hybrids among other entries. F_3 seeds were harvested from plants selected within F_2 populations in the pedigree nursery established (dela Cruz 2004). After several generations of selection within and between each line, promising stable lines were gradually included in our source nursery (SN) for use in testcrossing. For example, F_9 generation inbred selections from the initial hybrid varieties (IHVs) were grown in the 2007 wet season SN and used extensively in crosses.

In the 2006 DS, several of the selected lines were included in our regular observational nursery (ON). Plot size for this unreplicated trial was 8 rows × 19 hills per row. Spacing between hills and rows was 20 cm × 20 cm. The number of hills harvested for grain yield demonstration was 102. The materials were sown on 17 December 2005 and transplanted on 12 January 2006. The following scoring systems were used in evaluating the ON materials for grain quality: milled rice sample (raw), G1 = excellent, G2 = good, G3 = fair; cooked sample, G+ = better than IR64, G = comparable to IR64, G– = poorer than IR64; aroma, 1 = strong, 2 = slight, 3 = fair. A promising hybrid that we bred using one of the ON selections as a restorer already reached the national cooperative test (NCT) for hybrid rice in the 2008 DS. Identifying superior hybrids and inbreds and recommending their release to farmers as accredited varieties is the role of the NCT, which is implemented by the Rice Technical Working Group of the National Seed Industry Council.

Results and discussion

Figures 1A, 1B, and 1C show some of the restorer lines selected from PR36248 that were included in the 2008 DS source nursery at CES. These lines descended from the IHV Rong You Hang 1. One outstanding restorer line selection from this lineage in combination with an elite CMS line produced the very promising hybrid PR36541H, which was nominated to the 2008 DS NCT for hybrid rice as a new entry (photo and data not shown). Selections designated as PR36243, PR36244, PR36245, and PR36246, on the other hand, are related to the IHVs Rong You 1, Rong You 2, Rong You 3, and Rong You 4, respectively.

Restorer lines are mainly screened by testcrossing existing rice varieties. In addition to this screening method, restorer line × restorer line, maintainer line × restorer line (or restorer line × maintainer line), male sterile line × restorer line, and multiple crosses are also made to develop new elite restorer lines (Li and Yuan 1985). Good restorer lines do not only develop outstanding hybrid combinations. Good restorer and maintainer lines can be deployed as conventional varieties as well. To this end, some of the inbred products resulting from this breeding activity have been nominated to the inbred general yield trial for transplanted irrigated lowland rice at CES under the favorable environment program.

Table 1 shows the agronomic characteristics of some of the new inbred lines selected from the five IHVs. Typically intermediate in plant height, medium to very high in tillering ability, and late and slow in leaf senescence, the lines selected were also of the medium-early to medium maturing types in general. Late and slow leaf senescence, or the stay-green trait, has long been considered as a desirable plant character for realizing higher yield potential. Moreover, this trait has been associated with postflowering drought-resistance mechanisms that enable plants to withstand premature senescence under severe moisture stress. In some genotypes with slower senescence (stay-green), Rubisco degradation is slower, which results in longer duration of canopy photosynthesis and higher yield (Khush 2004).

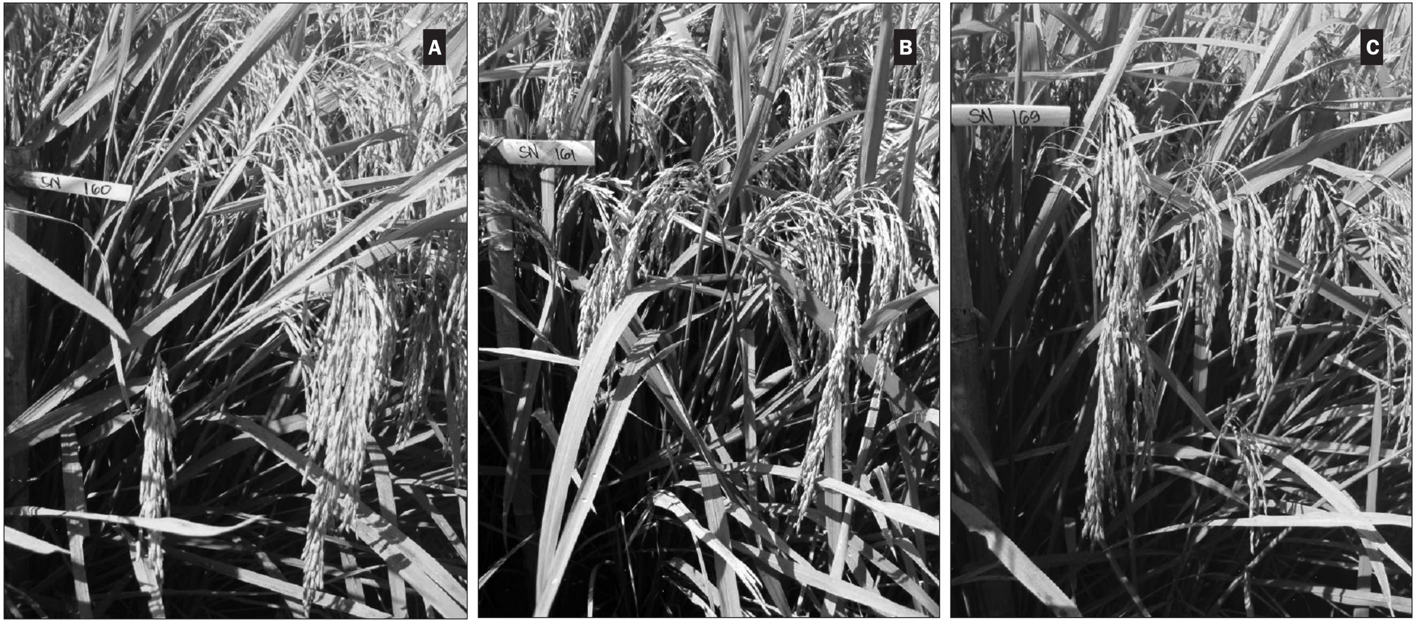

Fig. 1A-C. Some promising restorer lines selected from the IHV Rong You Hang 1 (photos courtesy of J.C. de Leon).

Table 1. Agronomic characteristics of some new restorer lines from IHVs used in the source nursery at CES.

Pedigree	Plant height		Tillering ability		Leaf senescence
	Av	SES	Av	SES[a]	SES
PR36248-HY-1-7-2-1	100.6	Sd	18.2	M-H	Late & slow
PR36248-HY-1-7-4	119.2	Inter	8.8	L	Late & slow
PR36248-HY-2-3-3	125.0	Inter	12.6	M-H	Late & slow
PR36248-HY-2-4-2	118.2	Inter	11.8	M	Late & slow
PR36248-HY-3-6-5	120.8	Inter	12.6	M-H	Late & slow
PR36244-HY-1-8-1	113.8	Inter	11.4	M	Intermediate
PR36244-HY-3-1-4	103.8	Sd	15.4	M-H	Late & slow
PR36246-HY-1-16-2	120.2	Inter	11.4	M	Late & slow
PR36246-HY-1-17-1-1-1	119.8	Inter	12.0	M	Late & slow
PR36246-HY-18-1-1	121.4	Inter	12.4	M	Late & slow
PR36246-HY-1-19-2-2	110.0	Sd	13.4	M	Late & slow
PR36243-HY-2-3-1	130.0	Inter	12.8	M-H	Late & slow
PR36243-HY-2-3-2	116.4	Inter	17.2	M-VH	Late & slow
PR36245-HY-2-2-1	133.8	Tall	18.8	M-VH	Late & slow
PR36245-HY-1-1-2	120.6	Inter	13.8	M	Late & slow
PR36247-HY-1-21-2	92.0	Sd	18.8	M-VH	–
PR36247-HY-2-22-1	118.4	Inter	13.0	M	Late & slow

[a]Based on range of recorded values. Sd = semidwarf; Inter = intermediate; L = low; M = medium; H = high; VH = very high.

Rice breeders have recognized that further improvement would not come from continued crossing and selection within semidwarfs. Consequently, they elaborated various strategies in order to exceed the yield potential established by the best semidwarfs. These included selection for yield components, hybrid rice, recurrent selection, the new plant type proposed by scientists at IRRI, the introgression of quantitative trait loci (QTLs) from wide crosses involving *Oryza* species, and the extension of the active photosynthesis period through stay-green (Jennings et al, nd).

The information provided in Table 2 and in Figure 2 shows the other characteristics of the homozygous line selections, particularly for traits associated with economic productivity such as panicle length, number of spikelets, and grain density. The higher yielding ability of these selections relative to the best commercial inbreds and hybrids was demonstrated in the 2006 DS. More than 120 lines were included in the ON that season at PhilRice-CES. The highest yield harvested from these lines was 11.2 t ha^{-1}. Several lines outyielded the check varieties used in the trial as shown in Table 3. The

Table 2. Panicle characteristics of some new restorer lines from IHVs used in the source nursery at CES.

Pedigree	Panicle length (cm)		DUS[a]	Spikelets panicle^{-1}	
	Av	Range		Av	Range
PR36248-HY-1-7-4	29	28–29	M	335	297–381
PR36248-HY-2-3-3	28	26–29	M	306	268–343
PR36248-HY-2-4-2	28	26–29	M	337	288–380
PR36248-HY-3-6-5	28	27–30	M	292	267–311
PR36244-HY-1-8-1	22	20–25	S-M	246	216–280
PR36246-HY-1-16-2	27	22–30	M	341	307–378
PR36246-HY-18-1-1	29	26–32	M-L	319	258–414
PR36243-HY-2-3-2	25	24–26	M	312	271–380
PR36245-HY-2-2-1	27	26–29	M	234	218–278
PR36245-HY-1-1-2	25	23–29	M	301	263–329
PR36247-HY-2-22-1	28	26–29	M	259	180–359

[a] S = short; M = medium; L = long. Adapted from the Guidelines for the Conduct of Distinctness, Uniformity, and Stability. 2000. BPI-PVPO.

Fig. 2. Panicle characteristics of some of the new restorer lines (photo courtesy of J.C. de Leon).

Table 3. Yield and other agronomic traits of new restorer line selections in the observation nursery at CES, 2006 dry season.

Agronomic trait	R line selections[a]		Standard varieties[b]	
	Max.	Min.	Max.	Min.
Yield (in kg ha^{-1})	11,248	8,468	8,967	6,624
Maturity (days)	130	116	126	113
Phenotypic acceptability	1	5	3	5
Seed set (%)	92	59	93	60
Panicle exsertion	1	5	1	1
Plant height (cm)	125	106	109	97
Productive tillers	17	10	17	14
1,000-grain weight (g)	39	23	30	23
Grain quality				
Raw sample	G1	G2	G2	G2
Cooked sample	G+	G+	G+	G
Aroma	1	3	2	3

[a] Data presented are based on 29 new restorer line selections evaluated.
[b] Standard varieties were PSB Rc18, PSB Rc72H, PSB Rc82, and NSIC Rc116H.

same varieties are used as checks in the NCT. Furthermore, the breeders' assessment of grain quality generally favored some of these high-yielding lines over the IR64 control. One or two lines were also found to be aromatic or to have a slight aroma.

One method for examining yield performance is to break yield into its components. A computation of yield components would be meaningful for designing a blueprint of the target yield. It is also useful for examining the defects of a given crop if a comparison is made with another crop that has already achieved a good yield under a similar environment (Yoshida 1981). In relation to this, selections included in our source nursery in the 2008 DS were examined in detail for yield potential. The average yield performance of the lines was compared to the model yield component reported for IR8 by Chandler in 1969 as cited by Yoshida. In this analysis, grain yield was estimated as grain yield (t ha^{-1}) = panicle number m^{-2} × spikelet number per panicle × % filled spikelets × 1,000-grain weight (g) × 10^{-5}. Using this method, the maximum yield of the inbred selections was 12.2 t ha^{-1}. Among the yield components, spikelet number per panicle showed the greatest potential for increasing the realizable yield of the selections (Table 4). Data for filled spikelets actually ranged from 51% to 88%, whereas 1,000-grain weight ranged from 15.7 to 25.1 g. Grain density, or the number of grains per panicle per panicle length (Sasahara 1997), was 8 based on the

Table 4. Comparison of model yield components and the performance of promising selections.[a]

Component	Dry season	Wet season	Selections (DS)
Panicles per m^2	375	250	275
Panicles per hill	10	10	11
Spikelet number per panicle	100	100	286
Total number of spikelets per m^2	37,500	25,000	78,650
% filled spikelets	85	85	74
1,000-grain weight (g)	29	29	21
Expected grain yield (t ha^{-1})	9.24	6.16	12.22

[a] Data on model yield components are for IR8 and based on the analysis of R.F. Chandler (1969).

number of filled spikelets averaged across selections and 11 based on total number of spikelets (data not shown).

Conclusions and recommendations

Collectively, the genotypes discussed herein represent a new generation of restorer lines for experimental hybrid breeding at PhilRice-CES. Judging form the results presented (and from the good heterobeltiosis observed from related hybrids grown in our 2008 DS testcross nursery), we see potential in these lines that can advance the exploitation of heterosis in our breeding program. Thus, more detailed studies involving these selections will be pursued by PhilRice.

Inbred lines are developed for various purposes in genetic research and applied plant breeding programs, for example, for direct use as line cultivars or as parents of hybrid and synthetic varieties. The expected contribution of either parental line to the genome of an inbred line derived from a bi-parental cross with Mendelian inheritance is 0.5 for an F_2-derived inbred, 0.75 for the recurrent parent of a BC_1-derived inbred, and 0.25 for the donor parent of a BC_1-derived inbred. Experimental studies show a considerable variation in the parental genome contribution around these mean values. Selection and genetic drift during selfing may cause differences between observed and expected parental contributions to inbred progeny (Wang and Bernardo 2000). Until recently, no theoretical concept for describing the variance of the parental genome contribution to homozygous inbred lines existed. Frisch and Melchinger (2007), however, showed that in wheat and maize, for example, the 0.99 quantile of the parental genome contribution to F_2-SSD lines is 0.638 and 0.709, respectively.

In the midst of the current jitters, and even confusion about a rice “price spike” and food price crisis, hybrid rice has established itself in the minds of our policymakers, agriculture-sector movers, and farmers in the Philippines as a clear way out of this conundrum. The breeding of the new restorer lines reported herein, which started in

2004, shows immense potential for hybrid variety development that can help address our concerns about increasing productivity, attaining self-sufficiency, and ensuring the availability of enough rice that is affordable to all.

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Author's addresses: I.A. dela Cruz, L.S. Baldedara, S.M.F. Ablaza, C.D. del Rosario, T.F. Padolina, R.T. Cruz, L.S. Sebastian, and J.C. de Leon, Plant Breeding and Biotechnology Division, Philippine Rice Research Institute (PhilRice), Central Experiment Station, Maligaya, Muñoz 3119, Nueva Ecija; E.D. Redoña, now with the Plant Breeding, Genetics, and Biotechnology Division, International Rice Research Institute (IRRI), College 4031, Laguna, Philippines.

Practice and thoughts on developing hybrid rice for super high yield by exploiting intersubspecific heterosis

Zou Jiang-shi and Lu Chuan-gen

Since the breakthrough in grain yield owing to improvements of dwarf rice and the three-line system of hybrid rice, rice breeding for high yield showed little significant progress in the next two decades. It was considered that using heterosis between subspecific varieties (*Oryza sativa* L.) would be an effective approach to increase yield further. During 1987-93, an indica-japonica hybrid, Yayou 2, yielded as high as 10.5 t ha^{-1}. However, it failed to be commercialized because of a seed purity problem due to nonuniform emasculation by chemical agents in seed production, and sensitivity of seed set in F_1 plants to environmental conditions. In the past decade, the authors released two intersubspecific hybrids, Liangyoupeijiu (Peiai64S/9311, javanica/indica) and LiangyouE32 (Peiai64S/E32, javanica/japonica), both of which exhibited grain yield higher than 10.5 t ha^{-1}, and were widely considered as pioneers of super hybrid rice. Of these two, Liangyoupeijiu has been successfully popularized over 7 million ha in wide climatic areas and it has occupied the top position in rice-planting area for several years in China, while LiangyouE32 had a record yield, and offered a model of plant ideotype for super hybrid rice. It was considered that, in combination with plant ideotype, active physiological functions, and wide-range adaptability to ecological conditions, the exploitation of indica-japonica heterosis would be a key approach for super hybrid rice breeding.

Keywords: rice, breeding, intersubspecific hybrid, plant type, two-line approach

Rice is a major source of food worldwide, and especially in Asia, which features a growing population and food shortages. Increasing rice yield will be extremely important as the population grows and arable land decreases in a highly populated country such as China. Rice improvement for high yield in China can be divided into three phases: dwarf rice, hybrid rice, and super rice breeding.

In the early 1950s, rice varieties usually produced grain yields below 6 t ha^{-1} due to their high stature and weak resistance to nitrogenous fertilizer. In 1956, a dwarf mutant was found in an indica variety, Nantehao, in Guangdong Province. Since then, dwarf rice breeding was initiated by Huang et al in Guangdong and southern China.

A series of indica semidwarf varieties such as Guangluai 4 and Guichao 2 were successfully bred and released. They displayed a yield potential of 7.5 t ha^{-1}, or 20–30% higher than that of the traditional varieties owing to improved resistance to more fertilizer and plant lodging (Huang and Lin 1994). In addition, another semidwarf variety, IR8, once honored as "miracle rice," was released by IRRI in the 1960s. It had high grain yield and was widely popularized all over the world. Dwarf rice breeding was regarded as the highlight of the so-called Green Revolution in rice breeding.

A breakthrough for hybrid rice breeding, in fact, was made in China during 1976. Later, a series of indica hybrids such as Nanyou No. 2 and Shanyou 63 were released and widely planted, covering half of the rice area in China. Generally speaking, hybrid rice showed yield potential of 9 t ha^{-1} or 10–20% higher than that of indica or japonica pure lines.

Since 1984, rice production in China has shown no significant improvement in both yield potential and breeding technique. For national food security, a super rice breeding project was established by the China National Science and Technology Council. It was headed by the China National Hybrid Rice Research and Development Center (CNHRRDC) and China National Rice Research Institute (CNRRI), with cooperation from agricultural institutes and universities. Grain yield potential aimed at 10.5 t ha^{-1} for the first five-year plan (1996-2000) and 12 t ha^{-1} for the second five-year plan (2001-05). Several super rice varieties such as Liangyoupeijiu and Xieyou 9308 had been bred. They exhibited grain yields above 10.5 t ha^{-1}, or 15% higher than those of traditional hybrids (Lu et al 2002).

The history of rice breeding in China can also be divided into three phases in which breeding was characterized by the exploitation of favorable genes, heterosis, and improved plant type.

The significant increase in grain yield was brought about by dwarf rice improvement, which benefited from using a dwarf gene, *sd1*. The successful use of rice heterosis relied on the discovery of a wild abortive (WA) gene. In the 1980s, a wide compatibility gene (WAG), *S5-n*, was discovered. It can overcome indica-japonica hybrid sterility, the main obstacle in the use of intersubspecific hybrids, and it became an important favorable gene in super hybrid rice breeding through intersubspecific heterosis (Ikehashi and Araki 1987, Ikehashi et al 1994).

The use of indica-indica heterosis was a fundamental principle of rice improvement during the 1970s-1980s. In the past two decades, a stronger heterosis of intersubspecific hybrids between indica and japonica varieties showed importance for super rice breeding. A wide cross between rice and other cereal crops would provide rice yield and other traits with tremendous heterosis. Rice scientists have hoped and expected to make an incalculable breakthrough in rice improvement.

Improving plant type is another important objective in high-yield rice breeding. In the past half-century in China, rice varieties showed a great shift from high plant stature and drooping leaves to semidwarf height and erect upper leaves. Rice improvement transferred paying attention only to morphological traits gradually into paying attention to both morphological and functional traits. These practices not only improved rice morphological traits to avoid lodging but also improved the efficiency of receiving solar light and leaf physiological functions such as photosynthetic rate.

Practices in developing hybrid rice for super high yield by exploiting intersubspecific heterosis

Breeding pure lines from progenies of indica × japonica

Japonica rice is popular in areas around Tai Lake. However, planting area decreased gradually with lower yields of japonica pure lines than indica hybrids in the 1970s. We bred elite japonica pure lines from indica/japonica//japonica to increase yield by introducing advantageous traits from indica. In the late 1970s, we bred and released varieties such as Nanjing35, Zijinruo, etc. Those japonica varieties were well known by their yield of 9 t ha^{-1} in production fields over 100 ha and 10.8 t ha^{-1} in plot yield tests. The two varieties were widely planted in areas around Tai Lake during the 1980s.

Exploiting indica-japonica heterosis with chemical emasculation during 1987-93

In 1987, an elite line, 02428, which later received an award from the Ministry of Agriculture, China, was identified as a line carrying wide compatibility genes (WCG). This line was developed at the Jiangsu Academy of Agriculture Sciences (JAAS) in a progeny population from F_1 between two M_1 derived from ^{60}Co-irradiated japonica varieties, Pangxiegu and Jibangdao (Zou et al 1989). For its wide compatibility, the line was later widely used as a parent or donor of the *S5-n* gene in intersubspecific hybrid rice breeding. The author's research group has attempted to use 02428 to develop intersubspecific hybrid rice with the aid of a chemical agent. One of the hybrids, Yayou 2, showed a high yield of 10.5 t ha^{-1} and was planted on 5,000 ha. However, it failed to be widely commercialized because its seed purity was only 60–80% even under favorable seed production conditions due to nonuniform chemical emasculation. Furthermore, as a typical indica-japonica hybrid, it showed sensitivity to planting conditions, for its seed setting fluctuated drastically as temperature and other conditions changed adversely around flowering and grain-filling stages (Lu 1999).

Super hybrid rice breeding with the two-line approach in the past decade

After the failure of practices using indica-japonica F_1 by a chemical agent, the authors had to adjust the technical pathway. We considered that using an indica-javanica or japonica-javanica hybrid might avoid such sensitivity in a typical indica-japonica F_1. On the other hand, a two-line approach using an environment-sensitive genic male sterile (EGMS) line showed high efficiency and was successfully being used in rice breeding. If an EGMS line possessed a WCG, it would be beneficial in intersubspecific hybrid rice breeding, for it could be crossed to both indica and japonica varieties. The safety of seed production could be secured by selecting a suitable climatic area, and by using a so-called warm-water irrigation technique if the female was attacked by cool weather during the sterility-sensitive stage (Zou et al 1999). With persistent practice, we successfully released two hybrids, Liangyoupeijiu and LiangyouE32.

Liangyoupeijiu was developed with a cross of Peiai64S/9311 (65002). The female parent Peiai64S was a javanica EGMS line bred by CNHRRDC with its WCG from Padi, a variety from Indonesia, and temperature-sensitive character from an EGMS, Nongken58S, a mutant from a japonica variety, Nongken58. The male parent was an elite line developed from an indica variety, Yangdao 6, released by JAAS (Zou et al 2003, Lu and Zou 2000).

Liangyoupeijiu exhibited an improved plant type with erect uppermost three leaves, a higher light transmission rate, and a lower light extinction coefficient in its middle to late growth phases in comparison with an indica hybrid, Shanyou 63, which had been widely popularized in China and tropical areas (Lu et al 2004). For a combination of both heterosis and plant ideotype, Liangyoupeijiu showed high yield potential, fine grain quality, and strong resistance to rice bacterial leaf blight and blast diseases. The hybrid had a growth period of 150 ± 24 d, with 15–16 leaves, and an average grain yield of 8.4 ± 2.6 t ha^{-1} according to data of 19 testing sites in a national regional yield trial (northern latitude 23–33°) of southern China in 1999 and 2000 (Lu et al 2004). During 1999 to 2000, in a national test for super hybrid rice, Liangyoupeijiu had an average grain yield of more than 10.5 t ha^{-1} at 38 sites, over 6.7 ha for each site in Hunan and Jiangsu provinces. A grain yield as high as 18.5 t ha^{-1} was obtained in Yunnan Province in 2006. The grain quality of the hybrid was evaluated to be of good grade 2 and 50% of the traits evaluated reached the standard of grade 1 by comparing the general investigation results from CNRRI with the *China Rice Quality Standard.* As a two-line system for seed production, the hybrid had seed yield of 2–2.5 t ha^{-1}, with purity of about 98%.

In terms of both high yield and good grain quality, Liangyoupeijiu is well known as the pioneer of "Super Hybrid Rice," and it was awarded the China National Prize on Technique Innovation, Dupont Innovation Prize, and First Class Prize of Science and Technology in Jiangsu Province. It made headlines in "The top ten news of science and technology" in China in 2000. The hybrid has been registered in six provinces and nationally registered as the first two-line hybrid, and was popularized over 7 million ha during 1999-2008 in wide climatic areas from 0 to 35°N in southern regions of China to southeastern Asia, for example, Vietnam, the Philippines, and Indonesia.

LiangyouE32 (Peiai64S/E32, 65396) was bred by JAAS and CNHRRDC in a two-line hybrid system using Peiai64S as a female parent and elite line E32 as a male parent. The hybrid had been planted from 18 to 35°N and registered in Vietnam in 2001. In 1999, it yielded 17.1 t ha^{-1} in a 720-m^2 plot in Yongsheng County, Yunnan Province, China, which had a record yield of hybrid rice for the time, and reached a daily grain yield of 100 kg ha^{-1}, which was one of the targets for super hybrid rice breeding. For both hybridization and morphological improvement, the hybrid has been praised as a pioneer of "Super Hybrid Rice," and a plant ideotype for super hybrid rice as proposed by Yuan (Dennis 1999).

Thoughts on rice breeding for super high yield

The use of heterosis

As rice F_1 hybrids showed heterosis in their yield potential and other traits, hybrids from indica or japonica varieties have been tested and used in China since 1976. In the past decade, they have been extended to India, Vietnam, and tropical countries, and shown a yield increase of 10–20% over indica or japonica pure lines. The heterosis of intersubspecific hybrids between indica and japonica varieties could be as much as 30% higher than that of indica or japonica cultivars in biomass, but the use of heterosis has been difficult because of partial sterility of panicles in F_1 hybrids, which is known to be the main barrier to the use of pronounced heterosis. Spikelet sterility in indica-japonica hybrids has been attributed to an allelic interaction at locus *S5* on chromosome 6. An allele, *S5-n*, was detected in some varieties, which are referred to as wide-compatibility varieties (WCV), and was known to overcome the sterility in indica-japonica hybrids (Ikehashi and Araki 1987, Ikehashi et al 1994). This created a possibility to increase rice yield significantly by using indica-japonica heterosis.

Since the detection of the *S5-n* allele, several indica-japonica hybrids with a WCV as parents have been tested for their yield potential. Most of these hybrids showed normal spikelet fertility and a pronounced heterosis in grain yield. Although WCVs were used as parents, some of the hybrids showed an unstable seed-setting rate ranging from 20% to 90% in varied environmental conditions, especially at low temperatures. Some indica-japonica hybrids could generally attain a seed-setting of 75–90%, but rarely over 90%, whereas, for their parents or inbred varieties, this seed-setting rate is common. For example, Yayou 2 (3037/02428), a typical indica-japonica hybrid, had a seed-setting rate of about 90% and displayed very strong heterosis in grain yield under favorable conditions. However, its seed-setting rate could decline to 20% when daily average temperature is below 25 °C during temperature-sensitive stages. In addition, its seed-setting rate was seldom above 90% even under favorable conditions. Thus, it was not desirable to apply such hybrids in areas beyond 34°N, where temperature is unstable during rice heading. In these cases, it was found that the female gametes possessed viability while partial pollens could lose viability at lower temperature. It was also found that, even under favorable conditions, pollen fertility was lower than 90% by morphology check, and, of those morphologically normal pollens, a portion of them still lacked functions for fertilization. This trait was observed in many indica-japonica hybrids. It was assumed that pollen abortion caused a reduction in pollen fertility and unstable fertilization, resulting in a low and unstable seed set (Lu 1999).

To develop indica-japonica hybrid rice with high and stable fertility, it is necessary to improve the parents with wider compatibility (Yuan et al 1997). Neutral alleles *S5-n*, *S7-n*, *S8-n*, *S9-n*, *S15-n*, and *S16-n* at the corresponding sterility loci, and pollen sterility-neutral genes, *ga11-n* and *ga14-n*, have been detected in some varieties, such as Akihikari, Dular, and Ketan Nangka (Wan 1995, Lu et al 2000). From a three-way cross, Akihikari//IR36/Dular, neutral genes at *ga11* and six sterility loci (*S5*, *S7*, *S8*, *S9*, *S15*, and *S16*) were combined and elite lines were developed after successive progenies in the author's previous paper. It was confirmed that some elite lines have carried seven or six neutral alleles at the sterility loci and male gamete abortion locus. By testing the performance of F_1s, which used the breeding lines and various testers as parents, these lines actually increase seed set of their F_1s through mitigated spikelet sterilities by six sterility loci and gamete abortion by a gametophyte gene, *ga11*. These lines could be used as parents or potential donors to increase the width of compatibility of rice varieties for improving fertility in intersubspecific hybrid rice breeding (Lu et al 1999, 2004).

Model for plant ideotype

There are several models for rice plant ideotype. For example, a semidwarf and early-growth methodology was proposed by Huang et al, based on practices in dwarf rice breeding (Huang and Lin 1994). A model of large and erect panicles suggested by Yang et al was successfully practiced in northeastern China (Yang et al 1996). A new plant type emphasizing large panicles and less tillering capacity was proposed by IRRI. Recently, Yuan put forward a model for a super hybrid, in which a key trait suggested was having long, erect, narrow, and thick uppermost three leaves (Yuan 1997). These models supported rice breeding with a solid basis for both theory and practice.

From the models mentioned above, the authors noticed that a rice plant ideotype may include two aspects, a basic type and ecotype (Zou et al 1999, Lu and Zou 2003). The basic type contains common traits in all models of the ideotype, such as a short and strong basal internode, and erect uppermost leaves, while an ecotype contains the traits corresponding to environmental conditions, such as plant height, leaf length, and width (Lu and Zou 2003). Furthermore, we realized that a suitable plant type should be varying to correspond with growing stage. Thin leaves and a loose plant type were beneficial for enlarging leaf area and rapid growth during the early stage, while thick and erect leaves increased biomass and seed set by improving the ecological and physiological traits of the rice colony during middle and late growing stages (Lu et al 1991).

According to climatic and ecological conditions, along with the author's thinking and breeding practices on rice plant type, a model of ideotype for super hybrid rice (indica) in the lower reaches of the Yangtze River Valley was proposed as a length of 35–40 cm and width of 2 cm for the top leaf, and 50–55 cm and 55–60 cm in length, respectively, for the second and third leaves from the top. In addition, the ideotype would have an angle of 5°, 10°, and 15°, respectively, and a curvature of 1–1.5 cm^{-1} for leaf face at the heading stage; uppermost three leaves keeping their activities as long as 70 d, which leads to an LAI of 3 at full-ripening stage; loose plant type by thin (SLW = 2.5–3 mg cm^{-2}, dry weight) and curved-slanted leaves during the early growing stage, and compact plant type with thick (SLW = 4–5 mg cm^{-2}) and erect leaves during middle and late growing stages; with a coefficient of light extinction as 0.3–0.4, which allows the plant to have an optimal LAI as high as 8–10 during the middle growing period; plant height of 110–120 cm, with a 2–4-cm basal internode and a long uppermost internode occupying 45% of total stem length; 25–28-cm panicles with 8–10 spikelets per centimeter and showing bend-type in ripening; rich chlorophyll, which leads to a high net photosynthetic rate; and tolerance of light shading and photooxidation, which help increase adaptability to varying light conditions. These characteristics are all important. Yield components are 2.4–2.7 million panicles per ha, 200–250 spikelets per panicle, and a seed-setting rate of 85% (Zou et al 1999, Lu and Zou 2003, Cheng and Zhai 2000).

Rice breeding based on ecological conditions

The yield potential of a rice plant growing in an environment can be expressed according to its adaptability to the ecological conditions. Rice breeding should be based on ecological resources. The lower reaches of the Yangtze River Valley belong to a subregion of the Central China Double and Single Rice Cropping Regions in Chinese regionalization. Rice is commonly sowed there in May and harvested in October. The climatic and ecological conditions are as follows: during May and the first half of June, temperature, light, and water are sufficient for rice sowing and growing; the second half of June and the first 10 days of July used to be rainy and wet, with deficient light intensity and less daily temperature fluctuation; mid-July to mid-August was always accompanied by high temperature and strong solar light; mid-August to mid-September used to be attacked two or three times by a typhoon accompanied by heavy rain; September has a suitable temperature and light for growing and grain filling; October has a suitable temperature, light, and water for rice grain filling, except for occasional cool-dry weather (Lu and Zou 2003).

According to such conditions, rice breeding there should pay attention to tillering ability in the early growing stage to ensure adequate tillers under lower temperature and light intensity during June. In the middle growing stage, high chlorophyll content, which led to high net photosynthetic rate, and tolerance of shading and photo-oxidation are important to mitigate the damage caused by high temperature and intensive light during July and August. A plant height of 110–120 cm favors biomass accumulation and lodging tolerance when attacked by a typhoon accompanied by heavy rain. Resistance to early senescence under cool weather is necessary as well for a long-growth hybrid (Lu and Zou 2003). We might use heritability and heredity of leaf morphological and physiological factors to select suitable parents for a combination.

For a released hybrid, suitable planting area, favorable and safe temperatures for flowering and fertilization, and suitable seasons for sowing and heading for various cropping systems could be determined according to its biological and ecological characteristics (Lu and Zou 2004). Those data would be helpful for the hybrid to express its maximum yield potential.

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Notes

Authors' address: Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China; email: rb8@jaas.ac.cn.

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Creating varieties by fixing the heterosis effect in rice hybrids

J.K. Goncharova

Although heterotic hybrids are widely used, the nature of heterosis has not been completely studied. Data obtained by means of molecular markers are also quite controversial. V.A. Strunnikov in 1999 proposed a theory that not only explained the nature of heterosis but also answered the main questions of heterosis breeding: how gene complexes, which provide a heterosis effect, are created, and how the heterosis effect can be maintained in the following generations. This theory united all the theories that were offered earlier. Data confirmed the theory of the nature of heterosis and also the possibility of maintaining a heterosis effect in the following generations (in rice in particular) according to his method. By using backcross generations of 13 rice hybrid combinations, analyses of the productivity of the received backcross generation were carried out in comparison with the initial hybrid, doubled haploid line, the female parent, the second generation of the hybrid, and parents. The absence of segregation in the backcross generation confirmed the opportunity of fixing the heterosis effect by this method. As methods develop, it is possible to obtain varieties with a yield equal to that of the initial hybrid in 3–4 years.

Hybrids not only exceed the yield of the best traditional varieties by an average of 20–50%, but they have increased protein in grain and are highly adaptive to unfavorable environmental conditions. At the same time, the productivity potential of released hybrids increases as it increased in the parents. Super rice hybrids, recently released in China, exceed the yield of "first-generation" hybrids by 15–20% (Yuan 2002, Ma and Yuan 2002, Bastawisi et al 2002).

The price for hybrid seeds is high because of the labor-intensiveness in seed production. As a result, developing countries do not have enough resources for developing hybrid crops and farmers cannot afford the seeds of the most adapted hybrids. The maintenance of heterosis in the next generations will help to use the heterosis effect. Now, several approaches have been suggested for maintaining the heterosis effect: the creation of an apomictic hybrid, a vegetatively propagated plant, and transferring genes responsible for the formation of the corresponding traits from other types of plants (Bennett et al 2002, Eckardt 2003, Tucker et al 2003). But none of these has been realized in production. In addition, the genotype of the initial hybrid is not improved by using these methods.

Although hybrid crops are widely used, the nature of heterosis has not been completely studied. Experimental data obtained from different theories were controversial. In recent years, some experiments on heterosis have been carried out by using molecular markers (Xiao et al 1995, Xu 1988, Yu et al 1997, Zhang et al 1995, Zhao et al 1999); however, the results were also somewhat controversial:

1. Heterozygosis does not guarantee heterosis, but can provide an advantage over homozygotes.
2. All types of genetic effects influence the development of heterosis (partial, complete, or extra domination) at the allelic level as well as among different loci.
3. The additive effect plays a key role in developing heterosis (Hua et al 2002).

Strunnikov's theory of the nature of heterosis

There are still lots of discussions about the nature of heterosis. Strunnikov in 1999 proposed a theory that not only explained the nature of heterosis but also answered the main questions on heterosis breeding: how a gene complex that provides the heterosis effect is created and how the heterotic effect can be maintained in the following generations (Strunnikov and Strunnikova 2000a,b). According to Strunnikov, the heterosis effect develops because of inheritance from a parental compensation complex of favorable genes as a result of selection in conditions of unfavorable genetic and ecological factors. If parental lines of a hybrid carry unfavorable genes, they could only survive (and also have good productivity) if they also have a complex of favorable genes that are strong enough to compensate the effects of the unfavorable genes. In a hybrid, an unfavorable gene must be in the recessive form and not decrease productivity. A complex of favorable genes from one or both parental lines provides a manifestation of the heterosis effect. This theory united all the theories that were suggested earlier. There is no big difference between heterotic hybrids and a highly productive variety. In both cases, the same genetic mechanisms exist, and the application of the heterosis effect only allows finding and using more effectively parental lines with naturally created gene complexes.

Heterosis maintenance in successive generations

The idea of possible heterosis maintenance in successive generations appeared after the study of the nature of heterosis. In anther culture, it was found that a lethal gene in a haploid pro-nucleus leads to the death of an embryo at one of the development stages. Only those genotypes that have minimum lethal genes and maximum favorable genes can survive. The frequency of the surviving individuals usually does not exceed 0.5% during development from a pollen grain to a doubled haploid plant. The survival percentage is high if these genotypes have minimum lethal genes. The genotypes with lethal and half-lethal genes were eliminated, but a complex of favorable genes is preserved with surviving plants. It is clear that backcrossing a hybrid to a DH line without harmful genes in homozygous and heterozygous conditions generates an F_1 progeny with a genotype of no lethals in a homozygous condition. This gene complex and a great number of gene modifiers helped to not only maintain but also strengthen heterosis in all successive backcrossed generations. Thus, heterosis maintenance can be achieved by means of getting hybrids without lethals and half-lethals. At the same time, the genotype of the initial hybrid is not only maintained but also improved. By backcrossing, the number of genes with no productive function in each new progeny is two times less than that in the previous generation.

The high viability of backcross generations is of great interest because these generations come from self-propagation that is usually less viable. High viability is explained by the fact that backcrossing does not, unlike simple bisexual propagation, lead to the appearance of lethal genes in a homozygous condition. A backcross genotype consists of one set of genes with lethals from one parent and another set with only a favorable gene complex that allows pollen grains to survive in anther culture. The unfavorable genes in a backcross genotype will be in a heterozygous condition without function and the complex of favorable genes will provide a manifestation of a heterosis effect.

The method of heterosis maintenance in successive generations proposed by academician V.A. Strunnikov was tested on hybrids of silkworm and *Drosophila,* and we tested it in plants at our Institute with more success than expected because it not only maintained the heterosis effect in backcross generations but also improved hybrid genotypes by eliminating lethal and half-lethal genes that reduce productivity. With this method, we can develop varieties with yield similar to that of outstanding hybrid combinations in a short time.

The method of maintaining hybrid heterosis in future generations is by using recurrent crosses, including the generation of doubled haploids from pollen through anther culture and backcrossing. Data showed that the process of obtaining doubled haploids purified the genotype of the initial hybrid from lethal and half-lethal genes brought by the parents. Backcrossing increases dramatically the possibility of obtaining a variety with the high productivity of the initial hybrid.

Results and discussion

Our data showed that backcross generations obtained from doubled haploids increased productivity without segregation, which justified the efficiency of technologies for creating rice varieties. By this method, it is possible to develop a variety with a yield equal to that of the initial hybrid in three to four years. This will help to increase rice productivity potential to 20–50% and in a short time to release varieties with a yield of 12–15 t ha^{-1}. In addition, unique germplasm with a complex of compensation genes will be obtained and provide a basis for the development of new-generation hybrids. The genetic basis of heterosis learned from rice gives us the possibility to use this method for other types of crop plants, especially in cereals.

Table 1. Panicle weight of backcross progeny, hybrid, and F_2 in 13 hybrid combinations obtained in 2005.

Statistical data	Backcross generation	F_1	F_2
Mean panicle weight (g)	2.22	2.39	1.79
Variance	0.06	0.06	0.16
Standard deviation	0.24	0.24	0.40
Coefficient of variation (%)	11.49	10.17	22.40

Table 2. Agronomic traits of backcross generations in 13 hybrid combinations obtained in 2006.

Traits	Mean		Standard deviation		Standard error of mean	
	F_1	F_1/DH	F_1	F_1/DH	F_1	F_1/DH
Plant height (cm)	76.2	79.9	10.43	11.08	1.71	1.81
Panicle length (cm)	14.7	15.1	1.91	1.81	0.31	0.46
Grains per panicle (no.)	91.7	88.1	39.58	41.22	4.86	6.77
Sterile spikelets per panicle (no.)	9.3	8.6	8.83	5.61	1.45	0.92
Spikelets per panicle (no.)	100.9	96.7	39.91	41.47	4.91	6.81

From 13 hybrid rice combinations, we obtained two kinds of backcross generations: (1) progenies derived from a DH line backcrossed with any plant in a hybrid population that was used for generating a DH line; (2) progenies derived from a DH line, which was generated from the best plant in a hybrid population, backcrossed with the original hybrid plant. Their productivities were analyzed in comparison to those of initial hybrids, doubled haploids, the parents used for backcrossing, F_2s of hybrids, and parents. Many segregated lines were generated from the first type of progenies because different genotypes were used for backcrossing. However, segregation was absent in the second type of backcross progeny, which confirmed the possibility of fixing the heterosis effect by the backcross method. The grain productivity of backcross generations was even higher than that of the original hybrids because of many opportunities to select highly productive DH lines for backcrosses to create high-yielding progenies (Tables 1, 2, 3).

The methods proposed by Strunnikov allowed us to improve hybrid genotypes by eliminating lethal and half-lethal genes with less productivity. The productivity of the plants generated by backcrossing a DH line in hybrids of Khazar/Liman was higher than that of the original hybrid and the better parent, Khazar (Fig. 1). Through the doubled haploids, genotypes from the initial hybrid with unfavorable genes were eliminated in anther culture. But some lines with unfavorable genes survive; therefore,

Table 3. Productivity of plants obtained by backcrossing hybrids with doubled-haploid (DH) lines generated from hybrid pollen.

Combination	Plant height (cm)	Panicle length (cm)	Grains per panicle (no.)	Sterile spikelets per panicle (no.)	Mean of main panicle weight (g)
Khazar/Liman					
F_1/DH	84.25	15.25*[a]	135.5	3.25	3.88*
F_1	77	14.25	106.25*	3.75	3.04*
F_2	74	14	86.3	10.2	2.47
Pegasso/Viragh					
F_1/DH	76.5	15*	92*	8	2.94*
F_1	59	12	69*	2	2.21
F_2	58.1	11.7	56.9	12	1.82
Muiya/Sneghinka					
F_1/DH	79	17	112.3*	8.3	3.21*
F_1	83	17	86*	15	2.46*
F_2	74	16.3	74	21	2.12
VNIIR 7718/VNIIR 7887					
F_1/DH	88	15	181*	12	4.26*
F_1	77.67	13.00	120.7*	7	2.87*
F_2	77.30	14.40	112.3	16	2.59

[a]* = Significantly different at the 5% level.

Fig. 1. The productivity of plants obtained from backcrossing hybrid Khazar/Liman with a DH line, hybrid F_1 (Khazar/Liman), and the best parental forms of hybrid Khazar.

Table 4. Characteristics of doubled haploid lines.

Hybrid combination	Line no.	Plant height (cm)	Panicle length (cm)	Grains per panicle (no.)	Sterile spikelets per panicle (no.)	Main panicle weight (g)	1,000-grain weight (g)
Khazar/Liman	1	75	14.5	98	54	2.61	26.6
Khazar/Liman	2	83	15	89	27	2.90	32.6
Khazar/Liman	3	85	15	70	18	2.59	37.0
Khazar/Liman	4	65	12	71	5	2.08	29.3
Khazar/Liman	5	86	15	96	12	2.27	23.6
Liman/Khazar	1	76	13	93	22	2.28	24.5
Liman/Khazar	2	74	13	106	10	2.36	22.3
Liman/Khazar	3	83	16	117	13	2.60	22.2
Liman/Khazar	4	75	13	120	13	3.00	25.0
Liman/Khazar	5	79	10	97	11	2.59	26.7
Liman/Khazar	6	78	12	86	17	3.08	35.8
Liman/Khazar	7	81	14	93	24	2.62	28.2

Table 5. Chlorophyll mutation in a population of doubled haploid lines at seedling stage.

Hybrid combination	White blade[a]	Yellow leaf	Striped leaf	Brown spot
VNIIR 7718/VNIIR 7887	*	*		
Khazar/Phontan		*	*	
Serpantin/Khazar	*	*	*	
Khazar/Izumrud	*	*	*	*

[a] * = Presence in population of doubled haploid lines generated from the hybrid combination.

it is necessary to produce a large quantity of DH lines, as some of these lines have low productivity and are sterile (Table 4). In this case, we can use the best DH line for backcrosses.

Another observation was that a large quantity of mutations occurred during the process of anther culture, including both nuclear and somatic tissues (Tables 5, 6). In addition, some of these lines carrying the lethal genes also survive (Goncharova et al 2003, 2005). It is easy and necessary to screen and reject those lines by selecting for productivity among DH lines, as all lethal genes in these lines are in a homozygous

Table 6. Chlorophyll mutation in a population of doubled haploid lines developed from hybrid combination Khazar/ Izumrud.

F_1 plant	Size of population (no.)	White blade (no.)	Striped leaf (no.)	Yeallow leaf (no.)	Brown spot (no.)
c.39	59	13	2	10	–
c.5, p.2	91	7	2	–	–
c.27, p.5	102	12	–	–	–
c.32, p.5	97	–	2	1	7
c.5, p 5	70	2	–	2	–
c.5, p.3	131	10	4	2	–
c.2, p.3	60	1	–	2	–

status. In recurrent backcrosses, only the best DH lines with nonlethal genes will be used, and this helps to preserve the heterosis effect.

Conclusions

The absence of segregation in backcross generations proved the theory of the nature of the heterosis effect, which is determined by a complex of favorable genes instead of their heterozygotic condition.

Generating a DH line allowed us to purify the genotype of initial hybrids from parental lethal and half-lethal genes that cause reduced productivity in the subsequent generations. Backcrossing a hybrid to a DH line allowed us to create varieties with productivity equal to or higher than that of the original hybrid. By the methods developed, it should be possible to develop varieties with yield similar to that of the original hybrid within 3–4 years and increase the productivity potential of a crop by 20–50%.

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Notes

Author's address: All-Russian Rice Research Institute, email: serggontchar@mail.ru.

Development of high-quality cytoplasmic male sterile rice lines

Guangjun Ren, Fangyuan Gao, Xianjun Lu, Zhihua Li, Guangchun Liu, Shuxia Sun, and Haojie Li

Improving the quality of hybrid rice varieties, especially the quality of indica hybrids, is one of the most important goals in rice production for many rice-producing areas of the world. In our breeding program, two cytoplasmic male sterile (CMS) lines, Chuanxiang-29A (Ch-29A) and Chuanmei-102A, as well as some good-quality maintainer lines were developed by combining classical breeding with molecular marker-assisted selection (MAS) to obtain desirable alleles. We introduced the aromatic trait from Xiangsimiao-2, an aromatic rice landrace native to southern China, into the CMS maintainer line II-32B by crossing. From the progenies, we obtained an elite CMS maintainer line named Chuanxiang-29B (Ch-29B) and the corresponding sterile line, Ch-29A, which had a high-quality appearance, large panicles, and high general combining ability (GCA). Subsequently, we generated a series of hybrid rice combinations to be released for production. Of all the combinations, ten aromatic hybrid rice combinations were developed and released by Sichuan, Chongqing, Fujian, Anhui, and Guangxi Provincial Variety Examination Committees. Moreover, a total of three combinations were approved by the National Variety Examination Committee during the past five years. Having early flowering and pollination times, a high percentage of exserted stigmas, and a high outcrossing rate, Ch-29A also proved to be a new CMS line that could yield about 4.5 t ha^{-1} in hybrid rice seed production. Further linkage analysis between simple sequence repeat (SSR) markers and the aroma locus for aromatic F_2 plants mapped the Ch-29B aroma gene to a chromosome 8 region flanked by SSR markers RM23120 at 0.52 cM and RM3459 at 1.23 cM. In an effort to specifically improve the rice quality of the CMS line, G46A, Lemont was used as a donor parent to introduce resistance to preharvest sprouting (PHS), a low percentage of chalkiness, and intermediate amylose content (AC) into G46B, the maintainer line of G46A that was used as a recurrent backcross parent. Based on the evaluation of agronomic traits and grain quality, MAS with the SSR markers RM3754, RM447, and 484/485 (linked to PHS and AC, respectively) was used for screening the plants in the backcross population between G46B and Lemont. Some elite lines obtained from the backcross generation were then backcrossed to G46A to improve breeding of the CMS lines. A new CMS line, Chuanmei-102A, was successfully developed using a strategy similar to that employed to generate G46A, but this line was specifically improved in PHS, AC, grain shape, and chalkiness. Furthermore, we used major maintainer lines currently used in rice production (II-32B, F-32B, Bo-B, Ch-29B, Zhong-9B, and IR58025B) as recurrent parents, and Basmati370, KDML 10, and Lemont as donor parents to develop more than 2,000 maintainer lines with intermediate AC, good appearance quality, and a high percentage of exposed stigmas. These lines could be useful for developing new CMS lines and good-quality hybrid rice in the future.

Keywords: hybrid rice, cytoplasmic male sterile line, rice quality, aroma, intermediate amylose content, preharvest sprouting, outcrossing rate

The three-line system of hybrid rice breeding and production is an important milestone in the process of rice breeding following the use of dwarf varieties. It not only widened rice genetic resources and changed breeding technology but also promoted technology innovation and the development of the rice seed industry. The successful cultivation of three-line indica hybrid rice increased yield more than 20% over that of conventional semidwarf varieties, which has made tremendous contributions to Chinese and world food security. In 2006, the area growing three-line hybrid rice reached 12.92 million ha accounting for 84% of the total hybrid rice-growing area in China (Agricultural Technology Service Center, Ministry of Agriculture, People's Republic of China, 2007). This shows that three-line hybrid rice in southern China remains the major type.

Gangyou 725, Gangyou 527, II you 838, and II you 7 are representative of the new hybrid rice varieties and they have become the four leading varieties of medium hybrid rice in China. Additionally, they made an important contribution to the stability and development of rice production. However, most of them are criticized for their poor appearance and bad eating quality, caused primarily by the poor quality of their cytoplasmic male sterile (CMS) lines, G46A and II-32A.

Aroma is one of the most important characteristics for good-quality rice. Aromatic rice is extremely popular and highly valued for its flavor and palatability. However, most traditional fragrant rice varieties have taller plants and poor yield. Although the successful breeding of fragrant semidwarf varieties has been accomplished, their yield and adaptation are still worse than those of hybrid rice. Therefore, good-quality aromatic hybrid rice with a high yield would need to be developed by adding an aroma gene.

Amylose content (AC) is one of the most important determinants for the cooking and eating quality of rice grains. It is established that granule-bound starch synthase (GBSS), encoded by the *Wx* gene, plays an important role in determining AC in plants (Shure et al 1983, Preiss 1991, Smith et al 1997). Bligh et al (1995) found the presence of a (CT)n repeat tightly linked to the coding sequence of GBSS in *Oryza sativa* and designed the simple sequence repeat (SSR) primer pair 484/485, which allows for the detection of forms of the (CT)n repeat for different rice varieties. Furthermore, some studies have verified that 484/485 accounts for a large portion of AC variation (Ayres et al 1997, Shu et al 1999). Improvement of AC by marker-assisted selection

(MAS) with 484/485 has been successfully implemented in our breeding program (Li et al 2004). Lemont, Basmati370, and KDML 105, which are characterized as having intermediate AC (Kenneth and Webb 1997, Gao et al 2008b), were used as donors to introduce intermediate AC into G46B, II-32B, and Ch-29B, which were used as recurrent backcross parents. MAS with the SSR marker 484/485 was used for detecting the genotype of intermediate AC in the backcross population, aiming at exploring effective methods for improving the eating quality of indica hybrid rice.

In the rice-growing area of Sichuan, China, the preharvest sprouting rate (PSR) of G46A in hybrid rice seeds was about 10%. In some years, especially in 2005, when rainy weather lasted for a long time, the preharvest sprouting (PHS) of G46A had strong effects on hybrid rice seed quality due to a more than 50% PSR. Therefore, hybrid rice seed production requires CMS maintainer and sterile lines with good resistance to PHS.

Materials and methods

Aroma evaluation and development of aroma with CMS maintainer lines

Aroma in leaves was determined according to the method described by Sood and Siddiq (1978). The samples were classified into two categories according to the presence or absence of aroma. In addition, all important materials will be identified by a panel consisting of 3–4 members with differential abilities for distinguishing between aroma and nonaroma. Samples were also scored again through tasting individual grains if there was no consensus on the evaluation of aroma.

From 1992 to 2000, a CMS maintainer line (II-32B) was selected to cross with aromatic rice variety Xiangsimiao-2. The pedigree method was used to select for fragrant materials, which would then be used to testcross with CMS lines and backcross with aromatic CMS lines.

Evaluation of PHS

PHS was determined as Gao et al (2008) described. Briefly, three random branches of panicles were harvested from each plant. Within 1 h, the harvested branches of panicles were immersed into de-ionized water for 4 h in 150-mL Erlenmeyer flasks. Extra water was then removed to keep the samples in about 1 cm of water covered with a plastic film to prevent water loss through evaporation. The preharvest sprouting rate was scored after 7 days of incubation.

DNA extraction and SSR analysis

DNA was extracted from fresh leaves according to a modified CTAB method (Murray and Thompson 1980). Out of 800 SSR primer sets distributed on the 12 rice chromosomes (from public data at Cornell University), 136 SSR markers were used for genetic background selection. The SSR marker 484/485 is linked to the *waxy* gene and was designed by Bligh et al (1995). All the primers were synthesized by TaKaRa Company in Dalian, China. PCR analysis was carried out as described previously

(Gao et al 2008a). The bands specific to G46B and Lemont were designated as “G” and “L,” respectively; the bands specific to F_1 were recorded as “H.”

AC measurement

Experiments for measuring AC were conducted following the simplified procedures described in National Standards NY 147-88 (NY is the Chinese abbreviation for “agricultural”). Amylose content standards (premeasured following ISO 6647-1987 or GB 7648) were brought from the Rice Product Quality Testing Center at the Ministry of Agriculture in Hangzhou, China.

Evaluation of grain quality for appearance

Simple visual estimation of the shape, chalkiness, and transparency of brown rice was used to select plants and lines with good PHS resistance and intermediate AC. Furthermore, plants with poor appearance were discarded.

Results

Breeding and application of an aromatic rice CMS line, Ch-29A

In the summer of 1992, a three-line maintainer (II-32B) with a high outcrossing rate was used as the female parent and crossed with Xiangsimiao-2, an aromatic rice landrace native to southern China. In the winter of 1992, F_1 hybrids were planted in an experimental field in Hainan. The generated F_2 seeds were grown in an experimental field in Chengdu in the summer of 1993. After morphological identification, a total of 17 plants with aromatic fragrance, good shape, and abundant pollen were selected for further self-pollination. A total of 14 plants with good agronomic traits were screened, and the F_4 plants were planted at Hainan in the winter of 1994. Then, 12 plants were selected and crossed with Zhenshan 97A. All resultant F_1s were sterile males, which were planted at Chengdu in the summer of 1995. Plants with the desired paternal traits were selected to backcross with sterile lines. The BC_1F_1 lines were planted in the winter of 1995. The pollen abortion lines with paternal tendency were used as the female parent in a pair backcross. After continued backcrosses at Chengdu and Hainan from 1996 to 1998, a female line (12595) was obtained from the BC_6F_1, which has stable sterility, a high yield, and good GCA. Based on the results of paired testing, some combinations were included in a multiregion test in Sichuan Province, and most of them indicated good grain quality and high yield. Based on the investigation of heterosis, pollen sterility, outcrossing rate, and agronomic traits, the sterile line 12959 was designated as Ch-29A.

Ch-29A has an earlier flowering time. In sunny weather, the beginning of flowering time occurs before 1000, with full bloom at 1140. Seventy-five percent of the florets opened before noon, which is better than the flowering time of restorer lines (Fig. 1). In addition, the exposed stigma rate of Chuanxiang-29A is as high as 78%, of which 46% of the stigmas are double-exposed. Because of the high outcrossing rate, 4.5 t ha^{-1} of seed production can be achieved with Ch-29A as a female parent. Seed setting on F_1s between Ch-29A and eight restorer lines, Minghui63, CDR22, Chenghui177,

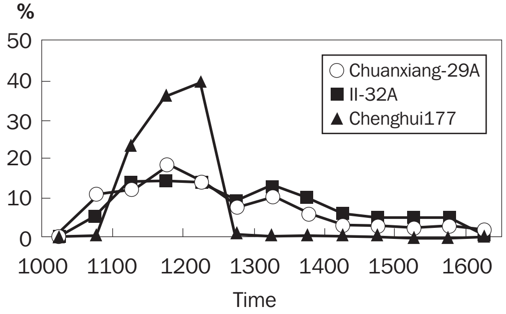

Fig. 1. Comparison of flowering time among Ch-29A, II-32A, and restorer line Chenghui177.

Fuhui838, Shuhui527, Mianhui725, Chenghui448, and R402, ranged from 77% to 83% and averaged 80.5%. Based on the data from the Ministry of Agriculture rice product testing center, Chuanxiang-29A had a seed setting that was 80.2% of the brown rice rate, 74.1% of the milled rice rate, and 59.4% of the head rice rate. The grain had a length of 5.7 mm, a length/width ratio of 2.4, a chalky grain value of 48%, a chalkiness of 7.6%, a transparency of 2, a gel consistency of 54 mm, an amylose content of 21.6%, and a protein content of 12.0%. The segregation ratio of F_2 nonaromatic to aromatic plants was 3:1, which indicated the inheritance of a single recessive gene in aromatic rice variety Ch-29B. Linkage analysis between SSR markers and the aroma locus for the aromatic F_2 plants mapped the Ch-29B aroma gene to a chromosome 8 region flanked by SSR markers RM23120 at 0.52 cM and RM3459 at 1.23 cM. Our molecular mapping data from the two populations indicated that the aroma locus occurs in a 142.85-kb interval on BAC clone AP005301 or AP005537, implying that it might be the same gene reported by Bradbury et al (2005). In rice grains, aroma was controlled by the endosperm (3n). Nonaroma was a dominant character just like the behavior of the unscented parent. The fragrant and nonaromatic grains in the hybrid rice of Ch-29A crossed with unscented restorer lines are a natural rice mixture because only 25% of the rice grains are fragrant.

A total of 10 fragrant hybrid rice combinations bred by Ch-29A were released by the Sichuan Provincial Variety Examination Committee, of which Chuanxiangyou-2, Chuanxiangyou-6, and Chuanxiangyou-8 were released by the National Variety Examination Committee. In the middle and downstream area of the Yangtze River with better light and temperature conditions, Chuanxiangyou-2 and Chuanxiangyou-6 can achieve higher yield. A higher percentage of head rice and a lower percentage of chalky grains were generally observed in the fragrant hybrid rice based on the results of rice quality analysis. The quality of appearance, processing, and eating also improved significantly (Table 1). Two hybrid combinations, Chuanxiangyou-2 and Chuanxiangyou-993, reached the second grade of the national standard for the main quality parameters. Up to 2008, the cultivation area for those combinations from Ch-29A totaled more than 800,000 ha in southern China.

Table 1. Preharvest sprouting resistance (PSR), amylose content, and appearance quality of some near-isogenic introgression lines of G46B in BC_2F_5.[a]

Line	PSR (%)	Genotype of 484/485	AC (%)	Chalky grains (%)	Chalkiness (%)
G46B	95.40	G	26.13	97.06	11.16
Lemont	2.98**	L	20.10**	2.64**	0.13**
K06	18.47**	L	20.03**	14.02**	1.11**
K23	10.85**	L	19.87**	12.22**	0.84 **
K45	16.35**	L	20.89**	22.99**	1.32 **
K65	11.82**	L	19.50*	26.96**	1.62**
K73	0.12**	L	20.40**	25.33 **	1.59**
K81	7.22**	L	20.03**	13.09 **	0.81 **
K89	6.95**	L	19.43**	14.99 **	1.06 **
K135	9.37**	L	20.15**	12.33 **	1.00 **
K152	13.24**	L	20.11**	20.55 **	1.51 **
K156	19.79**	L	20.12**	20.67 **	0.33**

[a] Indicates significance at the 0.01 probability level. G and L represent the *Wx* genotypes of G46B and Lemont, respectively.

Pyramiding of good PHS resistance and good grain quality into an elite indica CMS maintainer line, G46B

To specifically improve PHS resistance and AC in CMS line G46A, Lemont, which is characterized as having intermediate AC and resistance to PHS (Kenneth and Webb 1997, Gao et al 2008), was used as a donor to introduce these elite traits into G46B, which was used as the recurrent backcross parent. Our previous study indicated that the difference in the *Wx* gene between those two varieties can be detected with the SSR marker 484/485 (Li et al 2004). In the winter of 2001, G46B was used as the recipient and crossed with Lemont at Hainan. The F_1 plants were backcrossed with G46B twice to create a BC_2F_1 population. PCR amplification with 484/485 was performed in BC_2F_1 and BC_2F_2, and plants heterozygous at the *Wx* allele were analyzed with 136 SSR markers evenly distributed on 12 chromosomes. The plants heterozygous for 484/485 and relatively similar to G46B in agronomic traits were selected for further backcross and self-pollination. From the BC_2F_3 generation, PHS, grain appearance, and AC were evaluated. Stable integrated character was observed until BC_2F_5. Some characteristics of the BC_2F_5 lines with the Lemont allele at the *Wx* locus and less than 20% of PSR are summarized in Table 2. The greatest difference in PHS resistance was observed among those lines with less than 20% PSR, which ranged from 0.12% to 19.79% and may be caused by the differential amount of minor QTLs for PHS in different lines. Furthermore, the AC for these lines was intermediate, with a variation

Table 2. The (CT)n of the *Wx* gene and amylose content (AC) of donor varieties.

Donor	Subspecies	AC (%)	CT repeats in exon 1 of *Wx* gene	Origin
KongYu131[a]	Japonica	17.2	18	Japan
Lemont	Japonica	20.0	20	America
KDML 105	Indica	15.8	17	Thailand
Basmati370	Indica	19.0	17	India

[a]One of the main varieties grown in the japonica rice area in northeast China.

of 19.43–20.40%. By discarding plants with a large percentage of chalkiness in the self-pollination generation, the chalkiness of those lines listed in Table 2 was obviously improved compared with that of G46B. The results indicated that the pyramiding of desired alleles from Lemont can effectively improve the PHS resistance, eating quality, and appearance of G46B.

Although the abovementioned traits have been improved in BC_2F_5 lines, most of the lines did exhibit some undesirable agronomic traits, such as taller plant height, lower seed set, and lateness for heading, compared with G46B because of their indica/japonica background. In addition, the genetic basis of PHS resistance is still unclear. Therefore, a near-isogenic line of G46B, K81, with good preharvest sprouting resistance, intermediate amylose content, and good appearance, was selected to backcross with G46B. An F_2 population of 164 plants derived from G46B and K81 was developed to detect the QTLs governing PHS. A total of three QTLs for PHS, qPSR2, qPSR5, and qPSR8, were identified on chromosomes 2, 5, and 8, respectively. Among these QTLs, qPSR8 resided in the interval between RM3754 and RM447 on chromosome 8 and was the major QTL controlling PHS because it had a relatively high LOD score and explained 43.04% of the phenotypic variation (Gao et al 2008). The SSR markers RM3754 and RM447 were closely linked to the major QTL qPSR8 and were used in MAS for resistance to PHS from the BC_3F_3 generation. In the subsequent self-crossed generations, about 80% of the plants showed lower PHS (PSR<20%) in the progeny of G46B/Lemont when homozygous Lemont alleles were amplified with both RM3754 and RM447. Therefore, it is quite plausible that homozygous Lemont alleles or G46B alleles can be used to screen PHS with SSR markers on chromosome 8 in a segregating population. To specifically improve PHS, lines with "L" amplification bands at both RM3754 and RM447 markers should be targeted.

From BC_3F_2, MAS for PHS and AC was combined with the investigation of appearance, plant height, and seed setting for brown rice. Furthermore, 28 BC_3F_5 lines with good preharvest sprouting resistance, intermediate amylose content, and lower percentage of chalky grain were selected and backcrossed with the sterile line G46A five times to develop new CMS lines. Following this procedure, we obtained two stable sterile lines, 101A and 102A, and their respective maintainer lines, 101B and 102B. The yield of F_1s between the two CMS lines and the three restorer lines (Chenghui 727, Mianhui 725, Fuhui 838) is higher than that of other combinations. G46B displayed a high AC, around 26.1%, whereas 101B and 102B showed 21.0% and 19.4% AC, respectively. In 101B and 102B, 7.0% and 10.3% chalky grains were observed, respectively, while the chalkiness of G46B was 97.1% (Gao et al 2009). In 2008, several combinations from Chuanmei-102A were selected to participate in a multilocation test.

Using MAS to develop introgression lines with intermediate amylose content

To meet the demand for good-quality rice for eating and to adhere to the current use of the major male sterile lines in different ecological zones of China, the following characteristics should be included in a maintainer line selected as a receptor parent: (1) F_1 combinations should have strong heterosis. Some of them have been broadly used in rice production or they performed excellently in regional trials; (2) a higher head milled rice rate should be observed in hybrid rice; and (3) they should have good outcrossing rates and high yield of seed production and reproduction. Therefore, a total of 8 maintainer lines were chosen as key receptors for our improved breeding program: II-32B, Ch-29B, Chuan358B, and F-32B from the region of medium indica hybrid rice cultivation; Zhong-9B from double-cropping rice cultivation area; Bo-II B and Bo-III B from the late hybrid rice cultivation region in southern China; and IR58025B, an aromatic indica maintainer line bred by IRRI and widely used in Indian rice production.

Although different standards were adopted to evaluate grain quality in different countries, most consumers prefer soft rice with an intermediate AC, ranging from 15% to 20%. Kongyu-131, the main japonica rice variety currently used in northeast China, is one of the two rice varieties whose cultivation area surpassed 670,000 ha in 2006. People favor it for its high yield, wide adaptability for different environments, good grain quality, and AC of 17.3%. The jasmine rice variety KDML 105 is a cultivar with the largest cultivation area in Thailand. It is also the most popular rice worldwide because it is aromatic, soft, and tender. Testing for grain quality at IRRI indicated that the AC of KDML 105 is about 15.7%; both Lemont and Basmati370 have intermediate AC of about 20% and a moderate degree of softness. Moreover, Basmati370 is especially appropriate for people who live in South Asia since it has low viscosity and stickiness.

In our study, Ch-29B, II-32B, Chuan358B, and other rice varieties were used as receptor maintainer lines to cross with donor varieties to produce F_1 seeds. These seeds were then backcrossed one to three times with the corresponding receptor line. Further self-pollination and selection generated K81, a near-isogenic introgression line of G46B with intermediate AC, which was selected as a donor line to introduce the intermediate allele of Lemont into other receptor maintainer lines. The strategies for backcross and selection are similar to those used for G46B. MAS for 484/485 was

Table 3. Comparison of rice quality among receptor maintainer lines and improved lines (2008, Hainan).

Line	Combination	Length/ width	Percentage of chalky grains	Chalkiness (%)	Amylose content (%)
Ch-29B	II-32B/Xiangsimiao-2	2.5	51.0	9.0	23.5
29309	92037/K81////Ch-29B	2.7	24.2	4.0	20.0
29311	92037/K81////Ch-29B	3.0	10.1	1.0	19.3
29981	K81/92037////Ch-29B	2.7	14.4	2.3	19.6
F-32B	You 1B/FeigaiB//L301B	2.7	23.2	6.3	23.1
32716	K81/92097////F-32B	2.7	13.1	2.4	18.1
32720	K81/92097////F-32B	2.7	9.1	1.6	18.5
32789	K81/92037////F-32B	3.1	8.2	1.7	20.0
Bo-B	(Bo-B/1441)F4/ Bo-B	2.4	12.0	2.1	14.4
II-713	K81////Bo-B	2.5	15.1	3.3	18.1

conducted in each backcross generation and plants with the Lemont allele of *Wx* were selected to backcross with receptors. The plants with good grain shape, high yield, and good appearance and grain quality were emphasized in the self-crossed population. The repeated identification of the *Wx* genotype and the evaluation of AC should be done on those lines with better genetic purity and fine comprehensive agronomic traits. On the other hand, some special methods should be explored for special receptors. For example, an improved line of Ch-29B (92037 or 92097) with slender grain shape and low percentage of chalky grains was used as an original receptor to cross with K81 instead of using Ch-29B because of its length-width ratio (2.5) and higher percentage of chalky grains. Subsequently, more than 2,000 introgression lines with intermediate AC have been developed in our study. Table 3 shows the AC and appearance quality of the introgression lines, which have been significantly improved compared with their corresponding receptors.

Discussion

Identifying and applying the aroma gene in rice

Elution of leaves with dilute KOH and testing of grain taste were done to evaluate aroma in our breeding program to develop aromatic rice maintainer lines, including Chuanxiang-29B. In several previous mapping experiments, the aroma gene *fgr* was localized on chromosome 8 (Ahn et al 1992, Sun et al 2008). Bradbury et al (2005) suggested that a gene encoding putative betaine aldehyde dehydrogenase (BADH2) is most likely the *fgr* gene, because of its sequence divergence between fragrant and nonfragrant rice varieties. 2-acetyl-1-pyrroline (2AP) has proved to be a key component of rice scent (Wongpornchai et al 2003). The accumulation of 2AP in fragrant genotypes may be explained by an 8-bp deletion in exon 7 of BAD2, subsequently resulting in a loss of function of the *fgr* gene product (Bradbury et al 2005). From our genetic and physical mapping data, the aroma gene in Ch-29B is presumably the same *fgr* gene encoding BAD2.

Because rice fragrance is a recessive trait, it will be difficult to use traditional methods for distinguishing the plants with an aromatic genotype, which will be used in further crosses or backcrosses, from all BC_nF_1 plants after crossing aromatic and nonaromatic varieties. For the coding region of the betaine aldehyde dehydrogenase 2 gene *BAD2*, Bradbury et al (2005) designed co-dominant primers, which revealed significant polymorphisms in aromatic rice genotypes versus nonaromatic genotypes, thus facilitating the identification of the aroma gene in a rice breeding program. MAS using this marker to introduce the aroma gene of Ch-29A into several good-quality indica maintainer lines, F32B, Zhong9B, and some others, has been successfully used in our breeding program (this will be published in another paper).

Identifying and applying the major QTL for PHS

Previous studies indicated that PHS is closely related to rice seed dormancy (Frank et al 2005, Groos et al 2002, Wan et al 1997). By using molecular markers, QTLs for seed dormancy or resistance to PHS have been identified on all rice chromosomes (Lin et al 1998, Miura et al 2002, Dong et al 2003, Gu et al 2004, Wan et al 2006). However, almost all the QTLs reported for PHS are able to explain only a small portion of the phenotypic variation. Therefore, there is still a question as to whether MAS can be efficient in improving rice PHS. In our previous study, a major QTL, qPSR8, was identified by using an F_2 population derived from G46B and its near-isogenic introgression line. This QTL can explain 43.04% of the phenotypic variation of PHS (Gao et al 2008). Because of our successful use of this locus in MAS for PHS, the PSR of improved lines of G46B dropped significantly to less than 20% compared with about 95% PSR in G46B, implying that MAS for qPSR8 is a highly efficient way to breed for PHS improvement.

Improving AC by using MAS for the SSR markers linked to the *Wx* gene

It is well established that granule-bound starch synthase (GBSS), encoded by the *Wx* gene, plays an important role in determining AC in rice grains (Shure et al 1983, MacDonald and Preiss 1985). The genetic basis for starch quality could be more complex for endosperm traits because these traits might be affected by quantitative genes of triploid endosperm, cytoplasm, and the maternal plant genome (Bao et al 2002). However, some studies indicate that the major genes for eating, cooking, and textural qualities, such as *Wx* and *alk* (or starch synthase IIa, SSIIa), were confirmed by QTL mapping (He et al 1999, Bao et al 2000, Wan et al 2004, Fan et al 2005). Two functional markers in the *Wx* gene, a (CT)n microsatellite (or SSR) and a G/T single nucleotide polymorphism (SNP), have been well characterized with different alleles differing in apparent amylose content (AAC) (Bligh et al 1995, Ayres et al 1997, Shu et al 1999). Thirteen (CT)n microsatellite alleles, (CT)8, (CT)10, (CT)11, (CT)12, (CT)13, (CT)14, (CT)16, (CT)17, (CT)18, (CT)19, (CT)20, (CT)21, and

(CT)22, were found at the *Wx* locus based on the identification of a large amount of rice germplasm (Ayres et al 1997, Shu et al 1999, Bergman et al 2001, Prathepha and Baimai 2004, Bao et al 2006, Mikami et al 2008). The analysis of the relationship between the (CT)n alleles of the *Wx* gene and AAC for 499 nonwaxy rice lines has clearly indicated that rice lines with (CT)8, (CT)10, (CT)11, or (CT)12 had higher than 28% AAC. The AAC of *Wx* alleles (CT)17 and (CT)18 was intermediate, ranging from 15.0% to 16.4%. The *Wx* SSR and *Wx* SNP alone could explain a large portion of the variation for all physicochemical properties. For example, *Wx* SSR and *Wx* SNP could explain 90.3% and 89.3% of the total variation for AAC, 77.2% and 75.5% of the total variation for gel hardness, and 82.8% and 82% of the total variation for gel cohesiveness, respectively (Bao et al 2006). Therefore, it is quite plausible to identify the genotypic difference of rice varieties with different eating quality by MAS since the markers are tightly linked to the *Wx* gene.

In our study, several major maintainer lines currently used in rice production, II-32B, F-32B, Bo-B, Ch-29B, Zhong-9B, and IR58025B, were selected as recurrent parents to cross with Basmati370, KDML 10, Lemont, and Kongyu131, which have intermediate AC. MAS with 484/485 was used to identify AC in the backcross and self-crossed generations. Subsequently, more than 2,000 maintainer lines with intermediate AC, good appearance, and a high percentage of exposed stigmas have been developed. Further breeding for corresponding sterile lines was successful in our program.

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Notes

Authors' address: Crop Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China.

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Research on the development of CMS-D1-based japonica hybrid rice with high yield potential for the Yun-Gui Plateau

Xuelin Tan, Ruke Hong, Yaling Tan, Shoulin Jin, Hongyun Xu, Ai Guo, Lijuan Chen, Dajun Huang, Zhonglin Zhang, Guanxi Tao, Xi Wang, Shuhua Zhang, Changjun Shi, and Zhengyou Li

Japonica rice is grown widely in fields with altitudes from about 1,400 to 2,700 meters in the Yun-Gui Plateau in southwestern China, where climatic conditions are unfavorable for the growth of landrace rice varieties. To improve hybrid vigor for rice grain production, research based on cytoplasmic male sterility (CMS) of Dian type 1 (D1) has been carried out in the following areas in recent years. (1) The development of japonica CMS lines with high and stable sterility under various temperature conditions. (2) Genetic characterization and molecular genotyping of fertility revertants discovered in CMS-D1 populations. (3) Identification of major *Rf* loci in various CMS systems with DNA markers, in which restorers of several CMS systems showed the same genotype at the *Rf-1* locus. (4) Screening of new *Rf* sources in landraces with the DNA markers located within the *Rf-1* locus. (5) Enriching of genetic diversity of restorers and maintainers of the CMS-D1 system through development of indica-japonica lines, which possess characteristics of indica or wild rice and the genetic background of japonica cultivars. (6) The development of japonica hybrid varieties possessing high yield potential, in which two hybrid varieties released possess disease resistance, good grain quality, and yield potential above 12.75 t ha^{-1} averaged on a 6.67-hectare field.

Keywords: CMS, Dian 1 type, japonica hybrid rice, fertility revertant, super rice

Rice is the most important food crop in the Yun-Gui Plateau, a region diversified in geography, climate, rice germplasm, and culture. In this region, rice is grown in fields varying in altitude from 100 to 2,700 meters, and japonica cultivars are grown mainly in fields with altitude above 1,400 m. Because of the special climatic conditions in japonica rice areas, which occupy about 60% of the rice fields in Yunnan Province, all the varieties used in production are developed in Yunnan, as landrace varieties introduced from other regions, such as northeastern China, Japan, and Korea, do not fit the climate.

To improve hybrid vigor for grain production of japonica rice, two cytoplasmic male sterility (CMS) systems, boro II type (BT) and Dian 1 type (D1), are used in China. CMSCMS-BT, which was developed in Japan in 1965 (Shinjyo 1975) and introduced in China in 1972, is mainly used in the northeast and a few other provinces in China (Yuan 2002). CMS-D1, which was developed in 1969 at Yunnan Agricultural University in China, is used in Yunnan and other provinces (Li et al 1980). Since the first CMS-D1-based hybrid combination was developed in 1973, breeding activities have been carried out for the development of japonica hybrid varieties (HVs). However, the commercial application of japonica hybrid rice is very limited because of the lack of *Rf* genes in japonica rice, the unstable sterility of CMS lines, and low hybrid vigor of HVs. These are three important factors in the development of japonica HVs for commercial application.

Besides these three factors, the use of japonica HVs in the Yun-Gui Plateau faces more difficulties, such as serious blast disease and low temperature during rice growth. To overcome these problems, research has been carried out for the development of japonica HVs to be used in the special environmental conditions of the Yun-Gui Plateau. This article explains some research done recently based on the CMS-D1 system.

The identification of stable sterility of CMS-D1 lines

Some CMS lines of BT and D1 types will become partially self-pollinated in warm conditions, which will cause a reduction in hybrid seed purity in seed production. Over time, several HVs with strong hybrid vigor, such as Liyou 57 and Xuanza29, which were japonica HVs based on the BT and D1 system, respectively, failed because of poor seed purity in the extension of commercial HVs. Therefore, the development of CMS lines with stable sterility is important for hybrid-seed-based japonica rice production. Evaluating the stable sterility of CMS lines has become an essential step during the development of CMS-D1 lines. For this evaluation, CMS lines were grown in areas with diverse altitudes, which makes temperature conditions much different. All of the CMS-D1 lines were separated into three sets and grown continuously for two years at Yuanjiang, Mengzi, and Kunming, where altitudes are 400, 1,250, and 1,960 m. Daily average temperatures in the month during rice flowering are 28.3, 22.8, and 19.7 °C, respectively. Through evaluation, we found that most of the CMS-D1 lines had stable sterility under environments varying in temperature. Anthers of these CMS lines were not dehiscent and did not pollinate at the two sites. Although some of the CMS lines did not have stable sterility, their anthers became dehiscent in warm conditions at Mengzi. Those CMS lines showed partial pollination, and had a low seed set, around 0.5–1%, under bagging. Those sterile unstable CMS lines could not be used in the development of hybrid rice.

Genetic characterization of CMS fertility revertants

Besides the stable sterility of CMS lines, the existence of fertile plants (FPs) in CMS populations is another common and serious problem in seed production of CMS-based hybrid rice. FPs are similar to CMS plants in phenotype, except for fertility, which is supposed to be caused by contamination by mechanical or biological factors (Lu et al 2000, Yashitola et al 2004), but its cause is a puzzle to be solved.

It was reported that some FPs could have the *Rf* gene, and they were assumed to have resulted from a reversion of CMS lines (Tan et al 2001). To study FPs in detail, a population with 11,000 plants of CMS-D1 (Dian type 1) line Hexi42A was generated through bagging, which is an effective method to prevent CMS lines from being pollinated by off-type varieties. In this population, 0.073% (eight) FPs were found, in which two types of FPs were distinguished through test-crossing and DNA marker genotyping.

One type of FPs had six plants, accounting for two-thirds of the total FPs. They had the N(*rf/rf*) genotype, the same as that of CMS maintainers. They could have been derived from contamination of maintainers in the production of CMS seeds. They could be reduced, even eradicated, by a restriction procedure in CMS seed production.

Another type had only two FPs. They produced about 50% fertile pollen, and had the sterile cytoplasm of CMS-D1 type. The FPs had the *Rf* gene, designated as *Rfr(d1),* in heterozygous genotype *Rf/rf*, which made the fertility restoration of CMS-D1 plants in a gametophytic mode. The frequency of FPs was 0.0182% in the CMS population. They should be generated through fertility reversion of CMS genotypes. These plants were designated as fertility revertants. An allelic test showed that *Rfr(d1)* was allelic to CMS-D1 restorers, and it was located within the *Rf-1* locus, which was identified by CAPs marker M45461*Taq*I and M49609*Bst*UI located within the *Rf-1* locus developed by Komori et al (2004). *Rfr(d1)* could be generated through spontaneous reversion of an *rf* genotype in CMS lines. This is the first demonstration of fertility revertants in rice CMS populations. The details were reported by Wen et al (2008a,b).

Mapping the relationship of major *Rf* genes of CMS systems

CMS-D1 and CMS-BT both share the same characteristics of pollen abortion, and share the same maintainers and restorers. Fertility restoration is controlled by a single dominant gene in both systems, and the *Rf* genes for these two systems are allelic, which was deduced based on results of testcrosses (Tan et al 2004). Recently, our studies showed that *Rf-d1,* the *Rf* gene of CMS-D1, was located in the middle of the long arm of chromosome 10 (Tan et al 2004). Just in the same region, *Rf-1*, an *Rf* gene identified for the CMS-BT system, has been cloned (Komori et al 2004, Wang et al 2006). Even so, it is still unclear whether *Rf-d1(t)* and *Rf-1* are located at the same position or just adjacent positions. DNA markers M45461*Taq*I and M49609*Bst*UI located within the *Rf-1* locus (Komori et al 2004) provide a convenient tool to study the relationship. These two markers showed that restorers of CMS-D1, such as Nan34, and restorers of CMS-BT, such as C418, had the same genotype. All the restorers had the same *Rf* gene located within the *Rf-1* locus, whereas all the maintainers of CMS-D1 lines showed another genotype. Nucleotide sequence of the band amplified from restorers of the CMS-D1 system had 99% similarity with the corresponding region of *Rf-1* genes. The results indicated that *Rf-d1(t)* identified in the CMS-D1 system by Tan et al (2004) was located within the *Rf-1* locus. In fact, evidence from mapping and cloning indicated that major *Rf* genes of other CMS systems, such as the CMS systems of wild abortive (WA), Honglian (HL), Dissi (D), and Yinshui (Y), detected on the long arm of chromosome 10 (Tan et al 1998, Liu et al 2004, Li et al 2007, Sattari et al 2008), could be the same one located within the *Rf-1* locus, although these genes were mapped at positions with minor variations in the region, which could be due to a size limitation and difference in mapping populations.

Screening of *Rf* sources in landraces with DNA markers

Exploring new sources of *Rf* genes for the development of restorers used in hybrid japonica rice would benefit the diversity of japonica hybrid rice production. A testcross between CMS lines and candidate lines is a classical method of identifying *Rf* genes, but it is a time-consuming procedure and it is limited by environmental conditions.

The feasibility to identify new sources of *Rf* genes by using the DNA markers located within the *Rf-1* locus developed by Komori et al (2004) was tested in the development of a CMS-D1 system based on hybrid rice. On the marker M49609*Bst*UI, restorers were distinguished from maintainers of CMS-D1. The restorers showed a 810-bp band, whereas the maintainers did not possess this fragment, but showed two bands with 610 bp and 200 bp. Thus, the *Rf-1* markers were used for screening lines derived from multiple crosses between indica and japonica lines in breeding programs, and landraces recently collected from mountainous areas in Yunnan Province in southwestern China. When 466 traditional cultivars collected from mountainous areas in Yunnan were surveyed with a CAPs marker, the *Rf* gene was detected only among indica cultivars, but not in any japonica cultivars.

Developing indica-japonica lines by wide crosses

Less genetic diversity among japonica lines used in japonica hybrid rice was a cause of weakness of hybrid vigor of CMS-D1-based japonica hybrid rice, which was recognized by analysis of the genetic distance between maintainers and restorers of CMS-D1 based on morphological traits (Tan 1988). To improve the genetic diversity of restorers and maintainers used for developing CMS-D1-based japonica hybrid rice, multiple crosses between japonica lines and indica cultivars or wild accessions were made to develop the lines used as restorers or maintainers. As the genetic backgrounds of japonica and indica or wild accessions have been combined into a line, these genetic materials are called "indica-japonica" lines. For restorers, besides possessing *Rf* genes, these indica-japonica lines also possess other genetic characteristics donated by indica or wild accessions, as well as characteristics such as cold tolerance donated by japonica cultivars. In developing indica-japonica lines with tolerance of low temperature and able to be grown in diverse areas varying from 1,400 to 2,200 m in the Yun-Gui Plateau, indica landraces grown at 1,400–1,700 m, or japonica cultivars grown at 2,650 m, which is the highest rice-growing area in China, were used in multicrosses. To overcome the low fertility of the progenies derived from the crosses between intersubspecies or interspecies, backcrosses were carried out using one or several japonica lines as recurrent parents. In so doing, a lot of indica-japonica lines carrying *Rf* genes have been developed and used as restorers, such as Nan34, Nan36, Nan43, and Nan46. For example, Nan46 was derived from the multicrosses involved in four japonica and three indica lines from several provinces in China and at IRRI. These restorers have a high ratio of indica genome background.

The possibility of developing high-yielding and resistant japonica hybrid varieties

High yield potential, resistance to diseases, and good grain quality are the main characteristics required in rice production in Yunnan. There was a general belief that it was not possible to combine high yield, strong resistance, and good grain quality into a variety. In rice production, a lot of varieties had only one of the characteristics as an advantage, such as possessing high yield but poor resistance to diseases and poor grain quality, or possessing excellent grain quality but poor resistance and low yield. For example, Yuza29, a CMS-D1-based japonica HV developed in the 1990s, had a very high yield potential, but it failed in commercial production because of poor resistance to blast disease, a fatal factor for japonica rice in Yunnan. A lot of such field examples made people speculate on the possibility of developing rice varieties possessing high yield potential, strong resistance, and good grain quality together.

The development of indica-japonica lines of CMS lines and restorers increased the possibility of developing japonica hybrid rice with hybrid vigor. Using these indica-japonica lines, a few D1-based combinations have been released for production. These HVs showed high grain yield, good grain quality, resistance to disease, and tolerance of unfavorable environmental conditions.

To develop HVs with disease resistance, materials with strong resistance were used as parents in developing indica-japonica lines. Moreover, the disease resistance of breeding materials was evaluated in the lab or by growing them in fields suffering from serious blast and other diseases annually, such as in fields at Yilian County in Yunnan. Through evaluation, a few lines with good resistance to blast have been identified. By using these resistant accessions, a few restorers and CMS lines with good resistance have been developed, such as Nan34 and Dianjingnuo1A.

With the restorers, several HVs with high yield and good resistance have been developed, such as Dianza31, Dianza32, Dianyou34, Dianza35, and Dianza40, in recent years. Of these HVs, Dianza31 and Dianza32 have super-rice yield potential. Harvested grains of these two varieties annually ranged from 12.8 to 14.4 t ha^{-1} during 2004-07 at Baoshan in Yunnan, where the major rice area is at 1,700 m. The yield surpassed the standard of super rice in China. The high yield of these two HVs resulted from large genetic distance between their parents. Their CMS lines and restorers are highly heterozygous, possessing an indica and japonica background denoted by several cultivars from various ecological environments. For example, the parents of Dianza31 were derived from multicrosses involved in both indica and japonica. The CMS maintainer of Dianza31 was involved in four japonica lines and one indica cultivar from Yunnan, Taiwan, Japan, and Korea. Its restorer, Nan34, is more divergent, being derived from crosses involved in two indica lines (GEA2and IR8) and five japonica lines (KMBJM, MDJ4, TZ31, KQ3, and YTLB) from Yunnan, Guangdong, Taiwan, Helongjiang, Italy, and IRRI.

The complexity of genetic backgrounds makes the HVs not only have high yield potential but also good fitness to various environmental conditions. Dianza35, a combination made by Nan34 with another CMS-D1 line, also has very good fitness to various environmental conditions. This HV grows well from the south to the north, and from the east to the west, at altitudes from 1,000 to 2,100 m in Yunnan. This HV also grows well in japonica rice areas of Sichuan, Guizhou, and a part of the mountainous area in Longhui County in Hunan. Therefore, developing indica-japonica lines is an efficient approach for making japonica hybrid combinations with strong hybrid vigor.

Developing HVs possessing excellent grain quality is more difficult than for inbred varieties since HV grains are seeds of F_2 progenies, which are under genetic segregation. Therefore, both restorers and maintainers should have similar characteristics of good grain quality. Thanks to the good grain quality of the restorers and maintainers of Dianza31 and Dianza32, the HVs also have good grain quality. These two HVs have 12 out of 13 quality traits for the National Standard of the People's Republic of China: Good Quality of Rice Grains (GB/T17891-1999, 1999), which makes the two HVs ranked as excellent grain quality varieties in rice in the third evaluation of rice grain quality in Yunnan.

Although diseases are serious problems in rice production, disease resistance is controlled by a dominant gene in general (Wang et al 1989, Wu et al 1999), which makes it relatively easy to develop HVs possessing resistance. Therefore, HVs will have resistance to disease, if the restorer or CMS line, only one of the parents, has resistance, which is well demonstrated by HVs based on the CMS-D1 system. CMS lines Yumi15A and LiyuA of Dianza31 and Dianza32 do not have disease resistance, but the HVs have strong disease resistance because the restorer Nan34 has resistance to all major pathogens of blast disease in Yunnan (Table 1). In addition, Dianyou34 and Dianza35 also have strong resistance to blast. In fact, these HVs also have good resistance to leaf blight. Therefore, this is an advantage for developing rice HVs with disease resistance. In addition, these HVs have good tolerance of unfavorable environmental conditions, such as cold weather and less water.

Table 1. Blast resistance of HVs and restorers.

Line	Main pathogen strains in Yunnan[a]							
	Y34	Y88-278	TH77-1	TH74-9	Y69	Qing92-06-b	88A	IW81-04
Nan34	R	R	R	R	R	R	R	R
Yumi15 A	S	S	S	S	S	S	S	S
Liyu A	S	S	S	S	S	S	S	S
Dianza31	R	R	R	R	R	R	R	R
Dianza32	R	R	R	R	S	R	S	R

[a]R = resistant, S = susceptible.

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Authors' address: Rice Research Institute, Yunnan Agricultural University, Kunming 650201, China.

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Advances in japonica hybrid rice breeding

Hua Zetian and Wang Yanrong

The development of japonica hybrid rice has been limited by a lack of restorer genes among local varieties, the low yield of hybrid seed production, insufficient heterosis, and poor quality. With efforts over 30 years, breakthroughs have been achieved, and theories and methods of japonica hybrid rice breeding are becoming more mature. The technology of "bridging between indica and japonica" rice was created to breed japonica restorer lines with wide affinity, which solved the problem of a lack of restorer genes, and japonica restorer lines such as C57, C418, and C2106 were mainly bred. To increase the yield of hybrid seed production, japonica sterile lines with a high stigma exsertion rate were bred by the method of crossing and backcrossing between japonica and indica or wild rice. Ecological heterosis groups for japonica hybrid rice breeding have been determined, that is, sterile lines should come from early japonica rice varieties that are tolerant of low temperature, and restorer lines should be derived from indica rice varieties in Southeast Asia, such as IR8 and Miyang23. Because rice quality traits are controlled mainly by additive effects, the quality of both parents should be improved at the same time in order to improve the quality of hybrids, especially that of the male parent, which makes a greater contribution to hybrid quality than the female parent.

Keywords: japonica hybrid rice, bridging between indica and japonica, stigma exsertion rate, seed production, heterosis, ecological heterosis group, rice quality

With the finding of wild-abortive (WA) male sterile rice plants in the 1970s (Yuan et al 1998), hybrid rice could be successfully bred by the three-line method and used widely for production. Then, especially in indica hybrid rice, quick development was achieved. Deng et al (2006) reported that the planting area of indica hybrid rice has accounted for more than 80% of the area of indica rice. However, the development of japonica hybrid rice has been relatively slow. Up to 2003, the planting area of japonica hybrid rice had accounted for only about 3% of the area of japonica rice. As indicated by Hua et al (2006), the lack of its own restorer genes, the low yield of hybrid seed production, insufficient heterosis, and poor rice quality were the main factors limiting the development of japonica hybrid rice. The use of heterosis in japonica is still a severe problem.

Since 1970, research on japonica hybrid rice has been done at the Rice Research Institute of Liaoning Province, and the first japonica hybrid rice combination was bred and used widely in production, which became the basis for japonica hybrid rice research. With efforts over 30 years, breakthroughs have been made, and theories and methods of japonica hybrid rice breeding are becoming more mature. Some aspects are described as follows.

Basic theories and methods of japonica hybrid rice breeding

The technology of "bridging between indica and japonica"

Regarding the use of heterosis in japonica rice, in the 1960s, the first japonica sterile line of BT was bred in Japan, and then other types such as Dian were bred. But, because of a lack of restorer genes of its own in japonica rice and the difficulty in using indica restorer lines directly, japonica hybrid rice did not succeed with the three-line method until the technology of "bridging between indica and japonica" was created. Early in the 1970s, to solve the problem of a lack of restorer genes, the Rice Research Institute of Liaoning Province first created the technology of "bridging between indica and japonica" to breed japonica restorer lines, through which the first japonica restorer line, C57, was bred successfully, as reported by Yang (1999). C57 formed the basis of japonica hybrid rice. The first japonica hybrid rice combination, Liyou57, was bred with strong heterosis and used widely in production. The development of japonica hybrid rice peaked in the 1980s. The technology of bridging breeding aims to transfer restorer genes from indica rice into japonica rice by crossing and backcrossing between indica and japonica varieties. It also partially uses the heterosis between indica and japonica. After C57 was bred, more japonica restorer lines, such as C418 and C2106, were bred by the technology (Hua et al 2006). C418 has become the backbone of restorer lines in both japonica and indica rice. C2106 is also a promising new restorer line with high combining ability. In C57, C418, and C2106, only one-third or less of the pedigree is of indica. The technology of bridging is a general guide for both japonica and indica hybrid rice breeding, and some indica restorer lines have also been bred by the method.

Improving stigma exsertion in japonica sterile lines

The low yield of hybrid seed production is another main factor behind the development of japonica hybrid rice. Tian et al (1990) and Dai et al (1999) found that stigma exsertion rate is one of the main factors affecting the yield of hybrid seed production. Because stigma vitality can be kept for 3–7 days (Li et al 2004), stigma exsertion can increase the probability of pollination not only on the flowering day but also after flowering, which can make up for losses in the difference in flowering time between the male and female parent to pollination. Li (1995) considered that the seed-setting rate of glumes with exserted stigma accounted for 70–80% of the whole seed-setting rate in hybrid seed production. According to Yang (1997), with an increase of 1%

Table 1. Characteristics of the main sterile lines of japonica hybrid rice in northern China.

Name	Year	Flowering time	Percentage of exserted stigma				Seed-setting rate (%)	Seed yield (t ha^{-1})
			Glume opening	Glume closing				
				Total	Single	Double		
XiuA	1977	1030–1330	55.9	28.9	25.4	3.5	34.8	1.5–2.0
TiA	1988	1100–1230	50.2	29.3	26.4	2.9	36.4	2.0–2.5
Liao326A	1993	1010–1230	42.3	15.2	13.3	1.9	28.6	1.5–2.0
Liao151A	1996	1120–1330	30.6	9.8	8.6	1.2	16.2	1.0–1.5
Liao5216A	1998	0930–1200	65.2	33.6	22.9	10.7	42.3	2.5–3.0
Liao02A	2000	1030–1210	60.2	37.9	33.5	4.4	37.8	2.5–3.0
Liao99A	2000	1000–1300	78.4	54.5	46.8	7.7	44.9	2.5–3.0
Liao105A	2000	0930–1210	85.3	57.9	49.0	8.9	50.2	3.0–3.5
Liao30A	2001	1000–1240	80.1	54.5	43.9	10.6	44.5	3.0–3.8
Liao39A	2003	1000–1230	87.3	55.8	48.3	7.5	50.5	3.0–3.5
Liao60A	2005	0930–1230	95.7	87.1	54.5	32.6	63.8	3.5–4.5
Liao40A	2005	0930–1300	92.4	63.2	46.3	16.8	56.7	3.5–4.0
Liao846A	2005	1000–1200	93.4	87.6	54.1	33.5	60.6	3.5–4.5

in stigma exsertion rate of sterile lines, the seed-setting rate of hybrid seed production would increase by 0.74–0.92%, and the yield of hybrid seed production would increase by 47–68 kg ha^{-1}. Therefore, the stigma exsertion rate of sterile lines is very important for achieving high yield in hybrid seed production. According to Xu and Shen (1986), the stigma exsertion rate of japonica rice is generally less than 30%, whereas that of indica rice is more than 60%, so it is necessary to improve the stigma exsertion of sterile lines to solve the problem of low yield of hybrid seed production in japonica hybrid rice.

Since 1996, we have been focusing on improving the stigma exsertion of japonica sterile lines by crossing and backcrossing between japonica and indica rice and have made progress. Over the years, the stigma exsertion rate of new sterile lines increased from 9.8% to 87.6% (Table 1), at the same time the flowering time became earlier, which resulted in an increase in seed-setting rate and seed production yield (Wang and Hua 2008).

Ecological heterosis groups for japonica hybrid rice

In crop breeding, the phenotype is always affected by environment as well as genotype, so ecotypes have been formed for different ecological environments. With the accumulation of breeding experience, ecological heterosis groups of parental materials

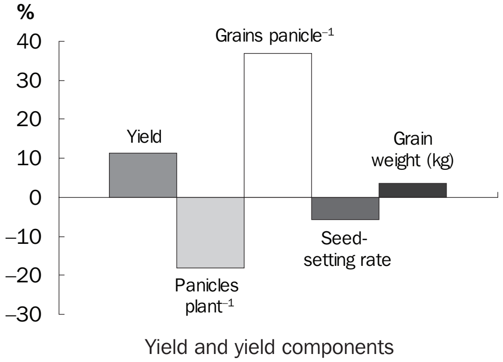

Fig. 1. The heterosis of japonica hybrid combinations and a control variety in yield and yield components.

have been constructed and used for breeding, which makes breeding more effective. The construction of ecological heterosis groups must take into account the relation between exogenous varieties and local varieties.

For japonica hybrid rice breeding, the ecological heterosis groups should have sterile lines coming mostly from early japonica rice, which is tolerant of low temperature, and restorer lines should have some consanguinity with indica rice in Southeast Asia such as IR8 and Miyang23, which are more helpful for achieving japonica hybrid combinations with strong heterosis and are fit for the cool climate in northern China (Hua et al 2006).

Achieving high yield

High yield is the most important competitive ability for hybrid combinations in conventional varieties, especially for japonica hybrid rice. The development of hybrid combinations in rice production depends on higher and higher yield.

Balancing yield components

Yield is composed of panicles per plant, glumes per panicle, seed-setting rate, and grain weight, and yield components are closely related to each other, so it is crucial to balance yield components for high yield. Figure 1 compares hybrid combinations and a control variety in yield and yield components in Liaoning Province. Compared with the control, the hybrid combinations have positive heterosis in yield, glumes per panicle, and grain weight, and negative heterosis in panicles per plant and seed-setting rate. For northern China, though, there is huge potential yield in japonica hybrid combinations, and potential yield cannot be brought into play for fewer panicles and low seed-setting rate because low temperature at the late growth stage affects grain filling. So, it is necessary to decrease glumes per panicle and increase panicles and seed-setting rate properly.

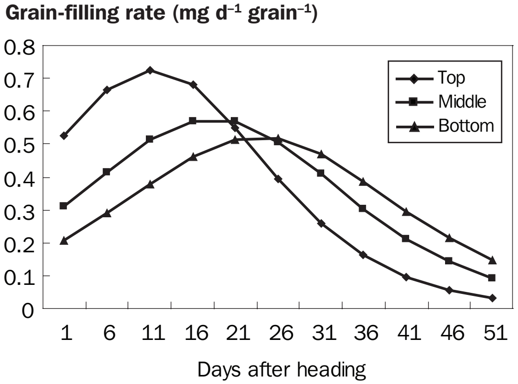

Fig. 2. Comparison in grain-filling rate among different positions on the panicle of japonica hybrid rice combinations.

Panicle characteristics for high yield

The flowering and grain filling of glumes on a panicle are on a time sequence from the top to the bottom and from the first branches to the secondary branches. In our research, we discovered that, for japonica hybrid rice, because of large panicles, there is a big difference in seed-setting rate among glumes in different positions on the panicle. The seed-setting rate of glumes on the top is higher than that of those on the bottom, and the rate on the first branches is higher than that on the secondary branches. Therefore, it can be concluded that "inverted-triangle" panicles should be of benefit for japonica hybrid rice to retain a high seed-setting rate, that is, the bottom of the panicle is mainly composed of first branches, and the top has more secondary branches.

At the same time, there was a significant difference in grain-filling rate and grain-filling duration among the different positions on the panicle, as seen in Figure 2. On the top of the panicle, grain filling was quicker and stronger than on the middle and bottom. So, to achieve high yield in japonica hybrid rice, the difference in grain filling among the top, middle, and bottom of the panicle should be reduced to as little as possible whether for grain-filling rate or for grain-filling duration, which depends on further optimization of panicle structure and yield components.

Enhancing the general combining ability of parents

In using heterosis, combining ability (CA), including general combining ability (GCA) and specific combining ability (SCA), of parents is usually considered an important parameter for forecasting heterosis. Table 2 shows the results of research on 100 hybrid combinations from 10 female parents and 10 male parents in Liaoning Province. The GCA of parents was significantly related to yield and yield components of hybrid F$_1$, but SCA was not significantly related. Further, in Figure 3, among high-yielding combinations, 63% were combined by both parents with high GCA, 28% were combined

Table 2. Correlation coefficients between parent CA and yield traits of hybrid F_1.

Trait	Yield	Panicles plant^{-1}	Glumes panicle^{-1}	Seed-setting rate	Grain weight
GCA	0.605	0.723	0.688	0.609	0.904
	**[a]	**	**	**	**
SCA	0.068	0.122	0.034	0.115	0.068
	ns	ns	ns	ns	ns

[a] ** and ns represent $P<0.01$ and $P>0.05$.

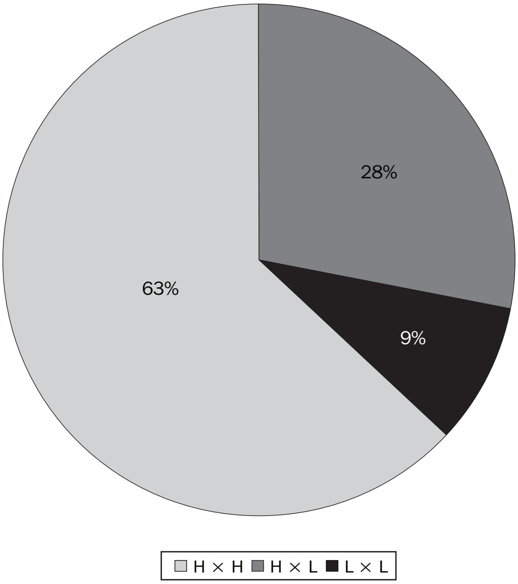

Fig. 3. The probability of high yield combinations combined by parents with different general combining ability.

Table 3. ADi of parents of japonica hybrid rice.

Female	ADi	Male	ADi
L105A	0.32	C2106	0.48
L5216A	0.27	C418	0.38
L95A	0.25	C414	0.32
L30A	0.22	C4115	0.30
L99A	0.18	C190	0.28
L20A	0.18	C258	0.25
L02A	0.16	C01	0.22
L24A	0.15	C332	0.20
L91A	0.12	C746	0.16
L326A	0.12	C238	0.15
L151A	0.12	C52	0.12

by one parent with high GCA and another parent with low GCA, and only 9% were combined by both parents with low GCA. So, it is easier to obtain a high-yielding hybrid combination by choosing parents with high GCA.

Optimizing the difference in the index of indica blood (ADi) between parents

Heterosis in rice has to use the heterosis between subspecies of indica and japonica because rice is a self-pollinating crop. The difference in ADi between the female and male parent should be positively related to heterosis. But, because of the problem of affinity between indica and japonica rice, heterosis between indica and japonica can only be used partially. The difference in ADi between the female and male should be moderated according to the ecological environment.

Table 3 shows the ADi of the main parents in japonica hybrid rice. The ADi was determined by using the method of simple sequence repeats. Fifteen pairs of primers were used for polymerase chain reaction and 52 polymorphic markers were produced. Now, in japonica hybrid rice, the ADi of sterile lines ranges from 0.12 to 0.32, and that of restorer lines ranges from 0.12 to 0.48. In Table 4, the ADi of the male is more closely related to the yield and heterosis of hybrid F_1 than ADi-D and ADi-F by the range of ADi shown. The ADi of the female is even negatively related to heterosis to some extent. So, in breeding japonica hybrid rice combinations, the ADi of restorer lines should be increased and that of sterile lines should be decreased appropriately on the basis of the range of 0.12 to 0.48.

Table 4. Correlation coefficients between parent ADi and yield and heterosis of hybrid F_1.

Trait	ADi-F	ADi-M	ADi-D
Yield	0.037	0.119	0.070
Heterosis	–0.037	0.201	0.167
ADi range	0.12–0.32	0.12–0.38	0–0.26

Table 5. The probability of combinations whose rice quality trait reaches the standard among 100 japonica hybrid rice combinations.

Trait	Grade one		Grade two	
	Standard value	Probability (%)	Standard value	Probability (%)
Brown rice rate (%)	≥81	100	≥79	100
Polished rice rate (%)	≥72	59	≥70	89
Fine polished rice rate (%)	≥59	16	≥54	36
Chalkiness (%)	≤5	4	≤10	30
Chalkiness area rate (%)	≤5	85	≤5	85
Transparency degree	1	35	2	65
Alkali digestion value	≥4	97	≥4	97
Gel consistency (mm)	≥60	93	41–60	7
Amylose content (%)	17–22	20	≤25	100
Protein content (%)	≥8	86	≥8	86

Improving rice quality

Rice quality in japonica hybrid rice

Poor rice quality is another factor in the development of japonica hybrid rice, because conventional japonica varieties have strong competitive heterosis in rice quality.

Table 5 shows the status of rice quality in japonica hybrid rice combinations. In comparison with the standards promulgated by the Ministry of Agriculture, the brown rice rate of all combinations reached the standard of grade one; the alkali digestion value of 97% of the combinations reached the standard of grade one; and 93%, 86%, and 85% of the combinations reached grade one in gel consistency, protein content, and chalkiness. In polished rice rate, fine polished rice rate, chalkiness, transparency degree, and amylose content, the combinations reaching grade one accounted for 59%, 16%, 4%, 35%, and 20%, respectively, and those reaching grade two accounted for 89%, 36%, 30%, 65%, and 100%. So, for japonica hybrid rice, improvement in

Table 6. The genetic effect of variance of rice quality traits.

Effects	Polished rice rate	Fine polished rice rate	Chalkiness	Chalkiness area rate	Transparency degree	Amylose content
Embryo A	0.527**	14.37**	23.7**	0.674**	0.045**	0.131**
Endosperm A	0	0	17.1**	0.311*	0.012**	0.092**
Endosperm D	0.030*	0	0	0	0	0
Mother A	0.104	7.17**	18.4**	0.706**	0.031**	0.119**
Mother D	0.689*	27.36**	50.2**	1.72**	0.102**	0.317**
Embryo × mother A	–0.012	5.87	23.5**	0.926**	0.039	0.171**
Endosperm × mother A	0	0	41.9	1.09*	0.071	0.248
Endosperm × mother D	0.583**	0	0	0	0	0
A variance rate	0.239	0.336	0.629	0.59	0.575	0.619
D variance rate	0.502	0.335	0.187	0.207	0.214	0.192

chalkiness, fine polished rice rate, amylose content, transparency degree, and polished rice rate is more urgent.

The basis for improving rice quality

Any improvement should be made according to the genetic basis. The variance of all rice quality traits is composed of additive variance and dominant variance, and for most traits additive variance is bigger than dominant variance except for polished rice rate (Table 6). Polished rice rate is controlled mainly by embryo additive and endosperm dominant, mother dominant, and endosperm × mother dominant effects. Fine polished rice rate is controlled by embryo additive and mother additive and dominant effects, and is not affected by endosperm. Chalkiness, chalkiness area rate, transparency degree, and amylose content are controlled by additive effects from the embryo, endosperm, and mother, and dominant effects from the mother.

At the same time, rice quality and ADi of female and male parents are closely related to rice quality of hybrid F_1 (Table 7). The female value is positively related to polished rice rate and amylose content of hybrid F_1, and the male value is positively and significantly related to polished rice rate, chalkiness, chalkiness area rate, transparency degree, and amylose content of hybrid F_1. ADi of the female parent is negatively related to most quality traits except for transparency degree, and is especially significant for chalkiness. ADi of the male parent is negatively related to polished rice rate and positively and significantly related to amylose content. ADi-D is positively related to chalkiness and amylose content.

Table 7. Correlation coefficients between parents and hybrid combinations.

Trait	Female	Male	ADi-F	ADi-M	ADi-D
Polished rice rate	0.386**	0.302**	−0.108	−0.302**	−0.142
Fine polished rice rate	0.056	0.027	−0.059	0.055	0.015
Chalkiness	0.113	0.481**	−0.235*	0.133	0.238*
Chalkiness area rate	0.027	0.346**	−0.172	0.068	0.113
Transparency degree	0.043	0.262*	0.055	−0.040	−0.030
Amylose content	0.240*	0.560**	−0.184	0.219*	0.249*

So, in order to improve the rice quality of hybrids, both parents should be improved, especially the male parent, whose value is more closely related to that of the hybrids. It is helpful to adjust the difference in ADi between the female and male parent to improve rice quality. A decrease in ADi-F is of benefit for improving polished rice rate and chalkiness. An increase in ADi-M and ADi-D is of benefit for improving amylose content and chalkiness, but not for polished rice rate.

The ecotypic plant type

In theories of super high yield, Yang Shouren proposed that "combining ideal plant type and the use of heterosis was the main method for rice super high yield." The ideal plant type should be determined according to the ecological environment.

For southern China, in rainy, humid, and disease-prone areas, a vertical-bending panicle type with tall plant, long leaves, and long panicles is more popular, and it needs less nitrogen fertilization. However, for northern China, in dry and less diseased areas, an erect panicle type with medium plant height, little leaf angle, and short leaves and panicles is more appropriate, and it needs high nitrogen fertilization. A tall plant, large panicles, and no lodging should be notable features of super hybrid rice.

Prospects

Heterosis between varieties, between geographical varieties, and between subspecies represents the three stages of heterosis use. More attention should be paid to the use of heterosis between geographical varieties, which is easier for obtaining strong heterosis than that between varieties and also for improving rice quality than that between subspecies.

The three-line method, two-line method, and one-line method should be used at the same time in hybrid rice breeding. But japonica hybrid rice has mainly depended on the three-line method. Because of susceptibility of sterility to temperature, the use of the two-line method has been limited in japonica hybrid rice. However, with the two-line method, special maintainer lines and restorer lines do not need to be bred, which makes it easier to obtain hybrid combinations. So, research and the use of the two-line method should be enhanced in japonica hybrid rice, and breeding of sterile lines with a lower critical temperature would be the main road to success.

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Authors' address: Liaoning Rice Research Institute and the Engineering and Technology Center of Japonica Hybrid Rice in Northern China, Shenyang 110101, China.

New genes for brown planthopper, blast, and bacterial blight resistance, and marker-assisted breeding in rice

K.K. Jena and J.P. Suh

Major improvements made for increasing the yield potential of rice are threatened by new biotypes/pathotypes of insects and diseases. Rice breeders face serious challenges to develop high-yielding cultivars with a broad spectrum of resistance. Recent advances in rice genomics research and completion of rice's genome sequence have paved the way to precisely identify new resistance genes for the major insect (brown planthopper) and diseases (blast and bacterial blight). A new resistance gene, *Bph18,* has been identified from the breeding line IR65482-7-216-1-2 and this gene is tightly linked to the DNA marker 7312.T4A that is used efficiently for marker-assisted selection (MAS) for BPH resistance. The gene *Pi40* identified from the breeding line IR65482-4-136-2-2 confers broad-spectrum durable resistance to blast isolates of Korea. The *Pi40* gene tagged to the DNA marker 9871.T7E2b is used for MAS of blast resistance. The pyramided resistance genes *Xa4* + *xa5* + *Xa21* have shown effective resistance to a wide range of BB isolates and are integrated into elite rice cultivars. These genes have great potential for brown planthopper, blast, and bacterial blight resistance breeding not only for the development of inbred cultivars but also for hybrid rice.

Rice is the staple food for more than half of the world's population. It is mainly cultivated in Asian countries under a hot humid climate in both tropical and temperate regions. The yield potential of rice was very low until the discovery and introduction of the semidwarfing gene (*sd1*) by the International Rice Research Institute (IRRI), Los Baños, Philippines. Nevertheless, rice yield has remained on a plateau during the past decade because of several biotic and abiotic stresses as well as poor adoption of modern management practices. The increasing world population will require higher rice production from less cultivable land with less water and less chemical fertilizer (Khush 2005). Hybrid rice technology has shown great potential to increase rice production significantly compared with inbred rice in China and the technology is becoming adopted in other Asian countries and Africa (Virmani et al 2003). However, the stability of increased yield potential in high-yielding inbred and hybrid rice cultivars is not yet achieved because of their susceptibility to diseases and insects caused by new biotypes of insects and pathotypes of bacterial and fungal pathogens. The major insects and diseases that reduce rice yield are brown planthopper (*Nilaparvata lugens*), blast (caused by *Magnaporthe grisea*), and bacterial leaf blight (caused by *Xanthomonas oryzae* pv. *oryzae*). This paper discusses the identification of new resistance genes for brown planthopper (BPH), blast (Bl), and bacterial blight (BB) and their integration into elite japonica cultivars using resistance gene–specific molecular markers for those genes through the marker-assisted selection (MAS) approach.

Brown planthopper resistance

Brown planthopper is one of the most destructive insect pests of rice. During the most conducive environmental conditions, the BPH population builds up rapidly, causing hopper burn, and eventually rice production is affected severely. BPH also transmits two virus diseases, grassy stunt and ragged stunt, that also reduce rice yield. Some 19 major resistance genes and several quantitative trait loci for resistance have been identified by researchers using different biotypes of BPH, and by systematic genetic analysis (Jena and Mackill 2008). Advances in molecular genetics and biotechnology research have enabled scientists to associate 10 major BPH resistance genes with molecular markers.

A new BPH resistance gene designated as *Bph18* has been identified from an elite indica breeding line, IR65482-7-216-1-2, in a greenhouse bioassay using a Korean biotype of BPH. Genetic analysis confirmed the dominant nature of the *Bph18* gene. Molecular analysis of an F_2 mapping population was conducted with SSR and STS markers, and the *Bph18* gene has been localized on the long arm of chromosome 12. High-resolution mapping of the new resistance gene was conducted through *e*-landing and using SSR and STS markers derived from BAC/PAC clones present in the region. A new approach of selecting resistant and susceptible plants at six time points during bioassays and their association with molecular marker alleles suggested the most putative location of the target *Bph18* gene (Jena et al 2006). In this study, the *Bph18* gene was located near the STS marker R10289S on the BAC clone OSJNBb0076G11. However, a putative resistance gene locus was identified in a BAC clone, OSJNBb0028L05, and the derived marker, 7312.T4A, was tightly linked with the *Bph18* gene for resistance. An allelism test with other known BPH resistance genes suggested the nonallelic nature of the *Bph18* gene in relation to *Bph1* and *Bph10*.

The PCR marker 7312.T4A was validated as a complete R- and S-associated DNA marker by genotyping resistant BC_2F_2 progenies. The application of marker 7312.T4A on 97 resistant progenies did not detect any plant with homozygous-susceptible alleles of the recurrent parent (Fig. 1). Thus, marker 7312.T4A was specific for BPH resistance and was associated with the *Bph18* gene.

Blast disease resistance

Blast (Bl) disease is a serious threat to rice production in the water-stressed upland ecosystem in the tropics as well as in the irrigated temperate rice ecosystem. An outbreak

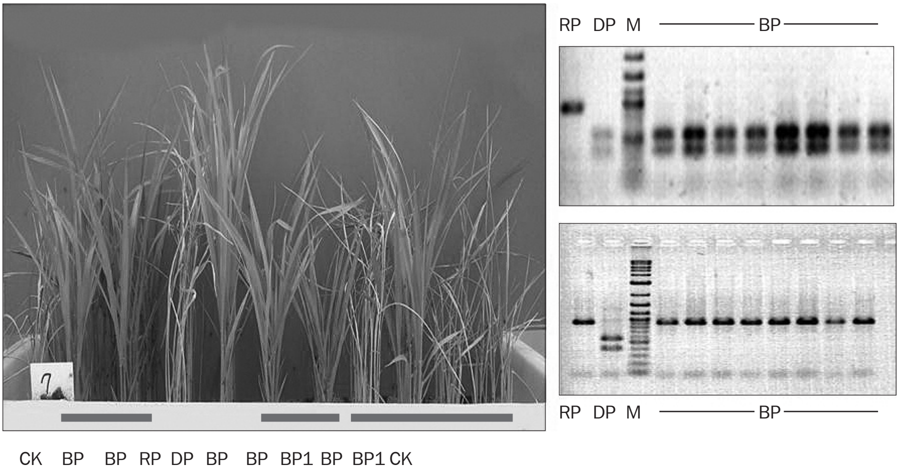

Fig. 1. MAS validity test of advanced BC progenies for the *Bph18* gene with marker 7312.T4A. RP = recurrent parent, DP = donor parent (*Bph18*), BP = backcross progenies with *Bph18* gene, BP1 = susceptible progenies, CK = check, M = molecular weight marker

of blast can devastate rice fields and, according to a conservative estimate, it destroys enough rice each year to feed 60 million people (Zeigler et al 1994). Blast resistance genes are not durable because of the virulence complexity of the blast fungus even though more than 40 resistance genes have been identified. It is imperative to identify new resistance genes with an understanding of their molecular mechanisms in host resistance to the pathogen to better design control strategies for rice blast disease. This paper highlights the identification of a novel blast resistance gene, *Pi40,* and the use of a tightly linked DNA marker for diagnosis of resistance to a broad spectrum of blast isolates and use of the resistance gene in blast resistance breeding through MAS.

An indica breeding line, IR65482-4-136-2-2, had strong resistance to a wide range of Korean blast isolates. Genetic analysis suggested that blast resistance in IR65482-4-136-2-2 was controlled by a major genetic factor and F_2 progenies were analyzed for precise molecular mapping of the resistance gene. Initially, the resistance gene (*Pi40*) was localized on the short arm of chromosome 6, flanked by the markers RM0527 and RM3330. Further molecular analysis with markers derived from candidate BAC and PAC clones using the *e*-landing approach in the target region of the resistance gene identified a PAC clone (P0649C11) that had six ORFs, which were annotated as NBS-LRR disease resistance protein homologs. Highly stringent primer sets derived from each NBS-LRR homolog were used for marker allele differentiation of a donor resistance allele and other genes (*Pi9, Piz, Piz-5,* and *Piz-t*) using blast monogenic differential lines through haplotype analysis. The locus 9871.T7 expressed seven types of marker alleles and differentiated all tested genotypes. The DNA marker 9871.T7E2b was tightly associated with the new resistance gene *Pi40* and was used for marker-assisted breeding for blast resistance (Jeung et al 2007).

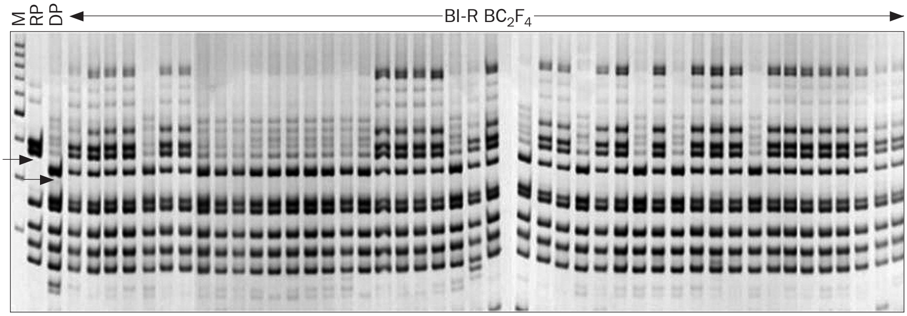

Fig. 2. Association of 9871.T77E2b marker alleles with the *Pi40* gene for blast resistance in BC_2F_4 resistant progenies. Genotypes: 57 BB type, 37 AB type, 0 AA type, RP = recurrent parent, DP = donor parent, M = molecular weight marker.

The linkage between the novel resistance gene *Pi40* and the marker 9871.T7E2b was validated as a complete linked marker to blast resistance by genotyping BC_2F_4 and BC_3F_3 progenies in the genetic background of two elite japonica cultivars. Some 192 blast-resistant individuals randomly selected for PCR amplification with 9871. T7E2b did not exhibit any homozygous-susceptible allele types of recurrent japonica cultivars and confirmed that the *Pi40* gene was the essential genetic component for resistance against blast infection (Fig. 2). The donor line IR65482-4-136-2-2 was evaluated for Philippines blast isolates and none of the tested isolates had compatibility to the genotype, suggesting that the *Pi40* gene indeed has broad-spectrum resistance and is different from other known genes.

Bacterial blight resistance

Bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)* is a destructive vascular disease that causes systematic infection of the rice crop at flowering stage and is reported to have reduced Asia's annual rice production by 60% (Dai et al 2007). As of now, 29 genes for BB resistance (R) have been found on different chromosomal loci (Tan et al 2004) and six R genes (*Xa1, xa5, xa13, Xa21, Xa26,* and *Xa27*) have been cloned using a map-based cloning approach (Song et al 1995, Yoshimura et al 1998, Iyer and McCouch 2004, Sun et al 2004, Gu et al 2005, Chu et al 2006). Unlike other R genes, *Xa4, xa5,* and *Xa2*1 showed a differential reaction to BB pathogen strains we used for BB resistance. We report here the cumulative effect of R-gene pyramids toward developing strong resistance to the dynamic BB pathosystem of Korea.

Korean BB isolates have been grouped into five races (K1 to K5) by using five rice cultivars as a *Xoo* differential system. In recent years, based on pathotyping results, it was reported that K2 and K3 races are prevalent and that a new race, K3a, has evolved. We have used a new strategy by quantitative analysis of the response of near-isogenic lines (NILs) carrying different R genes and gene combinations against the 16 most virulent isolates representing the Korean BB pathotype. IR24 NILs were

Table 1. New resistance genes, and primer sequences of markers with band size for MAS.

R gene	Chromosome	Marker	Primer sequences (5′→3′)	Band size (bp)
Bph18	12	7312.T4A	F: acggcggtgagcattgg R: tacagcgaaaagcataaagagtc	1,078
Pi40	6	9871.T7E2b	F: caacaaacgggtcgacaaagg R: cccccaggtcgtgataccttc	642
Xa4	11	MP1+MP2	F: atcgatcgatcttcacgagg R: tgctataaaaggcattcgg	175
xa5	5	10603.T10Dw	F: gcactgcaaccatcaatgaatc R: cctaggagaaactagccgtcca	300
Xa21	11	U1+I1	F: cgatcggtataacagcaaaac R: atagcaactgattgcttgg	1,300

tested for their effectiveness and durability against the 16 most virulent BB isolates. NILs with single R genes did not show resistance to a wide range of isolates except for *xa5* and *Xa7*. The NILs with *Xa10* and *xa13* genes were highly susceptible and NILs with *Xa1, Xa3, Xa4, xa8,* and *Xa21* expressed race- or isolate-specific resistance. The *xa13* gene also showed a higher degree of susceptibility to BB isolates even in combination with other R genes such as *Xa4* and *Xa21* because of quantitative compensation. Inoculation experiments on IR24 NILs with the newly evolved field isolate K3a showed high susceptibility for the R genes *Xa4* and *Xa7*, and moderate resistance in *Xa21*. However, strong resistance was observed for all BB isolates to R gene combinations (*Xa4* + *xa5, xa5* + *Xa21,* and *Xa4* + *xa5* + *Xa21*). The three gene combinations (*Xa4* + *xa5* + *Xa21*) caused quantitative complementation of individual R genes and expressed strong resistance to a broad spectrum of virulent isolates of Korea and the Philippines (Jeung et al 2006). It is recommended to use these gene pyramids for indica and japonica rice improvement programs with an effective protection against evolving strains of *Xoo* in Asia.

Marker-assisted breeding

Recent advances in rice genomics research and the complete sequencing of the rice genome have made it possible to precisely identify many valuable genes of agronomic importance. Noteworthy examples are the genes conferring resistance to BPH, Bl, and BB in addition to major QTLs for yield component traits and submergence tolerance (Jena and Mackill 2008). Using tightly linked markers for resistance genes *Bph18*, *Pi40*, *Xa4*, *xa5,* and *Xa21*, it has been possible to integrate these genes into elite japonica cultivars and develop advanced backcross breeding lines (Table 1). The advanced breeding lines have agronomic traits similar to those of their recurrent parents and they possessed a broad spectrum of resistance to BPH, Bl, and BB (Fig. 3).

Fig. 3. Morphological similarity of backcross progenies (BP) with the *Bph18* gene compared with a recurrent parent (RP). DP = donor parent (*Bph18*).

Conclusions

Biotic stresses in rice caused by BPH, Bl, and BB are the main cause of yield loss in rice cultivated in tropical and temperate regions of Asia. Even though several resistance genes were identified, most of those do not confer resistance to new biotypes of insects and pathotypes of diseases. The new resistance gene, *Bph18,* for BPH is a dominant gene and the source of resistance is an introgression line, IR65482-7-216-1-2, that inherited the gene from an EE genome wild species, *Oryza australiensis*. This gene has potential for use in indica and japonica rice improvement, including hybrid rice in South, Southeast, and Northeast Asia. Of the 40 blast resistance genes identified, *Pi40* has great potential for blast resistance breeding because it showed broad-spectrum resistance and has been fine-mapped on chromosome 6. The *Pi40* gene has linkage with nucleotide binding site (NBS)–leucine-rich repeat (LRR) protein sequence motifs. The gene has potential for broad-spectrum durable blast resistance breeding in inbred and hybrid rice breeding programs using marker-assisted backcross breeding. Exploitation of R gene NILs as a new strategy to understand *Xoo* pathogen populations in the field and the incorporation of pyramided genes into susceptible elite cultivars has proved successful for BB resistance breeding in indica and japonica rice. Among the R genes, the combination of *Xa4 + xa5 + Xa21* is recommended for incorporation into elite cultivars using MAS for durable BB resistance. These genes, being dominant in nature, will contribute significantly to a hybrid rice improvement program for biotic stress resistance.

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Authors' address: Plant Breeding, Genetics, and Biotechnology Division, International Rice Research Institute, c/o IRRI-Korea Office, National Institute of Crop Science, RDA, 209 Seodun Dong, Suwon 441-857, Kyeonggi Do, Republic of Korea. Tel: +82 31 290 6871; fax: +82 31 294 8185; email: k.jena@cgiar.org.

How to increase use efficiency of rice hybrids and their parental lines

Chang-xiang Mao, Yu-min Shi, Shan-fu Wei, Zhi-ping Song, Hang Zhou,
Wei-hao Wang, Li-ping Xie, and Bai-Long Liu

The use efficiency (UE) of a crop variety depends on how large its growing area is and how long it lasts in production. UE depends on how perfect rice hybrids and their parental lines are and on some nontechnical factors. This paper analyzes data and statistics of hybrid rice R & D in the past 30 years and notes that the UE of rice hybrids and parental lines has declined both inside and outside of China. This is a major constraint to the sustainable development of hybrid rice in the world. We suggest two ways to increase UE. The technical way includes breeding, a strict evaluation system for hybrids, improving cultural management, parental line purification, raising F_1 seed yield, avoiding a fast rotation of hybrids, and encouraging technical innovation. The nontechnical way consists of making policy adjustments, controlling malignant competition, organizing wide cooperation, removing local protectionism, and protecting farmers' benefits first, to increase UE for sustainable R & D of hybrid rice in China and globally.

Hybrid rice has made a great contribution to rice production and food security in China (Yuan 2003). Breeders' populations for hybrid rice are very large because of quick expansion and the total number of new rice hybrids developed and released has been increasing rapidly year by year. The number of rice hybrids planted (covering more than 6,666.7 ha) increased from 41 in 1988 to 199 in 2000, increasing four times within 10 years (China National Extension Service Center on Agricultural Techniques 1988-2006). From 217 rice hybrids in 2001 to 458 in 2006, annual growing area doubled within six years. However the percentage of hybrid rice in total rice area has not increased as fast as the number of varieties. The percentage first surpassed 50% in 1988, then hovered between 60% and 70%, never reaching or exceeding 70%. The highest was 68% in 1995. From 2001 to 2006, it went from 64% to 66%. For absolute growing area of hybrid rice in China, the total area in 1988 was 12.143 million ha, and the highest was 15.316 million ha in 1997. In 2006, the growing area was 15.199 million ha, similar to that in 1997. This means that the total number of rice hybrids increased, but not the percentage of hybrids in total rice-growing area.

Hybrid rice breeding is more complex than inbred rice breeding. It needs to develop three lines—A (CMS), B, and R lines—and then F_1 hybrids or two lines, S

(TGMS or PGMS) lines and R lines, and then F_1 hybrids that need much longer time and more inputs. Therefore, considering how to increase the use efficiency of rice hybrids and their parental lines would be helpful for the sustainable development of hybrid rice in China as well as globally in the future.

Encouraged by government policies and commercial benefits, hybrid rice breeding is now vigorous in China, the same as in some other countries. Thousands of new rice hybrids were bred and hundreds of new hybrid rice varieties passed regional yield trials and were released for commercial production every year throughout China. The total number of commercial varieties increased significantly, but the duration and growing area of the varieties released are much less than in the 1980s and 1990s. No doubt this is because of low UE. How to increase UE is a challenge for the sustainable development of hybrid rice.

History and current status of UE of rice hybrids and their parental lines

According to official statistics in China, there has been a great increase in the total number of hybrid rice varieties released for commercial production, while the average growing area of each hybrid is declining and the duration of hybrids is shortening. The total number of commercial hybrids increased from 41 in 1988 to 458 in 2006, while the average growing area for each hybrid (grown on more than 666.7 ha), was 296,160 ha in 1988 but dropped to 33,190 ha in 2006 (Mao 2005). However, compared with inbred varieties, the total number of commercial varieties has not changed much, and the total number of inbred varieties was 246 and average growing area for each variety was 46,360 ha in 1988, with 264 varieties and average growing area of 34,420 ha in 2006, which has kept relatively stable for about 20 years. In fact, more and more new rice hybrids were developed recently in each province in China. Breeders always want their hybrids to be able to pass regional yield trials and gain approval from the Varietal Committee for Release. Usually, several hundred new hybrids applied for regional trials every year, but only a few dozen were accepted. Most hybrids showed very limited heterosis compared with the checks, and, after approval for release, most hybrids had no growing area and disappeared soon.

A similar case happened with the parental lines of rice hybrids too. Most parental lines named and released in the 1970s, such as the CMS lines Nan A, Ai A, V41A, 71-72 A, Jin Nan Te 43 A, Nan Zao A, Chang Fu A, Zhen Long 13 A, etc., and some R lines, such as R-1 (Tai Yin- 1 A), R-2 (IR24), R-3 (IR661), R-4 (Gu 154), R-5 (IR665), and R-6 (IR26), were discarded gradually. Since the 1980s, hundreds of new MS lines, mostly "WA" type, and F_1 hybrids, had been used for a short time, with various disadvantages in China, and now nobody can remember their names.

Outside China, many countries started a hybrid rice program in the 1980s or 1990s, and some of them have announced the successful release of rice hybrids for commercial production. But, after many years, there was no big increase in growing area of these hybrids, or most of them disappeared from the name list, for example, hybrids APHR-1, APHR-2, MGR-1, DRRH-1, KRH-2, etc., in India; Magat, Mestizo, Panay, Bigante, etc., in the Philippines; Intani-1, Intani-2, Rokan, Maro, etc., in Indonesia; and UTL-1, UTL-2, HYT-56, HYT-57 VL20, VL24, etc., in Vietnam (Mao et al 2006).

No doubt, most of these rice hybrids and their parental lines showed strong heterosis and had been used for some time in commercial production with a specific growing area in their short life. Their low UE not only wasted large efforts by breeders but also brought some negative effects on hybrid R & D to these countries, or even gave a bad reputation to hybrid rice technology to the public.

Major reasons for lower UE

The reasons for the low use efficiency of parental lines and F_1 hybrids are quite complex. They can be classified into two major parts: technical and nontechnical. Either technical or nontechnical reasons are sometimes closely linked and could interact.

Technical reasons

Imperfect parental lines and hybrids. The parental lines and their hybrids showed high yield potential compared with checks in trials, but some of their traits are not acceptable to growers, such as susceptibility to major diseases or insect pests, unexpected growth duration, etc., that would cause their rejection by growers. Recently, many rice hybrids developed in and outside China released for commercial production had a low UE or short duration for this reason.

Hybrids unsuitable for the market and consumers. Many rice hybrids with perfect traits always give high yield to growers, but finally have low UE, mostly because they are not suitable for market demand or have low commercial value because their grain quality, especially eating quality, is poor, or they have a high broken rice rate and were rejected by the mill or the final market.

Genetically unstable parental lines. Some elite hybrids and their parental lines are good for both growers and markets, but they have a short life or low UE because their parental lines are genetically unstable. The stability of MS line sterility is easily affected by temperature during pollen cell formation stage, and some sterile pollens become fertile and F_1 seed purity becomes poor. The final result is not continuing with the hybrids because of unstable parental lines.

Poor GCA of parental lines. For breeding and selecting parental lines, if they have good general combining ability (GCA), MS lines could have more hybrids derived from mating different restorer lines; therefore, the MS lines will have long duration and be widely used for test-crosses. If they have only good specific combining ability (SCA), the MS lines may have limitations for producing more elite hybrids, so their UE will be lower than ones with good GCA. A good example is CMS line Zhi A with a very high outcrossing rate, whose F_1 seed production yield could reach 6–7 t ha^{-1}, but, because of poor combining ability, no heterotic hybrids were released for use in China.

Low yield of F_1 seed production. In many cases, F_1 hybrids with high and stable yield, and also suitable for the market, cannot be continued for commercial production due to very low yield of F_1 seed production. This is mainly attributed to the low outcrossing rate of the parental lines, especially the MS lines. The low F_1 seed yield would cause a high cost for seed producers as well as for F_1 growers. A good example is Wei You 35, an early-maturing and high-yielding hybrid suitable for the first rice cropping in Hunan Province in China, which farmers like very much. Unfortunately, its F_1 seed production is very low, which cannot bring profit to seed growers; so, it was finally eliminated from production.

Parental lines not purified. In the early stage of hybrid rice development in China, some perfect rice hybrids quickly lost their continuous production due to the purity problem of the parental lines. Seed producers did not pay attention to the purification of the parental lines, especially the MS lines. After a few seasons, the parental lines had more off-types or partially sterile plants in the population attributed to pollen contamination during parental-line multiplication. That was why the country then set up a strict parental-line purification system and procedures to control the purity of hybrid rice seeds.

Too-fast variety rotation. Some farmers thought that any new variety could bring higher yield than an old one and most seed companies or seed sellers always exaggerated the advantages of the new hybrids they were selling. That usually caused a fast and meaningless variety rotation of rice hybrids. Many good hybrids died out earlier, with a short life and very low UE.

Nontechnical reasons

Incorrect policy guidance. It is understandable that breeders and seed companies reasonably want to quickly release or produce a new rice hybrid in s short time. But some government policies greatly encouraged this trend. In China, new varieties as research achievements are closely linked to work promotions, salary increases, and extra income, and the sale of new hybrid variety seed with a high price could earn high profits for seed companies. Hence, thousands of new hybrids and dozens of new parental lines are developed every year in China, but market demand is limited. About 90% of the new hybrids and parental lines have no chance to enter commercial production. A newly released one can share only a limited market with a smaller and smaller portion. Eagerness for quick success and instant benefits is the major reason for the low UE of hybrid rice recently in China.

Incorrect funding support. In China, research funds mostly come from the government, which is decisive in guiding the trend of hybrid rice research. The government has long paid more attention to breeding with more funding support, meanwhile ignoring related techniques for new hybrids, such as agronomic management, parental line purification, and pest control. This leads nearly all scientists in multidisciplinary work at nearly all research institutes, either nationally or provincially, or at a county level, to undertake breeding as their first job. That is why thousands of new hybrids and dozens of new parental lines could be developed quickly. We call this phenomenon "all people breeding," which has resulted in a great waste of effort and very low UE.

Malignant competition. Malignant competition among breeders as well as among seed producers is another major reason for the low UE of hybrids and their parental lines. The competition is not based on the fair and equitable performance of hybrids or parental lines. Sometimes it is based on how much power breeders or companies have, so some elite hybrids or parental lines are replaced by some poor ones without any reason.

For their own intellectual property rights (IPR), more and more private seed companies started their own breeding program to develop parental lines and new hybrids with series names from which no one can know the parental lines' names and features, for example, recent hybrids named Xian-Nong, De-Nong, Long-Ping, Guo-Dao, Guo-Feng, Shen-Nong, etc. The number of series of Xian-Nong hybrids each growing on more than 666.7 ha reached 23, and four each for De-Nong, Long-Ping HiTec, and Shen-Nong in 2006. The rapid development of domestic companies in China and some multinational companies is a big challenge and competition to public institutes.

Local protectionism. Local protectionism (usually practiced by local government officials) permitted only the hybrids developed by local breeders or the F_1 seeds produced by local seed producers to be used in the area under their control regardless of the performance of the hybrids or parental lines.

Poor relationship between the public and private sector. The case of perfect hybrids developed by a public institute not being accepted by private seed companies for F_1 seed production was very common in the early stage of hybrid rice R & D in China and recently in many other countries.

For example, in Indonesia, only five hybrids (Intani-1, Intani-2, Rokan, Maro, and one other) have been released by the government for commercial production, although their performance and yield potential are nearly the same as those of introduced foreign hybrids that are still in the testing stage. In fact, companies in the country would rather introduce foreign hybrids and import their seeds from other countries, in order not to use local ones for seed production. When no large-scale seed production occurred after a public institute released hybrids, these hybrids gradually faded out, with much waste and a short life.

How to increase UE

Possibility for high UE

Though there are serious problems of low UE of rice hybrids and parental lines in and outside China, some exceptions have proved that there are some ways to increase EU. For example, CMS lines such as Zhen Shan 97 A and V20A and R lines such as MH 63 R, Gui 99 R, and Ce 64 R, named and released in the 1970s, till now have been used as parental lines. The most famous hybrid, Shanyou 63, is a good example with a very long life and high use efficiency. It has lasted more than 20 years, with its highest growing area of 6.81 million ha in 1990 and 0.15 million ha in 2006. Series hybrids, by using Zhen Shan 97A and V20A as CMS lines, are also good examples with high use efficiency. The total number and growing area of Zhen Shan 97A series hybrids was 27 and 9.568 million ha in 1992, and 20 and 0.445 million ha in 2006, respectively. The total number and growing area of V20 A series hybrids was 15 and

2.187 million ha in 1992 and 11 and 0.287 million ha in 2006. Another famous rice hybrid, Shan You Gui 99, developed by Guangxi, occupied 108,667 ha in 1990 and still 56,667 ha in 2005. All these good examples showed that if the hybrids had high yield and good adaptability to various ecological conditions, they could have a long useful life.

CMS lines IR58025A and IR62829A, with very high GCA, were developed by the International Rice Research Institute (IRRI) and have been widely used as parental lines for many commercialized rice hybrids with many good characters in tropical Asian countries. The serious problem for theses two CMS lines is their low outcrossing rate for F_1 seed yield. Breeders in different countries tried to not use them, but could not find replacements. However, in some countries, the outcrossing rate of these CMS lines became improved by either breeding or cultural management approaches, and by continuously being used. For example, for IR58025A, its outcrossing rate was improved by Chinese breeders in different provinces and they renamed it as a new CMS line in China.

Technical approaches

Although there are problems with low UE of hybrid rice, there are also some excellent parental lines with good GCA and SCA that have been used widely and for a long time. MS lines with high use efficiency in China are Zhen Shan 97 A, Jin23 A, II-32 A, Zhong-9 A, Pei Ai 64 S, V 20 A, Xie A, Gang A, Te A, Bo A, You-I A, etc. R lines are MH 63, MH 77, MH 86, Ce 64, Gui 99, 207 R, 253 R, 288 R,402 R, 527 R, 725 R, 838 R, etc. Outside China, the continuous use of IR58025A is a good example. This means that technical approaches could improve the UE of both parental lines and hybrids.

Breeding. The development of elite parental lines as well as suitable hybrids is the basis of high UE, which should have good breeding materials and germplasm, with rich experiences in how to select breeding lines based on the essential traits for good parental lines and F_1 hybrids. It is important to set up high standards and strict procedures to evaluate breeding lines and new hybrids or parental lines to ensure that the ones with best performance will be selected and the poor ones discarded. Multiple locations and seasons should be used for testing and demonstration for new hybrids to find hybrids with wide adoptability.

Breeding experience is very important for developing high-UE parental lines or F_1 hybrids. For example, among the excellent high-UE parental lines, II-32A, You-I A, and Zhong-9 A were bred by one well-experienced breeder, whereas MH 63, MH 77, and MH 86 were released by another senior hybrid rice breeder in China.

Recently in China, more and more new rice hybrids have been developed and released but most of these have a narrow genetic background, unlike those of the breeding program in the early stage of hybrid rice R & D in the 1970s and 1980s, when nationwide information, skill, and germplasm exchange were free and frequent. This kind of exchange should be encouraged again.

Setup of a strict evaluation system. It is important to strictly set up standards for new rice hybrids and critically evaluate new hybrids and parental lines. Comprehensively screening the traits and characteristics of each new hybrid, not only yield components but also quality, resistance, and adoptability, etc. For example, if all the characters, especially yield, are similar for some hybrids, the wider the adoptability, the better for approval. Evaluation and certification of parental lines should be more strict than for hybrids. The poor or short useful life of hybrids was usually caused by crossing poor parental lines. Parental line evaluation and certification are mostly based on the purity and stability of their sterility, not their combining ability. Combining ability is found by breeding practices later on. So, many newly released parental lines are the result of fast-developed near-sib parental lines from similar hybridizations. It is necessary to further raise the threshold for a high yield potential test under high-yielding conditions. For example, set up high-nitrogen plots, plots with artificial inoculation of some disease and insect pests, use more locations than the existing ones for yield trials to test wide adoptability, etc.

Improve cultural management. A related technical package for new parental lines and hybrids should be developed before their release for commercial production. For parental lines, indexes for high-yield F_1 seed production are important, whereas, for hybrids, high-yield cultivation, including fertilization, plant protection, etc., are important. Growers must manage new lines or hybrids with high and stable yield and get high UE for them. For instance, in China and in some tropical countries, some rice hybrids have very high yield and good grain quality, but the only problem is a high rate of broken rice when the hybrids are milled. When cultural practices were improved to obtain uniform maturity of panicles in the harvest season, such as increasing planting density, early fertilization, and controlling ineffective tillers, the head rice rate rose to the standards.

Parental line purification. Elite parental lines or their hybrids can possibly have high UE but this is not 100% assured, if attention is not paid to purification of the parental lines. China learned many lessons in the past. Some rice hybrids had very good performance and were welcomed by growers, but the hybrids did not have high UE, because of the F_1 seed quality problem, which was usually caused by contamination in parental line multiplication or in F_1 seed production. Parental line purification is a key step for high UE for some elite hybrids and parental lines.

Raising F_1 seed yield. Increasing F_1 seed production yield is a long-term strategy for seed companies to increase profits through high UE of some good hybrids. For example, IR58025A, developed by IRRI, is a key MS line for dozens of commercial hybrids in Asian countries, but it has a relatively low outcrossing rate, which is a barrier to high F_1 seed production yield. On the one hand, some countries tried to improve the outcrossing rate through breeding, and obtained some improvement, as in China new CMS lines are selected from the improved IR58025A population. On the other hand, some seed producers successfully tried to increase their outcrossing rate by field practices or cultural management approaches when these have to be continuously used for good commercial hybrid seed production. In these two ways, the UE of IR58025 A is becoming higher and higher.

Avoid fast rotation of hybrids. Don't give too high expectations for new hybrids to farmers, and don't expect to frequently change new rice hybrids to deliver high yield to farmers. By frequently changing hybrids, it is easy to cause low UE for both hybrids and parental lines. Any new rice variety or hybrid can obtain high and stable yield after its release. It should be continuously grown for several years or seasons as farmers become familiar with the features of the variety. F_1 seed producers need longer time to practice the approaches and skills for obtaining high and stable seed yield for a newly released hybrid, including getting good synchronization of parental lines, best row rations, best dosage and time for GA_3 application, the right time for supplementary pollination, etc. That is why recently in China no high seed yield records were announced and average seed production yield has not increased any more, because rotation of rice hybrids is too fast. In the 1990s, several record highs of 6–7 t ha^{-1} of F_1 seeds could be obtained by improving cultural management (Mao and Virmani 2003).

Encouragement of technical innovation. Technological innovation is an effective way to increase the UE of rice hybrids and their parental lines. A good example is the breeding and release of "Clearfield" hybrid rice in the United States by RiceTec recently. The so-called Clearfield hybrid rice can be resistant to herbicides when weed control is undertaken in the rice field. Biotechnology can be used to check the genetic diversity of parental lines and hybrids to classify whether they are significantly different from each other.

Nontechnical approaches

From a nontechnical viewpoint, government policy should guide the formation of a breeding team composed of multidisciplinary scientists, so that parental lines and hybrids will be more perfect, having not only high yield but also wide adoptability, suitable grain quality, resistance to major diseases and insect pests, and high F_1 seed yield with high UE.

Policy adjustment. Policy is the most important factor that can strategically affect the whole system of hybrid rice R & D in a region or entire country. The Chinese government released a series of favorable policies in the early stage of hybrid rice R & D in the 1970s to 1990s that guided the final success. Recent policies caused problems that negatively affected the UE for hybrid rice so R & D should be adjusted accordingly. The same is needed in other countries.

Controlling malignant competition. Malignant competition between breeders and seed producers should be controlled or stopped to not waste the common efforts of hybrid rice R & D in a country, region, or globally.

Organizing wide cooperation. Government policies should promote cooperation between competitors. They should encourage the formation of multidisciplinary expertise in a breeding team to increase the comprehensive design and breeding of new parental lines as well as hybrids. So far, most rice breeding programs in the public and private sector in China are based on yield components or morphological/visible traits. Some qualitative trait evaluations were based on tests in the later stage of breeding lines/varieties, and elite ones were selected mostly by chance. Good cooperation is one of the major factors for the success of hybrid rice R & D in China, but now it has become very limited. Definitely, it is important for increasing the UE of hybrid rice in an entire country or region.

Remove local protectionism. Local protectionism is a barrier to introducing the best rice hybrids to local growers. It is important to give up local protectionism set by some local government agencies and allow any elite rice hybrid to be grown in any region if it has wide adoptability and good performance.

Protect farmers' benefits first. Do not allow the use of a new variety's name to make money from farmers. Some seed companies use newly named hybrids to sell seeds with a higher price than their real value to make money from farmers. As Father of Hybrid Rice Prof. Longping Yuan said that hybrid rice technology is a commonwealth for its own IPR, more and more private seed companies started their own breeding program to develop parental lines and new hybrids with series names from which no one can know the parental lines' names and features, for example, recent hybrids named Xian-Nong, De-Nong, Long-Ping, Guo-Dao, Guo-Feng, Shen-Nong, etc. The number of series of Xian-Nong hybrids each growing on more than 666.7 ha reached 23, and four each for De-Nong, Long-Ping HiTec, and Shen-Nong in 2006.

We conclude that the UE of hybrid rice could decrease by technical and nontechnical factors, of course; it could also be increased by related approaches in the same way. For the sustainable development of hybrid rice, how to keep high UE of both rice hybrids and parental lines is an important issue to be considered and dealt with by not only research and extension experts but also by management and business organizations.

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Notes

Authors' address: Rice Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, 530007, China; email: cxmao@gxaas.net.

Association between pollen fertility and elongation of uppermost internode in TGMS rice

Manzhong Liang, Lixi Deng, Lijun Ou, Xiaojun Dai, Zhengfang Yu, and Liangbi Chen

Elongation of the uppermost internode (UI) of rice (*Oryza sativa* L.) is an adaptive characteristic that helps the panicle emerge from the flag-leaf sheath and complete anthesis. The elongated uppermost internode (*eui*) mutant Changxuan 3S (CX) of TGMS rice, which shows notably rapid elongation in UI during the heading phase and remarkable variation in UI length under different pollen fertility conditions, is a model plant for studying UI elongation. Dynamic elongation of CX plants revealed that detectable elongation growth of the UI in CX plants started 6 days prior to anthesis and ceased after anthesis. This growth was separated into three elongation phases: the exponential phase, linear phase, and slow-growth phase. Pollen fertility significantly influenced UI elongation growth during the linear and slow-growth phases. Temperature and pollen fertility during the pollen developmental phase in CX plants were significantly negatively correlated; moreover, the pollen fertility of the young panicle greatly affected the elongation growth of the UI. The variation in pollen fertility resulted in a significant difference in phytohormone levels in the UI and young panicle during the linear phase. Decapitation of the UI further confirmed that panicle fertility played a central role in UI elongation. We concluded that pollen fertility is mediated by the manipulation of phytohormones and that phytohormone levels are mediated by the manipulation of UI elongation.

Keywords: rice (*Oryza sativa* L.), thermosensitive genic male sterile, uppermost internode, pollen fertility, gibberellic acid

Elongation of the uppermost internode (UI) in rice (*Oryza sativa* L.) is an adaptive characteristic that helps the panicle emerge from the flag-leaf sheath and complete anthesis. The elongated uppermost internode (*eui*) mutant exhibits notably rapid elongation of the UI during the heading phase; this phenomenon is considered as a potential mechanism by which panicle enclosure is eliminated in male-sterile rice (Yang et al 2002, Luo et al 2006, Zhu et al 2006). Okuno and Kawai (1978) first reported recessive tall LM-1 mutants in Norin. Subsequently, Rutger and Carnahan (1981) isolated a recessive tall mutant designated as *eui*, which has the following characteristics compared with the wild type: the length of the UI is approximately double and panicle exsertion and length are greater. To date, many rice *eui* mutants have been reported (Maekawa et al 1989, Shen et al 1987, Virmani et al 1988, Yang et al 1999, 2001). The *eui* locus has been successfully incorporated into many cytoplasmic male-sterile rice lines through conventional breeding methods (Virmani et al 1988, Yang et al 2002). We have isolated a thermosensitive genic male-sterile (TGMS) *eui* mutant, Changxuan 3S (CX), from the seeds of the photoperiod and thermosensitive genic male-sterile [P(T)GMS] line Pei'ai 64 S that was irradiated by 350 Gy ^{60}Co γ rays (Zhou et al 2000). The TGMS *eui* mutant CX, which showed notably rapid UI elongation during the heading phase and remarkable variation in UI length under different pollen fertility conditions, is a model plant for studying UI elongation. Many studies have reported internode elongation in deepwater rice. Submergence induces rapid elongation of rice coleoptiles and deepwater rice internodes (Cho and Kende 1997, Kende et al 1998). However, UI elongation is one of the poorly understand factors that limit grain yield in hybrid rice seed and rice production. Our study aimed at elucidating the interplay between pollen fertility and elongation of the UI.

Plant materials

Changxuan 3S (CX) is a TGMS *eui* mutant selected from Pei'ai 64 S (PA) seeds that were irradiated by 350 Gy ^{60}Co γ rays.

The experiment was conducted at the Hunan Normal University farm, Hunan Province, China (28°45′N, 119°25′E), during the rice-growing season (April to October) of 2006 and repeated in 2007. Seedlings were raised in a field. Five-leaf-phase seedlings were individually transplanted into the field at a hill spacing of 20 × 20 cm, with single seedlings per hill. Five plants of each sowing time were transplanted into 20-cm-diameter plastic pots and 10 pots were planted for each treatment. All materials were grown under Changsha natural growth conditions until the treatments and materials at the same developmental phase were selected for the treatments.

Temperature treatment

The experiment was conducted in greenhouses under natural long-day conditions. The applied temperatures in the greenhouses were 20, 22, 24, 26, and 28 °C, and natural high temperature (greater than the mean diurnal temperature of 30 °C) was used as a control. When panicle initiation reached the phase of pollen mother cell formation (approximately 15 days before heading), 10 pots from each treatment were transferred to the greenhouses. Temperature treatments were continued for 10 days. After the treatment, the plants were transferred to the natural high-temperature conditions.

Decapitation treatment

At the linear phase, about 4, 3, 2, and 1 day prior to anthesis and anthesis day, 20 panicles of the main stem were excised from the panicle node on each day, respectively. UI length while being decapitated and final length decapitated were measured.

Measurement of panicle exsertion and length of the uppermost internode

For dynamic elongation of UI, 600 main stems were sampled from the mature-pollen phase about 6 days prior to anthesis to the end of anthesis. Thirty main stems at the same developing phase were tagged to be used as check stems for the following observations. Thirty samplings were carried out at 1000 at 6, 5, 4, 3, 2, and 1 day prior to anthesis to the end of anthesis, respectively. At each sampling, the length of the UI and panicle exsertion was measured.

At the end of anthesis, 30 main stems heading on the 15th day after treatment were labeled. At the mature phase, length of the UI and panicle exsertion was measured. Measurements of panicle exsertion were taken as the distance between the flag leaf cushion and panicle node.

Fertility observations

Single anthers from temperature-treated plants were sampled 1 day prior to anthesis and stored in Cannor (3 ethanol:1 acetic acid) solution for testing pollen grain fertility. The anther was squeezed completely on a marked square slide using 1% $KI\text{-}I_2$ solution and observed under a microscope. The irregularly shaped abortive pollen included typical unstained or yellowish pollen and spherical unstained or yellowish pollen; stained abortions were recorded as sterile, whereas all round and dark brown–stained pollen were recorded as fertile. Five replications for the CX and PA plants were conducted. At the time of maturity, 30 labeled and bagged panicles from each treatment were harvested and spikelet fertility was recorded. Pollen and spikelet fertility values (expressed as percentages) represent the means of 30 plants.

Measurement of plant phytohormones in panicle and uppermost internode

The endogenous hormone content was measured using the Agilent 1100 high-performance liquid chromatography (HPLC) system (Agilent, Palo Alto, Calif.). The panicle and uppermost internode at the linear phase (about 2 days prior to anthesis) were frozen in liquid nitrogen and then cold-dried under vacuum: 0.5 gram of dried leaves was added to 11 mL 80% aqueous methanol and immediately homogenized on ice. The homogenate was maintained for 15 h at 4 °C in darkness with continuous shaking. Afterward, it was centrifuged for 10 min at 4,500 *g* at 4 °C, and the supernatant was collected and evaporated under a vacuum. Dry residue was redissolved in 8 mL of ammonium acetic buffer (0.1 mol L^{-1}, pH 9.0). After centrifuging for 20 min at 15,000 rpm, the upper supernatant was purified sequentially through a polyvinyl polypyrrolidone (PVPP) column and DEAE Sephadex A225 column. Before HPLC analysis, the elution with 50% aqueous methanol was concentrated by a Sep-Pak C18 column (Waters Chromatography). Standard gibberellin and zeatin were purchased from Fluka Co. (Switzerland). All solvents and buffers were HPLC-quality.

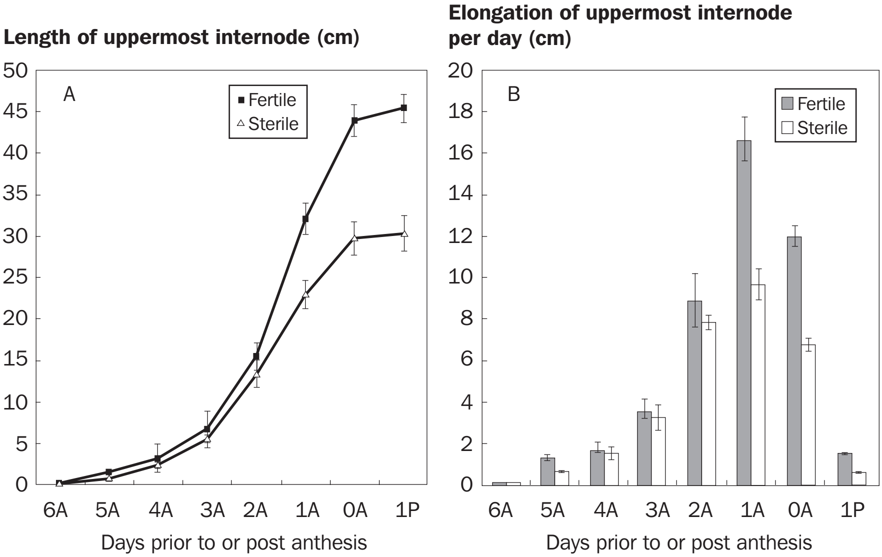

Fig. 1. (A) Elongation of the uppermost internode in CX plants. (B) Difference in elongation and rate between fertile and sterile pollen.

Statistical analysis

We presented the average values for the sampled UI length, panicle exsertion, and pollen fertility for statistical analyses using Excel and tested the significant difference by one-way ANOVA based on Duncan's test at $\alpha \leq 0.05$. Each value in Figure 1 and Tables 1 and 2 is the mean of 30 panicles ± standard error.

Results

Dynamic elongation of the UI in CX plants

Detectable elongation of the UI in CX plants began 6 days prior to anthesis (Fig. 1A). It was separated into three phases: the first phase showed concave curvilinear growth in the exponential phase of the earlier period at 3–6 days prior to anthesis. In the second phase, linear growth was recorded during the intermediate period at 3 days prior to anthesis until its completion, and convex curvilinear growth was observed in the third phase in the slow-growth phase during the later period, that is, from anthesis to 1 day post-anthesis. After anthesis, elongation growth ceased. In the exponential phase, no significant difference was recorded in the length and elongation rate of the UI between fertile and sterile pollen (Fig. 1B). However, a significant difference between fertile and sterile pollen conditions was recorded in the linear phase: the UI elongation rate under the fertile pollen condition was 16.5 cm and was approximately double that observed under the sterile pollen condition. In the slow-growth phase, the UI elongation rate under the fertile pollen condition decreased as the UI approached its final length; however, it was still higher than that observed under the stertile pol-

Table 1. Effects of temperature on the fertility and length of the uppermost internode.

Temperature[a] (°C)	Total pollens per anther	Abortion pollens per anther	Stained pollens per anther	Seed set (%)	Length of uppermost internode (cm)	Panicle exsertion (cm)
20	995.7 ± 37.38	130.1 ± 18.54	865.6 ± 20.26	33.7 ± 1.28	47.1 ± 2.67	12.2 ± 0.78
22	910.8 ± 41.76	714.7 ± 36.46	196.1 ± 2.80	5.7 ± 0.04	42.6 ± 2.06	8.2 ± 0.48
24	798.9 ± 32.84	798.9 ± 32.84	0.05 ± 0.00	0	37.8 ± 2.13	4.9 ± 0.24
26	756.9 ± 42.37	756.9 ± 42.37	0	0	32.2 ± 1.36	0.3 ± 0.02
28	689.1 ± 29.56	689.1 ± 29.56	0	0	30.8 ± 1.34	−2.0 ± 0.09
Check	600.0 ± 34.14	600.0 ± 34.14	0	0	28.8 ± 1.41	−4.2 ± 0.16

[a] The applied temperatures were 20, 22, 24, 26, and 28 ºC and natural high temperature (more than 30 ºC) from the forming pollen mother cell to meiosis in a climate chamber. Each value is the mean ± S.E. of 30 main panicles.

Table 2. Endogenous phytohormone levels (ng g^{-1}) in uppermost internode and young panicle of TGMS *eui* mutant CX.[a]

Materials		GA_1	GA_3	GA_4	IAA	Z	ABA
Young panicle	Fertile	14,674 ± 527.4	6,836 ± 411.0	5,105 ± 419.0	528.9 ± 16.4	691.8 ± 16.3	273.6 ± 12.7
	Sterile	7,785 ± 483.1	3,742 ± 413.5	957.6 ± 42.3	294.6 ± 13.5	314.3 ± 9.6	902.9 ± 29.7
Uppermost internode	Fertile	4,309 ± 1,214	8,901 ± 567.3	6,587 ± 479.7	996.1 ± 23.2	765.1 ± 49.6	64.0 ± 4.7
	Sterile	12,717 ± 991.6	5,742 ± 216.8	1,258 ± 55.1	600.5 ± 21.4	541.5 ± 8.7	668.6 ± 19.0

[a] GA_1, GA_3, and GA_4 = gibberellic acid 1, gibberellic acid 3, and gibberellic acid 4; IAA = indole acetic acid; Z = zeatin Z: ABA = abscisic acid.

len condition. It has been proposed that the significant difference in UI elongation is due to pollen fertility.

Effects of temperature and pollen fertility on UI length

The values shown in Table 1 indicate a significantly positive correlation between the total pollen count and UI length in CX plants under different temperature conditions ($R^2 = 0.9566^{**}$). Panicle exsertion showed a similar correlation with pollen fertility (ratio of stained pollen). Both the total pollen count and UI length increased with a decrease in temperature. Temperatures of 22 °C and below resulted in variable fertility since a few fertile pollens (iodine-stained pollen) were observed; moreover, all the panicles were well exserted. Similar positive correlations between pollen fertility and panicle exsertion in CX plants were recorded under temperatures ranging from 20 to 28 °C. The UI in the CX plants under fertile pollen conditions was 18.3 cm longer than under sterile pollen conditions. This suggests that pollen fertility of the young panicle greatly affects elongation growth of the UI.

Analysis of endogenous phytohormone levels in the UI and young panicle

Previous studies have indicated the involvement of plant growth substances in male sterility and normal stamen development (Sawhney and Shukla 1994, Chailakyan and Khryanin 1987) and the deficiency of phytohormones in male-sterile rice (Ashikari et al 1999, Spielmeyer et al 2002), and they have indicated that rice vegetative growth is primarily regulated by GA_1 (Kobayashi et al 1989, 1994). We initially compared the endogenous phytohormone contents in the UI and young panicle during the linear phase (1–3 days prior to anthesis) under fertile and sterile pollen conditions. The results (Table 2) indicated higher (approximately 3-fold) GA_s, auxin, and zeatin contents and a lower (approximately one-fifth) ABA content in the UI under fertile conditions compared with sterile conditions; the results were similar to those recorded for the panicle. A significant difference was observed between the contents of the bioactive gibberellins GA_1, GA_3, and GA_4. GA_1 showed the highest content in the UI under fertile pollen conditions. Remarkable GA_s accumulation and ABA deficiency were observed in the UI, which resulted in a significant difference in the UI elongation of CX plants under the two pollen conditions. Therefore, it is evident that phytohormones in the panicle and the UI mainly contribute to the elongation of the UI and that pollen fertility has a remarkable influence on UI elongation.

Effects of decapitation on UI elongation

Experiments were conducted with CX plants that were either left intact or decapitated. Decapitation involved the removal of the young panicle and flag leaf at different times during the elongation phase of the UI. When the young panicle in the CX plants was excised, it was observed that UI length in the decapitated plants was less than that in the intact plants (Fig. 1). The reduction in the length of the decapitated UIs at different times in the elongation phase was statistically significant. The final UI length after decapitation was determined by the UI length when the young panicle was excised.

We developed a model to predict the final UI length based on UI length when the panicle was decapitated [final UI length $Y_{F\ (\text{fertile pollen})}$ (cm) = $-0.0056x^2 + 1.0663x + 9.198$, $R^2 = 0.9818^{**}$, and $Y_{S\ (\text{sterile pollen})} = -0.0077x^2 + 0.901x + 8.4982$, $R^2 = 0.9674^{**}$, where x is the UI length at the time of decapitation]. It was shown that, on panicle excision, UI elongation ceased after a short elongation period and did not reach the final length when compared with the intact plants; however, removal of the flag leaf did not affect UI elongation. We concluded that the panicle and UI elongation are significantly correlated.

The pollen fertility of young panicles greatly affects UI elongation. The UI length under fertile pollen conditions was 47.1 cm, significantly longer than under sterile pollen conditions (28.8 cm). The average elongation rate of the decapitated UI under fertile pollen conditions was 7.40 cm; it was significantly higher than that under sterile pollen conditions (4.45 cm). The elongation rate of the decapitated UI was higher in the linear phase than in the slow-growth phase. This indicated that some growth substances in the panicle were transported into the UI, which resulted in UI elongation. On removal of the source of the growth substances in the panicle, UI elongation ceased after a short growth period. Increases in pollen fertility are mediated by manipulation of the levels of endogenous plant growth substances such as GA_s, auxin, zeatin, and ABA, which results in UI elongation. Therefore, we concluded that pollen fertility plays a critical role in UI elongation.

Discussion

Stem and internode elongation are important characteristics of many crop plants. Particularly in rice (*O. sativa* L.), UI elongation is an adaptive characteristic that helps the rice panicle emerge from the flag-leaf sheath and complete anthesis. In the case of deepwater rice, submergence induces rapid elongation of rice coleoptiles and internodes (Cho and Kende 1997, Kende and van der Knaap 1998). Detectable UI elongation growth in CX plants started 6 days prior to anthesis and ceased after anthesis. Pollen fertility significantly influenced UI elongation during the linear and slow-growth phases. It was proposed that the significant difference in UI elongation is due to pollen fertility.

In TGMS rice, pollen fertility is regulated by environmental temperature changes during the panicle development phase that starts from the formation of the pollen mother cell to the late uninucleate phase of the pollen grains (Sun et al 1993, Liu et al 2001). TGMS rice is sterile and undergoes panicle enclosure at high temperatures (diurnal mean temperature > 24 °C) during the pollen developmental phase. However, at lower temperatures (≤ 23.5 °C), pollens were fertile, and the panicle was well exserted. Our study showed a strong negative correlation between temperature and pollen fertility during the pollen developmental phase of TGMS rice CX plants; however, the pollen fertility of the young panicle greatly affected the elongation growth of the UI.

The young panicle and the UI act as growth centers during the UI elongation phase. Our study regarding the effect of decapitation suggested that young panicles played a critical role in UI elongation and that UI elongation growth was dramatically affected by the panicle. On excision of the young panicle, UI elongation was disrupted. Elongation was determined by UI length at the time of decapitation. It was proposed that some growth substances in the panicle were transported into the UI, which resulted in UI elongation. If the source of the growth substances in the panicle is removed, UI elongation would correspondingly cease after a short growth period. Pollen fertility was mediated by a manipulation of endogenous plant growth substance levels, which resulted in UI elongation. Excision of the flag leaf did not affect UI elongation. These results further confirmed the correlation between the young panicle and UI elongation.

Several temperature-controlled responses are mediated via the manipulation of endogenous phytohormone levels and/or signal transduction (Koornneef et al 2002). Internode elongation can also be influenced by phytohormones. In the case of deepwater rice, ethylene promotes rapid internode elongation by enhancing the amount and activity of endogenous GA (Hoffmann-Benning and Kende 1992). Rice internode elongation is not only modulated by GA level but also by GA sensitivity (Ashikari et al 1999, Spielmeyer et al 2002). In our investigation, we found that the UI and panicle in CX plants accumulated higher amounts of auxin, zeatin, and GA_s under fertile pollen conditions than under sterile pollen conditions; however, ABA deficiency was observed under the former conditions, resulting in notable elongation of the plants' UI. Therefore, we concluded that pollen fertility is mediated by the manipulation of phytohormones and that phytohormone levels are mediated by the manipulation of UI elongation.

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Notes

Authors' address: College of Life Science, Hunan Normal University, Changsha 410081, Hunan, China, 1962liang@163.com.

Genetics and improvement of resistance to bacterial blight in hybrid rice

Zhang Qi

Bacterial blight (BB) is one of the most devastating rice diseases in China. Since the 1980s, rice breeding for resistance research in China has been progressing rapidly and extensively. The gene *Xa4* was mainly used in three-line indica hybrids and two-line hybrid rice. This disease has been seen to be "quiet" for 20 years in China. However, it has gradually emerged, primarily in fields planted with newly released rice cultivars in the Yangtze River Basin in recent years. This situation concerns scientists and inevitably raises several questions: What causes the resurgence and what should we do next? Is the resistance still effective? With numerous new tools, what approach do we take for resistance breeding so that the resistance will be more durable, and the R-gene will be used more efficiently?

A combined strategy composed of traditional methods, molecular marker-assisted selection, and transgenic technology should bring a new era to hybrid rice breeding programs for BB resistance. This paper briefly discusses and deliberates on issues related to the broadening of BB resistance, controlled and suitable use of broad-spectrum resistance genes, and alternating planting of available resistance genes based on the monitoring of virulent populations of the bacterial pathogen in China and Asia.

Bacterial blight (caused by *Xanthomonas oryzae* pv. *oryzae, Xoo*) is one of the most serious diseases of rice in China. In the 1930s, the disease mainly occurred in Jiangsu and Zhejing provinces (Fang 1963). To date, it has spread widely nationwide, except in Xinjiang, Tibet, and the northern part of the northeast region of China (Zhang et al 2007). The disease is most destructive in the southern part of China, the major indica rice cultivation region. In the Ezhong rice cultivation area, in the middle region of the Yangtze River, BB epidemics occurred in 1974-75, and 292,260 ha of rice fields were affected, with yield losses of 67,700 t. In addition, 584,100 ha of fields were affected, causing 141,800 t of yield losses in 1980, 1983, and 1987 (Xu 1993). In Anhui, a BB outbreak caused yield losses of 205,400 t and total yield losses in some fields in 1982 (Ji 1989).

Improvement of resistance to BB in hybrid rice in China

In 1976, the Ministry of Agriculture of China established a national research project on "Resistances Toward Rice Fungal Blast and Bacterial Blight." In the subsequent prioritized research programs, "Breeding Rice for High Yield, Good Quality, and Disease Resistance" was enlisted as a main objective for rice improvement. Since the 1980s, breeding research in China has been progressing rapidly and extensively.

Three-line indica hybrid rice

The main cytoplasmic male sterile lines, such as those from wild abortive (WA), dwarf abortive (DA), Gang, D, Yinsui, and Honglian cytoplasm developed in China, are susceptible to bacterial blight, but many restorer lines, such as IR26, IR28, IR30, IR32, IR36, IR50, IR54, and Ce 64-7 (IR9761-19-1), are resistant; therefore, the hybrid rice combinations grown by farmers possessed various degrees of resistance to the disease.

From 1973 to 1986, 31 (35.6%) out of 87 three-line indica rice hybrids were moderately resistant to BB. Seventeen of the combinations covered a rice area of more than 0.27 million ha annually. Among them, Weiyou 64, Weiyou 6, Shanyou 6, Shanyou-gui 33, Shanyou-gui 34, Shanyou 30. Xuan, and Siyou 6 were also resistant to blast or brown planthopper. Together, the planted area of Weiyou 64, Weiyou 6, and Shanyou 6 had reached 1.34 million ha annually. Weiyou 64 had been grown for as long as 20 years. The area for other combinations increased until the mid-1990s, but gradually decreased (Zhang 2007).

In 1996, Chinese rice scientists initiated a project on developing "Super Rice," which boosted rice breeding programs nationwide. Numerous hybrid combinations with high yield and good quality were developed. In 1999, R218, a strong restorer line, was developed by marker-assisted selection (MAS) using conventional testing for resilience, and the line was used to make a hybrid of Xieyou 9308. It expressed super high yield, good grain quality, and resistance to both BB and blast. Up to 2004, its growing area had reached 0.713 million ha (Cheng et al 2004). The area of another outstanding super hybrid rice with resistance to both BB and rice blast, Zhongzheyou 1, had reached 6.7 million ha (Zhang et al 2008).

Up to 2005, 169 hybrids passed the national certification requirements, and 18 (10.7%) of these were resistant to BB or to both BB and blast (Yang et al 2004, Yang et al, personal communication 2005). Combinations composed of II you 084, Shanyou 77, Guofeng 1, and Teyouduoxi 1 covered a large rice area. In addition, in 2002-05, among the combinations with high yield or good quality that passed provincial certification requirements, Wandao 135, II you 118, Kangyou 98, II you 205, Fuyou 108, Nonghuayo 80, II you 1511, and Yixiang 2408 were resistant to both BB and blast, and Rongdao 1, 29 you 559, and II you 559 were resistant to BB, blast, and sheath blight.

The main combinations bred from the 1980s exhibiting resistance to diseases/ insects and having high yield or good quality approved and grown on a large area are listed in Table 1.

Table 1. Bacterial blight resistance in three-line indica hybrid rice and its restorers in China.

Hybrid rice	Combination	Reaction to[c]					Year approved/ bred	[d]Area (year)/ $\times 10^4$ ha
		BB	BL	SB	YD	BPH		
Weiyou 64[a]	V20A/Ce 64-7 (IR36)	MR	MR		MR	MR	1981	1983-2003, 1,227
Shanyou 6[a]	Zhenshan 97A/IR26	MR	MR				1976	1981-94, 933
Siyou 6[a]	V20A/IR26	MR				MR	1976	1981-95, 753
Shanyou-guei 33[ab]	Zhenshan 97A/Gui 33 (IR36/ IR20)	MR	MR				1983	1984-99, 466
Shanyou-guei 34	Zhenshan 97A/Gui 34	MR	MR				1984	1986-94, 152
Weiyou 6	V41A/IR26	R				MR	1977	1983-86, 15
Shanyou 77#[ab]	97A/Minghui 77	MR					1998	1992-2005, 250
II you 084#[a]	II-32A/Zhenhui 084	HR	HR				2003	2001-05, 76
Teyouduoxi 1#[a]	Longtefu A/Duoxi 1	MR	R				2001	1997-2005, 26
Zhong 9 you 838 selected# (Guofong 1)	Zhong 9A/838 selected	MR	R				2001	2002-05, 21
Fongyou-xiangzhan#[ab]	Yuegfeng A/R6547	HR	HR				2003	2002-04, 10
K you 047#[ab]	K 17A/Chenghui 047	HR	MR				2001	1996-2003, 10
Youyou 128#[a]	You IA/Guanghui 128	MR	R				1999	1997-2002, 4
K you402#[a]	K 17A/402	MR	MR				1998	1998-2004, 3
Zhongyou 223#[ab]	A/R223	MR					2001	
Zhongzheyou 1#[ab]	Zhongzhe A/Hanghui 570	MR	MR				2004	2004-06, 8
Xieyou 80#[a]	Xieqingzao A/R80	MR					2004	
Zhongyou 218#[ab]	Zhong 9A/R218-51	MR					2004	2005, 1

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Table 1 continued.

Hybrid rice	Combination	Reaction to[c]					Year approved/ bred	[d]Area (year)/ $\times 10^4$ ha
		BB	BL	SB	YD	BPH		
Zhongyou 85#[ab]	Zhong 9A/Puhui 85	HR					2005	
Xieyou 5968#[a]	Xieqingzao A/15968	MR					2004	2005, 0.01
Xinyougang 22#[b]	Xin A/752	MR					1999	
Zhong85#[ab]	Zhong 9A/Puhui 85	HR					2005	
Yixiang 9#[ab]	Yixiang I A/Yihui 9	MR					2005	2005, 1
Neixiang 2550#[b]	Neixiang-2A/Neihui 2550	MR					2005	
Yixiang 10#[b]	Yixiang I A/Yihui 10	MR					2005	
II you 363#[a]	II-32A/Shuhui 363	MR					2005	
Xieyou 9308[ab]	Xieqingzao A/C57//300/IR26	R	MR				1999	1998-2005, 25
II you 128[ab]	II-32A/Hui 128 (7 Gueizao 25/Ce64//Minghui 63)	MR	HR				1999	1998-2005, 53
Kangyou 63[a]	Zhenshan 97A/Kanghui 63	HR	HR				2002	
Fengyou 205[ab]	Feng 7A/D205	R	R				2005	
Xieyou 084[ab]	Xieqingzao A/Zhenhui 084	MR	HR				2004	2004-05, 8
Wandao 135[b]	Fengyuan A/YR909	HR	R				2004	
Tianyou 372[ab]	Tianghuei A/Guanghui 372	MR	R				2005	
Tianyou 290[ab]	Tianhuei A/Guanghui 290	MR	R				2005	
II you 118[ab]	II-32A/Huahui 118	R	HR				2003	2004-05, 3
II you 559[a]	II-32A/Huahui 559	MR	R	MR			2002	
29 you 559[a]	29A/Yanhui 559	MR	HR	MR			2003	

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Table 1 continued.

Hybrid rice	Combination	Reaction to[c]					Year approved/ bred	[d]Area (year)/ $\times 10^4$ ha
		BB	BL	SB	YD	BPH		
Xinxiangyou 906	Xinxiangyou A/Ronghui 906	R	MR	MR			2005	
K you 507[ab]	K17A/R507	MR	HR				2002	
Kangyou 98[ab]	II-32A/Kanghui 98	HR	HR				2003	
II you 205[ab]	Feng 7A/D205	R/ HR	R				2004	2005, 1
Hongyou 527[ab]	Hongai A/Shuhui 527	R					2004	
Fuyou 108[b]	Gang 46A/Jiahui 108	R	R				2005	
Nonghuayou 808[a]	M98A/MR0208	R	R				2005	
Zhongyou 250[b]	Zhong 9A/R2596	MR	MR				2005	
Ilyou 1511# ab	II-32A/Xinhui 1511	MR	HR				2007	
Dyou 986	D62A/Chihui 986	MR	R	MR			2007	
Tianyou 1120[ab]	Tianfeng A/Yanhui 1120	HR	HR				2008	
Gangxiang 707[ab]	Gangxiang 1 A/Shuhui 707	MR		HR			2008	

= registered at national level, and the others at provincial level. [a]High yield. [b]Good quality. [c]BB = bacterial blight; BL = rice blast; DF= rice yellow dwarf; BPH = brown planthopper. R= resistant; MR = moderately resistant; HR = highly resistant. d Planted area based on statistical data from National Agricultural Extension Service Center.

Source: Zhang et al (2008).

Additionally, several male sterile lines with BB resistance were developed and passed provincial certification requirements during 2003 to 2005, such as 29A, Zhong 1A, and H28A (Zhang et al 2007). Sterile line R106A exhibited high resistance to BB at all growth stages, and polymerized genes *Xa21* and *Xa23* by MAS (Luo et al 2005).

Three-line japonica hybrid rice

Up to the late 1970s, 43 out of 72 combinations and parents of three-line hybrids were moderately resistant to BB. Since then, most of the combinations bred also exhibited BB resistance. The main sterile lines, such as Reimei A, Toyonishiki A, Akihikari A, Liouqianxin A, Dangxuanwan 2 A, and Shurei A, were also resistant to BB.

From the 1980s to 1990s, three out of five japonica combinations cultivated in northern China were moderately resistant to BB. One of them, Liyou 57, has an accumulated area exceeding 0.66 million ha. Since 2001, some combinations exhibiting multiple resistances to pests had passed national and provincial certification requirements, including combinations of Yongyou 4, 69 you 8, Jialeyou 2, Jiayou 2, and others (Table 2).

Two-line hybrid rice (indica/japonica)

In general, the BB resistance of two-line hybrid rice is better than that of three-line hybrid rice because the parents could be selected widely and used, such as Teqing, Shanqing 1, Yuhong, Yangdao 6, Shuangqizhan, D100, 9311, Shuang 9, and Wanhuei 9, that exhibited moderate resistance to BB. Twenty sterile lines released during 1996-2000 were evaluated with six *Xoo* strains: C II (HB17), C IV (ZHE 173), CV (GD1358), GIV, GV, and P6 (PXO99). Most of the S lines were resistant to C IV only and were susceptible to the others (Zhang 2007).

Some combinations exhibiting high yield, good quality, and multiple resistances to BB, blast, rice bacterial leaf streak, or brown planthopper had passed national certification requirements after 2000, for example, the combinations of Peiza-shanqing, Peiza-maoxuan, Peiliangyou-teqing, and Yunguang 8. Up to 2006, six out of 21 combinations with high yield and good quality that passed national certification requirements were resistant to both BB and blast, such as Liangyou-peijiu, Peiza-shuangqi, and 70 you 9 (japonica). Up to 2005, the accumulated area of Liangyou-peijiu had reached 4.68 million ha, representing 50% of the total area of two-line hybrid rice in China. Recently, many new two-line hybrid combinations with BB resistance, high yield, and good quality were registered provincially in 2006-07, such as Fengliangyou 4, Xinliangyou 6, Xinliangyou 6380, Peiza 35, and Xinliangyou 98 (Table 3).

Rice breeders succeeded in achieving this objective as proven by the low incidence of BB throughout China for about two decades. Rice combinations grown by farmers possessed various degrees of BB resistance. But, in recent years, the disease has gradually emerged in fields planted with some rice cultivars and hybrid rice combinations in the Yangtze River Basin (Fig. 1). The resurgence of the disease after 20 years of “quietness” appeared to be a potential risk in China.

Table 2. Some three-line japonica hybrid rice with multiple resistance to diseases and pests, high yield, and good quality registered at national and provincial level in China since 2001.

Hybrid rice	Combination	Reaction to					Year approved and bred by institute
		BB	BL	SB	BS	BPH	
Yongyou 4#^{ab}	Yougeng 2A/K2001	MR					2004, Ningbo AAS
86you 8	863A/Ninghuei 8	MR	HR				2001, Jiangsu AAS
69 you 8^{ab}	69A/R11238	R	HR	MR			2001, Xuzhou AAS
86 you 242	863A/R242	MR	HR				2002, TaihuAAS
Jiayyou 1^{a}	Jia 60A/Jiahuei 40	MR	R			MR	2004, Jiaxin AAS
Jialeyou 2^{a}	151A/DH32	R	R				2004, Jiaxin AAS
Yongyou 6^{b}	Yonggeng 2 A/K4806	R	R				2004, Ningbo AAS
Yongyou 5^{b}	Yongnuo 2 A/K6926	MR		R			2004, Ningbo AAS
Yongyou 2^{ab}	Yonggeng 2A/K1722	MR	MR		MR		2001, Ningbo AAS
Wandao 76^{b}	Aizhixiang A/MC20518	MR		MR			2004, Hefei Xilong Rice Inst.
Yuza 34^{b}	Dianyu 1A/Nan34	R	R				2004, Yunnan AAS
Changyou 2^{b}	Wuyungeng 7A/C53	HR	R				2005, Changshu AAS
Suyou 22^{b}	Wuyungeng 7A/R16189	R	MR				2005, Zhongjiang Seed Company
Xuyou 201^{ab}	Xu 9320A/Xuhuei 201	MR	R	MR			2005, Xuzhou AAS
Xiuyou 5#^{ab}	Xioushui 110/andaYo 4	MR	R				2006, Jiaxin AAS

= registered at national level, the others at provincial level. [a]High yield. [b]Good quality. [c]BB = bacterial blight; BL= rice blast; SB = sheath blight; SR= rice stripe virus; BPH = brown planthopper. R = resistant; MR = moderately resistant; HR = highly resistant.

Table 3. Main two-line hybrid rice combinations with BB resistance in China.

Hybrid rice[#]	Type	Combination	Reaction to					Year approved and bred by institute
			BB	BL	BS	FS	BPH	
Liangyoupei 9#[ab]	Indica	Peiai 64S/9311	MR	HR				2001, JSAAS
Peizashuang 7#[ab]	Indica	Peiai 64S/Shuang7 zhan	MR	HR				2001, GDAAS
Peiza Mao 3#[ab]	Indica	Peiai 64S/Mao3	MR	R				2005, Maomin Hybrid Rice Center
Luliangyou 28#[a]	Indica	Lu 18S/Hua28	MR	MR				2005, Yahua Seed Research Inst.
Yangliangyou 6#[ab]	Indica	Guangzhan 63-4S/Yangdao 6	MR	MR				2005, Lixiahe AAS
Wandao 24#[ab]	Japonica	7001S/Wanhuei 9	MR	HR				2001, Anhuei AAS
Peiliangyou-teqing[a]	Indica	Peiai 64S/Teqing	MR	MR				1994, HN Hybrid Rice Research Center
Ejingza 1[ab]	Japonica	N5088S/R187	MR	MR				1995, HBAAS
Yunguang[8]	Japonica	N5088S/Yunhuei 11	MR	MR				2000, YNAAS
Peizashanqing[a]	Indica	Peiai 64S/Shanqing 11	MR	MR	MR			1997, Maomin Hybrid Rice Center
Peiliangyou-yuhong[b]	Indica	Peiai 64S/Yuhong I	MR	MR		MR		1997, HN Agricultural University
Peiza 6[ab]	Indica	Peiai 64S/G67	MR	MR				2000, HN Agricultural University
Tianliangyou 402[a]	Indica	Tianfeng S/R402	HR	MR				1998, Ganzhou AAS
Peiliangyou 275	Indica	Peiai 64S/275	R	MR				1999, GXAAS
Wandao 147[ab]	Indica	Xinan S/Anxuan 6	R	MR				2005, Yin Quan AAS
Peizamaoxuan[b]	Indica	Peiai 64S/Maoxuan	R	R				2000, Maomin Hybrid Rice Center
Ganya 1	Indica	Peiai 64S/HB-01	MR		MR		MR	2002, Binghu AAS

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Table 3. Main two-line hybrid rice combinations with BB resistance in China.

Hybrid rice[#]	Type	Combination	Reaction to					Year approved and bred by institute
			BB	BL	BS	FS	BPH	
Wandao 129[b]	Indica	Peiai 64S/Hong 98	MR	HR				2004, Xuancheng AAS
Wandao 119[b]	Indica	Xuan 69S/WH26	MR					2004, Anqing AAS
Liangyoupeijing	Japonica	Peiai 64S/94205	MR	R				2005, Xinyang AAS
Liangyou 108[b]	Indica	Peiai 64S/Ninghuei 108	MR	MR				2005, JS AAS
Fengliangyou 4#[ab]	Indica	Feng 39 S/Yandao 4	HR					2006, HF FL Seed Company
Xinliangyou 6#[ab]	Indica	Xinan S/Anxuan 6	HR					2006, HF YQF Seed Company
Xinliangyou 6380#[ab]	Indica	03S/D208	R	R	R			2006, Nanjing Agricultural University
Peiza 35#[ab]	Indica	Peiai 64S/Tehuazhan 35	MR	MR				2006, Hunan Agricultural University
Xinliang 98#[ab]	Indica	Xinan S/Hong 98	MR	MR				2007, HF Anquin AAS

= registered at national level, and the others at provincial level. [a]High yield. [b]Good quality. [c]BB = bacterial blight; BL = rice blast; BS = bacterial streak; BPH = brown planthopper. R = resistant; MR = moderately resistant; HR = highly resistant.

[d]Adapted partly from the report of Yuan QH (2001) and Yang SH (2006, private communication).

Fig. 1. Rice cultivars infected by bacterial blight in Donghai Anfeng, Jiangsu Province.

Discussion on the actual and potential damage situation

Reduced genetic variability of rice for BB resistance

Learned from the experience and research on rice bacterial blight from the last 20 years, a smart application of R-genes should be based on information on host-pathogen interactions. According to the studies on pedigree, inheritance, and gene analyses of BB resistance in 287 resistant cultivars, the gene *Xa4* has been widely adopted in the development of both indica hybrids and conventional cultivars in China, and it became the only source of bacterial blight resistance (Fig. 2) (Zhang et al 2007, Xie et al 1990, Shi et al 1993, Sha et al 1994, Yang et al 1994). Such genetic uniformity has made the rice crop vulnerable to disease epidemics that may cause great damage. It is well known that the emerging or re-emerging frequency of a disease and virulent pathogen populations depends on the inherent ability of the pathogen to adapt to the hosts. Introduction of a single resistance gene into cultivars often results in a shift of virulence in the *Xoo* population. Xu et al (1981) first reported that a new *Xoo* strain (Chinese pathtype V, CV) was virulent to IR26 (carrying *Xa4*). They warned that the host (carrying *Xa4*) had been cultivated extensively at for least 20 years. The rapid increase in genetic diversity of the CV pathotype indicated the breakdown of host resistance gene *Xa4* (Zhang et al 1997). The pathogen then spread from Guandong to Fujian, Guangxi, Zhejiang, Jiangsu, Yunnan, and Jilin provinces (Xu et al 1994).

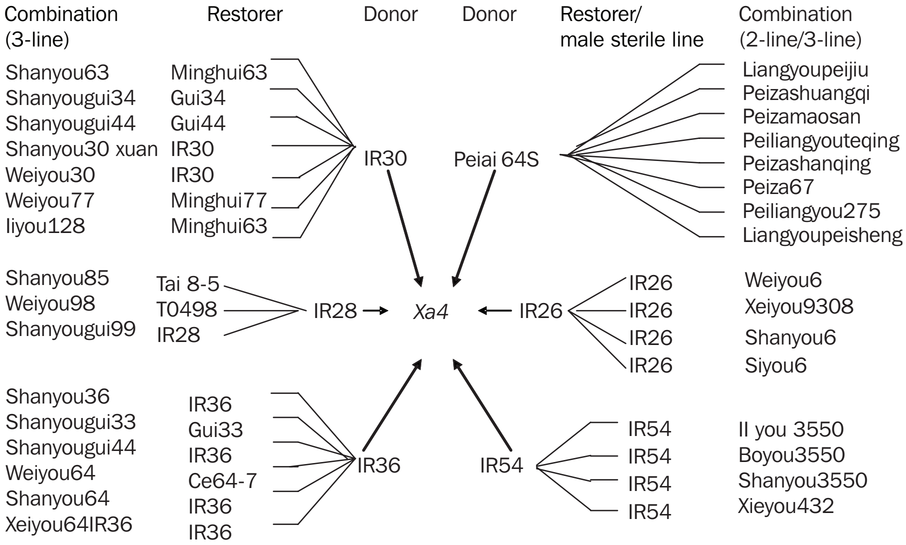

Fig. 2. Sources of BB resistance for main hybrid rice (all depend on *Xa4*).

The rice cultivated area has decreased for some combinations, such as the popular Shanyou 6, which declined from 3.3 million ha in the mid-1980s to 0.2 million ha in 2004 (Zhang et al 2007).

Many cultivars with *Xa4* have been cultivated widely in the Philippines and other Asian countries. The large-scale and long-term cultivation of cultivars carrying *Xa4* has resulted in significant shifts in the race frequency of *Xoo*. In many areas of India and Indonesia, rice cultivars with only *Xa4* have become susceptible to BB (Mew and Vera Cruz 1985, Mew 1987, Mew et al 1992).

The *Xa21*, originally reported to be a broad-spectrum gene with resistance to the South Asian *Xoo* strains, has been susceptible to some strains in India, Korea, Nepal, and China (Lee et al 1999, Adhikari and Basnyat 1999, Marella et al 2001, Muralidharan et al 2004, Zeng et al 2002, Ji et al 2003). This information suggests that we need to look into the pathogenic variability both nationwide and globally.

Population genetic diversity and pathogenicity of *Xoo*

Information on pathogenic variability is essential to formulating a strategy for disease management based on resistance deployment. Plant pathogens have an intrinsic mechanism to adjust to new cultivars and to a new crop production environment. Because of selection pressure, new strains of disease pathogens overcome the resistance developed, thus dominating the pathogen population and shortening the life of these cultivars. This “boom and bust cycle” continues (Mew 2004). So, we need to continue monitoring the pathogen population in response to the newly released varieties in order to be “a step ahead” of the disease problem.

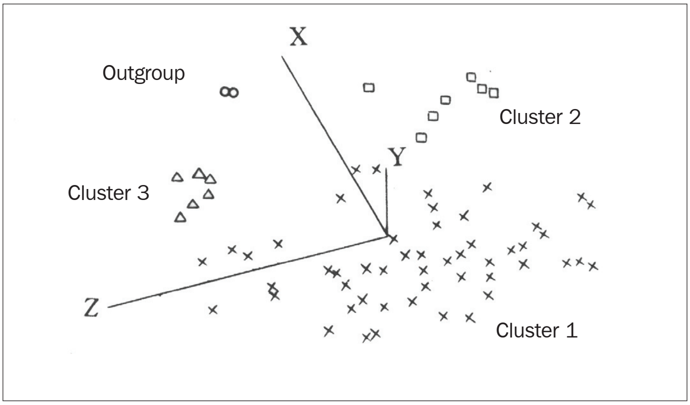

Fig. 3. Clustering analysis of 143 Chinese *Xanthomonas oryzae* pv. *oryzae* strains as derived from DNA band data obtained with rep-PCR (ERIC, BOX IS1112, and IS1113 primers) and RFLP (pBS*avrXa10* probe). The number of clusters that best fit the data was determined by the consensus of three clustering statistics. Positions of the strain on the three-dimensional graph were determined by multiple correspondence analysis (adapted from Zhang et al 1997).

The population of Xoo in China. Zhang et al (1997) and Wang et al (2001) analyzed the population genetic structure of *Xoo* strains collected from 1984 to 2000 in China. The results showed a simple population genetic structure of the pathogen. Most of the strains were grouped into clusters 1 and 2, and only strains from Guangdong (CV) were grouped into the "outgroup" as shown in Figure 3. This was due to the cultivation of host plants carrying a single R (*Xa4*) gene in China, as mentioned above. In addition, the molecular type of the pathotype V and Yunnan strains was distinct from that of the other collections. As expected, the *Xoo* population of Yunnan contained several new pathotypes that differed from the seven national pathotypes in China (Fang et al 1990). Nine and eight out of the 14 *Xoo* races were virulent to cultivars carrying *Xa4* and *Xa21* genes, respectively (Ji et al 2003). Zeng et al (2005) also found another new *Xoo* strain that has virulence to *Xa4*, *Xa21*, *Xa1*, *Xa2*, *Xa3, xa8*, *Xa10*, *Xa11*, *xa13*, and *Xa14*, and only *Xa23* is resistant and *xa5* and *Xa7* are moderately resistant to the strain in Guangdong Province.

In China, since the 1980s, the distribution of *Xoo* pathotypes in the major rice-growing areas has been stable: pathotypes II and I dominate in japonica rice in northern China; pathotypes IV and II are the major *Xoo* found in the Yangtze River Basin. It is important to note that the spreading threshold value of pathotype CV (affecting *Xa4*) is alarming and now approaching 20% (Xu et al 2004).

The distribution of Xoo and available resistance genes in Asian countries. In the Philippines, 10 races were identified, and *Xa7*, *xa5*, and *Xa21* were the most effective. In Japan, the predominant races I (59.5%), and II (33.5%) are distributed in all locations of the country; the effective R genes are *Xa3, Xa4*, and *Xa12* (Noda et al 1990). Adhikari and Basnyat (1999) reported that five virulence groups were clas-

Table 4. Main ineffective and effective genes with resistance to BB in Asian countries (resummarized based on Noda et al 1996).

Country	Race no. (isolates)	Main ineffective R gene	Effective R gene
Bangladesh	12 (74)	*Xa1, 2, 3, 4, 7, 10, 11,* and *xa5, xa8*	*Xa3, Xa12,* pyramiding line
India	5 (58)	*Xa1, 2, 3, 4, 10, 11, 12,* and *xa5*	Pyramiding line
Nepal	16 (45)	*Xa1, 2, 3, 4, 10, 11, 12,* and *xa5*	*xa5 , Xa7,* pyramiding line
Myanmar	7 (27)	*Xa1, 2, 4, 10, 11,* and *xa5*	
Tailand	5 (35)	*Xa1, 2, 10, 11,* and *xa5*	
Indonesia	8 (78)	*Xa1, 2, 3, 10, 11,* and *xa8*	*xa5, Xa7,* pyramiding line
Philippines	5 (61)	*Xa1, 2, 4,* and *11*	*xa5, Xa7, 12,* pyramiding line
Malaysia	2 (11)	*Xa1, 2, 10, 11,* and *xa8*	*Xa4, 7, 12,* and *xa5*
South China	6 (24)	*Xa1, 2, 3, 4, 7, 10, 11, 12,* and *xa8*	*xa5, Xa7, 22, 23*

sified in Nepal. The virulence of *Xoo* is very strong; all *Xoo* isolate collections were virulent to *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa14*, and *Xa21*. Only the pyramided lines with (*Xa4* + *xa5*), (*Xa4* + *xa5* + *xa13*), (*Xa4* + *xa5* + *Xa21*), and (*Xa4* + *xa5* + *xa13* + *Xa21*) were resistant to two, three, three, and five virulence groups, respectively. Noh et al (2004) reported that five races of *Xoo* were classified in Korea in the 1980s. A new race, K3a, was found and was virulent to cultivars carrying *Xa3*. The distribution frequency of K3a has been increasing throughout South Korea since 2003. In addition, cultivars carrying *Xa21* were susceptible to 15% of *Xoo* strain collections (1987-89) a few years after being cultivated, and 96% of the strain collections were virulent to *Xa21* in the mid-1990s in Korea (Lee et al 1999). Muralidharan et al (2004) reported the field performance of genotypes to BB in 11 locations in India. All the lines with a single R gene *xa5* or *xa13* recorded a resistant reaction in 5–7 locations, those lines with *Xa21* were found resistant at only 3–5 locations, pyramided lines possessing (*xa5*+*xa13*), (*xa13*+*Xa21*), and (*xa5*+*Xa21*) showed resistance at more sites, and genotypes possessing (*xa5*+ *xa13*+*Xa21*) showed resistance at 5–8 sites. Noda et al (1996) examined the variation in the pathogenicity of *Xoo* from nine countries in South and East Asia by testing Japanese and Philippine differentials. The data were resorted based on the results in Table 4.

Approaches to improve BB resistance in breeding hybrids

Broaden the genetic basis for BB resistance

Alternate BB resistance genes. Actually, since the early 1980s, rice breeders have been making efforts to use more genes to broaden the genetic basis for BB resistance. Zhu et al (1990) developed “TD” series rice lines carrying BB resistance gene *Xa7* derived

Table 5. Resistant three-line indica hybrid rice with BB resistance gene *Xa21* bred by MAS.

R combination or restorer	Restorer to be improved	Donor (gene)	Molecular marker	Bred by institute	Year approved
Xieyou 218	Fuhuei 838	IRBB21 (*Xa21)*	pTA248	CRRI	2002
Zhongyou 218	Zhonghuei 218	IRBB21 (*Xa21)*	pTA248		2003
Guodao 1[a]	R8006	IRBB60 (*Xa21)*			2004
Guodao 3	R8006	IRBB60 (*Xa21)*			2004
Zhongyou 1176	R1176	IRBB60 (*Xa21)*			2005
Shuhuei 527 improved	Shuhuei 527	IRBB60 (*Xa21)*	pTA248 MP12	SCAU	–
Minghuei 63 (carrying *Xa21*)	Minghuei 63	IRBB21 (*Xa21)*	pTA 248 pTA 21	HZAU	–
93-11 *(Xa21)*	93-11	Minghuei 63 (*Bt/Xa21*)			
93-11 *(Bt/Xa21)*			pTA 248		–
II you 8220	Miyang 46/ Minghuei 63	IRBB21 (*Xa21)*	pTA 248	ZJAU	2003
T71 T81	Minghuei 63	IRBB21 (*Xa21)*		ZJAU	–

[a] Registered at national level, and the others at provincial level.
Source: Xu (2007).

from TN1/DV85. The resistant restorer lines Kanghuei 63, Kanghuei 98, and D205 were developed using TD (as female) and Minhui 63 (as male). The combinations Kangyou 63, Kangyou 98, II you 205, and Fengyou 205 exhibiting resistance to both BB and blast were developed using these restorer lines with sterile line Shanyou 97A or II-32A. These combinations were popularized extensively in BB-damaged fields of Jiangsu, Zhejing, Anhuei, Henan, Yunnan, Hubei, Hunan, Jiangxi, and Fujian provinces, and they exhibited BB resistance, high yield, and wide adaptability. To date, the accumulated area of those combinations had reached 2.5 million ha (Ding 2005).

Up to 2006, a total of 30 BB resistance genes had been identified. Twenty-one and 9 out of the 30 *Xa* genes are dominant and recessive, respectively (Zhang 2005). Eighteen were mapped by molecular markers to facilitate reliable marker-assisted selection and application; tightly linked PCR-based markers have been identified for the common resistance genes *Xa4*, *Xa7*, *Xa21*, *Xa23*, *xa5*, and *xa13* (Table 5, Xu 2007). Six *Xa* genes were cloned: *Xa1*, *xa5*, *Xa21*, *xa13*, and *Xa26 t* (from Minghui 63), and *Xa27t* (from *Oryza minuta*). Since the cloning of the dominant gene *Xa21* (Song et al 1995), there has been major progress in applying marker-assisted backcrossing in crop improvement on BB resistance for both regular and hybrid rice (Xu 2007) (Table 5).

Among all designated *Xa* genes, the dominant genes *Xa7*, *Xa21*, and *Xa23* (from *O. rufipogon*) exhibit the broadest resistance spectrum and strongest resistance strength (Zhang et al 1998); *Xa3* and *Xa4* show broad-spectrum resistance against Chinese BB

Table 6. BB resistance combinations developed during 1973-2006.[a]

Period	Total	No of resistant combination.	Percentage of resistant combination
3-line			
1973-86	87	31	35.6 (indica)
	43	26	60.0 (japonica)
1996-2005	169	18	10.7 (indica)
2005	34	12	35.3
2006	6	4	66.7 (south)
	64	5	7.8 (north)
2-line			
1994-2000	32	17	53.1
2001-06	21	6	28.6

[a] Cultivars with an annual area of more than 6.7 ha

pathotypes, but cannot be used in regions where the prevailing pathotypes are C IV and C V in China.

Now, we must note that the new resistant hybrid combinations released have gradually declined since the late 1990s (Table 6). The question is, were the combinations not adequately screened prior to their release? Resistance breeding must still be one of the main objectives of rice improvement. Thus, we face two key challenges: (1) how to deal with the rapidly changing pathogens and (2) how to design and develop durable resistant combinations.

A combined strategy composed of traditional methods, molecular marker-assisted selection, and transgenic technology should bring a new era to a hybrid rice breeding program on BB resistance. To prolong the useful life of major resistance genes, we need to develop pyramiding lines carrying multiple resistance genes to broaden BB resistance, and make suitable use of broad-spectrum resistance genes such as *Xa7*, *Xa21*, *Xa22* (from a Yunnan variety), and *Xa23*. Refined strategies for alternate planting of available resistance genes and the adoption of "horizontal resistance" are also necessary. These measures will bring success if seriously carried out.

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Notes

Author's address: Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100081 China.

Developing new nonaromatic CMS lines and identifying restorers for hybrid rice breeding

R. Pushpam, S. Robin, S. Manonmani, K. Mohana Sundaram, and T.S. Raveendran

The successful use of hybrid vigor in rice largely depends on the availability of local cytoplasmic male sterile (CMS) and restorer lines. The CMS lines introduced from China are unsuitable to use as such in developing hybrid rice in India. To develop new CMS lines, it is important to screen locally adapted elite breeding lines for genetically diverse maintainers and promising restorers with a wide genetic base for developing commercial hybrids. The materials for our study consisted of 10 CMS lines—IR68897A, IR68885A, IR68886A, IR68888A, IR69616A, IR67684A, DRR2A, CRMS32A, APMS6A, Pusa5A—and 47 male parents. Crosses were made between CMS lines and male parents. The results of our investigation revealed that 131 male parents were identified as effective restorers, 43 were identified as potential maintainers, and 126 were grouped into partial restorers and partial maintainers. Among the 300 F_1 hybrids evaluated, 44 combinations showed higher standard heterosis than the check CORH 2. For the development of nonaromatic CMS lines, among the 43 potential maintainers identified, 23 backcross progenies are under a conversion program. The findings show that the fertility restoration of the cultivars varied with the genetic background of the CMS lines. To overcome difficulties in using the exotic nonadaptable CMS lines, the maintainer lines identified can be used to induce cytoplasmic male sterility in local germplasm by repeated backcrossing and effective restorers can be used in developing new hybrid combinations for commercial cultivation.

Among the strategies adopted to enhance further the use of heterosis to raise economic grain yield is the development of commercial F_1 hybrids. These hybrids have already become popular in China, giving a yield advantage of about 20–30% over high-yielding homozygous varieties. Of the various approaches for commercial application of heterosis, the three-line breeding method is the most commonly used method in China and elsewhere. It involves the use of a CMS line, its maintainer, and a restorer line. A high percentage of pollen fertility restoration, stable restoring ability over locations/season, and good combining ability are the important key attributes needed to ensure commercially viable hybrid technology.

The successful use of hybrid vigor in rice largely depends on the availability of local CMS and restorer lines. The CMS lines introduced from China are unsuitable to use as such in developing hybrid rice in India. Therefore, it is imperative to identify maintainers and restorers among the lines developed through conventional breeding procedures. Maintainers should have higher adaptability and restorers should have higher combining ability. To develop new CMS lines, it is important to screen locally adapted elite breeding lines for genetically diverse maintainers and promising restorers with a wide genetic base for developing commercial hybrids. Hence, our study was undertaken to develop new nonaromatic CMS lines and to identify the fertility restoration ability of different CMS lines of wild abortive cytoplasm for hybrid rice breeding.

Materials and methods

The materials for this study consisted of 10 CMS lines—IR68897A, IR68885A, IR68886A, IR68888A, IR69616A, IR67684A, DRR2A, CRMS32A, APMS6A, Pusa5A—and 47 male parents. Crosses were made between CMS lines and male parents during 2005 kharif and hybrid combinations were successfully made. Three hundred hybrid combinations were raised in a test-cross nursery during 2005-06 rabi, along with their parents and check CORH 2. Thirty-day-old seedlings were transplanted in the field at a spacing of 20 × 15 cm in 1.5-m rows in a randomized block design with two replications. Standard agronomic practices were employed. Maintainers and restorers were identified by observing pollen fertility at the time of flowering. The lines identified as effective maintainers can be further backcrossed with their respective F_1s to find completely sterile backcross progenies that can be further developed as new nonaromatic CMS lines.

Under the backcross breeding program involving 43 backcross progenies (BC_1F_1), seed set was observed in only 23 backcross progenies. These were raised in a backcross nursery (BC_2F_1). Plants showing good vigor, high spikelet number per panicle, good panicle stigma exsertion, 100% pollen and spikelet sterility, and high outcrossing rate were selected. Further backcrossing was done and the progenies were included in a conversion program to develop new nonaromatic CMS lines.

Results and discussion

Maintainers, restorers, and partial maintainers and restorers identified for the CMS lines are given in Table 1. Identified as effective restorers were 131 male parents. Forty-three were identified as potential maintainers and 126 were grouped into partial restorers and partial maintainers.

In our study, the same genotype behaved as a restorer for one CMS line and as a maintainer for other CMS lines. This kind of differential reaction of some rice genotypes with different CMS lines of the same cytoplasmic source has also been reported by other workers (Virmani et al 1986, Bijral et al 1989). This may be due to variations in sterility gene number or the presence of fertility genes that act in an ad-

Table 1. Restorers and maintainers for WA cytosterile lines of rice.[a]

	IR68897 A	IR68885 A	IR68886 A	IR68888 A	DRR2A	PUSA 5A	APMS 6A	IR67684 A	IR69616 A	CRMS 32A
IR3883-41-3-2-2R	R	M	R	R	R	R	R	PR	R	PR
IR62161-180-3-1-3-2R	R	R	R	R	R	PR	R	M	R	R
IR62124-83-3-2-1R	–	PR	R	R	R	R	R	R	R	R
IR63874-187-2-2-1-2R	R	PR	R	R	PR	PR	R	PM	R	PR
IR21567-18-3R	–	–	R	R	R	R	M	–	R	R
IR65489-11-AC2-2R	–	PR	R	–	R	R	R	PM	R	R
IR63079-195-2-2-3-2R	R	PR	R	R	PR	R	R	R	R	–
IR62036-222-3-3-1-2R	R	PM	R	PR	PR	R	R	R	PR	PM
IR62037-93-1-3-1-1R	R	PR	–	PR	R	PM	–	R	–	PR
IR63881-49-2-1-3-2R	R	–	M	–	–	–	M	PR	PM	PR
IR65597-143-2-3-1R	R	R	R	R	PR	PM	PR	PR	–	–
IR65483-14-1-4-13R	PR	R	R	R	–	R	R	R	–	–
W216	PR	R	–	R	R	PR	–	R	–	–
IR61614-38-19-3-2R	–	–	R	M	R	PR	PM	M	PM	–
IR72865-94-3-3-2R	R	PR	R	R	R	PM	R	R	PR	–
WCR21	R	–	R	–	R	R	PM	PR	PM	–
IR62037-129-2-3-3-3R	R	–	R	R	–	–	PR	R	–	PR
IR62030-83-1-3-2R	PM	M	M	PR	M	R	PM	PM	PM	PM
IR10198-66-2R	–	PM	R	R	R	PM	PR	–	R	R
IR62171-122-3-2-3-3R	R	R	R	R	R	R	PR	–	R	PM

Continued on next page

Table 1 continued.

	IR68897 A	IR68885 A	IR68886 A	IR68888 A	DRR2A	PUSA 5A	APMS 6A	IR67684 A	IR69616 A	CRMS 32A
IR59673-93-2-3-3R	R	R	R	R	R	PM	R	–	–	R
IR68427-8-3-3-2R	R	PR	R	R	PR	–	R	R	–	PM
IR68926-61-2R	R	PR	R	R	R	R	–	–	–	PM
RR363-1	PR	–	PR	–	–	–	PM	PM	–	M
RR361-3	PR	M	PR	–	–	–	–	–	–	–
RR354-1	M	M	M	M	M	–	PR	–	–	–
RR347-1	M	M	–	PM	M	M	PM	–	–	–
RR348-6	M	–	PM	PM	M	–	–	–	–	–
RR286-1	M	–	–	–	M	–	–	–	–	–
IR61608-213	–	–	M	PR	M	–	–	–	–	–
RR166-645	PM	–	R	R	PR	–	R	–	–	–
RR433-1	–	M	M	M	–	–	PM	–	–	–
RR434-3	–	PM	PM	R	–	–	–	–	–	–
ADT36	–	M	PR	R	M	–	–	–	–	–
ADT39	–	M	M	–	M	PM	–	–	–	PR
ADT43	–	M	M	R	–	–	–	–	–	–
ACK3002	–	PM	–	–	–	–	–	–	–	–
CO 43	–	R	–	–	–	PM	–	–	–	–
TKM11	–	–	–	–	–	–	PR	–	–	–
MDU5	R	PR	PR	PR	PR	–	–	–	–	–

Continued on next page

Table 1 continued.

	IR68897 A	IR68885 A	IR68886 A	IR68888 A	DRR2A	PUSA 5A	APMS 6A	IR67684 A	IR69616 A	CRMS 32A
I.W. Ponni	PR	PR	PR	PM	PM	–	–	PM	–	M
ACK99017	PM	PR	PR	PM	–	–	–	R	–	M
ADO 1259	R	PM	PR	R	–	PM	R	PM	–	R
ADO 1260	PR	R	PR	PM	M	PM	R	PR	–	PM
TP1021	PM	PR	PR	PM	M	PM	–	PM	–	M
CB99019	M	–	–	PM	PM	PM	M	–	–	PR
CB2001105	PR	PM	R	PR	PM	PM	PR	M	–	–

[a] R = restorer, M = maintainer, PR = partial restorer, and PM = partial maintainer, – = crosses not affected.

ditive fashion in restorer genes (Wilson 1968, Padmavathi et al 1997). The presence of these excess sterility genes inhibits pollen fertility restoration in the F_1 generation. Also, it was reported that environment has a major effect on fertility-restoring genes and sterile cytoplasmic genes (Virmani and Edwards 1983, Pandey et al 1990).

Earlier studies revealed that two pairs of major fertility restorer genes in the dominant state (Rf_1 Rf_1 Rf2 Rf_2 or RF_1 rf_1 Rf2 rf_2) impart complete and stable restoring ability in the sporophytic type, whereas, in the gametophytic type of pollen fertility restoration, the pollen is either fully fertile or partially fertile, depending on whether the fertility-restoring gene is homozygous or heterozygous dominant. The restoring ability of these two genes also differed, one being stronger than the other and showing dosage effects (Yang and Lu 1984, Virmani and Wan 1988). Furthermore, partial restoration also occurs if either of the two genes (Rf_1 Rf_1 or Rf_2 Rf_2) is present along with the modifier genes. The action of these genes in pollen fertility restoration is affected by the environment. In such a situation, except in the sporophytic type, single plant selection is suggested to stabilize the lines for fertility restoration.

In our study, the tester IR3883-41-3-2-2-2R behaved as an effective maintainer for CMS line IR68885A and as an effective restorer for CMS lines IR68897A, IR68886A, IR68888A, CRMS32A, IR69616A, DRR2A, Pusa5A, and APMS6A. The tester AD 01260 behaved as an effective maintainer for CMS line DRR2A and as an effective restorer for CMS line APMS6A. The tester AD 01259 was found to be an effective maintainer for CMS line IR67684A and an effective restorer for IR68888A, IR68897A, CRMS32A, and APMS6A. The variations in behavior of fertility restoration indicate that either the fertility-restoring genes are different or that their penetrance and expressivity varied with the genotype of the parent or the modifier of female background.

Among the 300 F_1 hybrids evaluated, 44 combinations showed higher standard heterosis than the check CORH 2 (Table 3). The heterotic hybrids showing more heterosis are IR68897A × IR68926-61-2R (82.3%), IR68897A × IR62036-222-3-3-1-2R (75%), IR68897A × IR59673-93 (60.4%), and APMS6A × IR62124-83-3-2-1R (60%).

When identifying the maintainers, a very low magnitude of pollen fertility was observed among the hybrids. With 43 potential maintainers identified, backcrossing was done with a recurrent parent and seed set was observed in only 23 combinations (Table 2). In the backcross progenies, the plants showing good vigor, high spikelet number per panicle, good panicle stigma exsertion, 100% pollen and spikelet sterility, and high outcrossing rate were selected. Further backcrossing was done and the progenies were included in a conversion program for the development of new nonaromatic CMS lines. New CMS lines developed through substitution backcrossing have been reported by Zaman et al (1996), Abraham et al (1998), and Ingale et al (2004).

The findings of our study reveal that fertility restoration of the cultivars varied with the genetic background of CMS lines. To overcome difficulties in using exotic nonadaptable CMS lines, the maintainer lines identified can be used to induce cytoplasmic male sterility in local germplasm by repeated backcrossing and effective restorers can be used in developing new hybrid combinations for commercial cultivation.

Table 2. Maintainers for different CMS lines.

CMS line	Effective maintainers
IR68897A	RR286 -1
	RR348 -6
	RR347 -1
	CB99019
IR68885A	RR354 -1
	RR433 -1
	ADT36
	IR3883-41-3-2-2-2R
	ADT39
IR6888A	RR286-1
	RR354-1
	ADT43
	IR62030- 83-1-3-2R
	IR61608-213
IR67684A	CB2001105
	IR61614-38-19-3-2R
CRMS32A	TP1021
	ACK99017
DRR2A	ADT36
	AD01260
APMS6A	CB99019

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Table 3. Standard heterosis for grain yield for new cross combinations.

Hybrids	Single plant yield (g)	Standard heterosis (%) over CORH 2
IR68897A/IR68926-61-2R	87.5	82.3
IR68897A/IR62036-222-3-3-1-2R	84.0	75.0
IR68897A/IR59673-93-2-3-3R	77.0	60.4
IR68897A/IR63881-49-2-1-3-2R	63.1	31.5
IR68897A/IR62171-122-3-2-3-3R	59.2	23.3
IR68897A/IR62037-129-2-3-3-3R	55.0	14.6
IR68897A/WCR21	54.3	13.1
IR68897A/MDU5	52.7	9.8
IR68897A/IR72865-94-3-3-2R	50.0	4.2
IR68888A/IR6892661-2R	67.5	40.6
IR68888A/AD01259	67.3	40.2
IR68888A/IR62037-129-2-3-3-3R	63.3	31.9
IR68888A/W216	63.3	31.9
IR68888A/IR72865-94-3-3-2R	60.2	25.4
IR68888A/IR21567-18-3R	60.0	25.0
IR68888A/IR62161-184-3-1-3-2R	58.5	21.9
IR68888A/IR3883-41-3-2-2-2R	54.0	12.5
DRR2A/IR62171-184-3-1-3-2R	59.7	24.4
DRR2A/IR72865-94-3-3-2R	52.0	8.3
DRR2A/IR62161-184-3-1-3-2R	48.0	–
IR69616A/IR62161-184-3-1-3-2R	60.8	26.7
IR69616A/IR62124-83-3-2-1R	58.5	21.9
IR69616 A/IR65489-11-AC2-2R	54.6	13.8
CRMS32A/IR 10198-66-2R	60.8	26.7
CRMS32A/IR 6548911-AC2-2R	55.5	15.6
CRMS32A/WCR 21	50.3	4.8
CRMS32A/IR21567-18-3R	48.0	–
CRMS32A/IR62161-184-3-1-3-2R	48.2	–
IR 68886A/IR62171-122-3-2-3-3R	67.0	39.6
IR68886A/IR68427-8-3-3-2R	60.8	26.7
IR68886A/AD 01260	60.2	25.4
IR68886A/WCR 21	56.0	16.7
IR68886A/IR62171-122-3-2-3-3R	54.3	13.1

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Table 3 continued.

Hybrids	Single plant yield (g)	Standard heterosis (%) over CORH 2
IR68886A/IR21567-18-3R	53.5	11.5
IR68886A/IR 3883-41-3-2-2-2R	50.6	5.4
IR68886A/W 216	50.0	4.2
IR67684A/IR 63079-195-2-2-3-2R	62.6	30.4
IR67684A/IR 65489-11-AC2-2R	57.3	19.4
IR67684A/IR 63881-49-2-1-3-2R	55.8	16.3
IR67684A/IR 72865-94-3-3-2R	54.6	13.8
IR67684A/W 216	54.5	13.5
IR67684A/IR 62036-222-3-3-1-2R	51.2	6.7
PUSA5A/TPI 1021	66.5	38.5
APMS6A/IR62124-83-3-2-1R	76.8	60.0
APMS6A/IR65489-11-AC2-2R	73.3	52.1
APMS6A/IR63079-195-2-2-3-2R	72	50.0
APMS6A/IR62171-122-3-2-3-3R	66.3	38.4
CORH 2 (check)	48.0	

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Notes

Authors' address: Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India. Email: pushpa_parthiban@yahoo.co.in.

Relationship between endogenous hormones and phytic acid concentrations of intersubspecific hybrid rice

Wang Ruo-Zhong, Zhou Min, Ding Jun-Hui, and Xiao Lang-Tao

In order to explain the relationship between endogenous hormones and phytic acid concentrations of intersubspecific hybrid rice, some parameters and variations of endogenous IAA, Z, ZR, and ABA concentrations of grains were studied in this paper, using intersubspecific hybrid rice combinations with different grain plumpness as materials. The results show that the phytic acid concentrations of intersubspecific hybrid rice with higher grain plumpness were expressed very clearly. Correlation analysis indicates that the IAA and Z concentration of grains from florescence to 9 days postflowering is significantly negatively correlated with phytic acid. The ZR and ABA concentration of grains from 12 to 30 days postflowering is significantly negatively correlated with phytic acid. The IAA concentration of poor grain plumpness is significantly negatively correlated with ABA. The IAA concentration of high grain plumpness is very significantly positively correlated with ABA. The ABA concentration of grain in different varieties from 30 to 41 days postflowering is significantly positively or negatively correlated with phytic acid, which is related to endogenous IAA and ABA concentration and their interaction to a certain degree.

Keywords: phytic acid, endogenous hormones, intersubspecies

Phytic acid (myo-inositol 1,2,3,4,5,6 hexakisphosphate $InsP_6$) is the hexaphosphoric ester of hexahydric cyclic alcohol meso-inositol. The molecular formula of phytic acid is $C_6H_{28}O_{24}P_6$, and the structural formula of phytic acid is $C_6H_6[OPO(OH)_2]_6$ (Johnson and Tate 1969). Phytic acid is present in substantial amounts in almost all plant and mammalian cells (Vucenik and Shamsuddin 2003). The applications of phytic acid in medicine, food, and metal anti-oxidation have been widely studied (Midorikawa et al 2001, Graf et al 1987, Katherine et al 1991). Phytic acid is also a major phosphorus storage compound in plant seeds, and the contents are constant (Hantman 1979). In developing seeds, phytic acid can accommodate ionic concentration and distribution to participate in the regulation of vital movement by its effects on the absorption and liberation of metal ions such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, Ba^{2+}, and Zn^{2+} (Batten 1986, Chen and Pan 1977, Strother 1980, Beecroft and Lott 1996). Phytic acid also participates in processes such as cell signal transduction, ATP synthesis, DNA repair, and RNA transport (Voglmafer et al 1996). As an information transfer substance, plant hormones have an important role in regulating rice growth and development, especially in the grain-filling process (Davies 1987, Brenner and Cheikh 1995). Kato et al (1993) observed that ABA content is higher in large grains than in small grains in the rice grain-filling process. Phytic acid and endogenous hormones both participate in the regulation of vital movement. A relationship of phytic acid and grain filling or endogenous hormones and grain-filling has been reported (Wang et al 2003, Wu et al 2007, Xiao et al 2002, Zhao et al 2006), but most research involves grain filling alone and is less involved in the interaction between phytic acid and endogenous hormones. In order to explain the relationship between endogenous hormones and phytic acid in the grain-filling process, some parameters and variations in endogenous hormones and phytic acid concentrations of grains were studied in this paper, using three varieties of rice in field cultivation with different grain plumpness as materials.

Materials and methods

Materials

ISHR1 (intersubspecific hybrid rice combinations with poor grain plumpness), ISHR2 (intersubspecific hybrid rice combinations with general grain plumpness), and ISHR3 (intersubspecific hybrid rice combinations with high grain plumpness) are all supplied from the Hunan Agricultural University Rice Research Institute.

Experimental design

Field cultivation was divided into three areas, in which 3 g of fertilizer was added before transplanting rice in the experimental rice fields in 2007.

Sampling

Sampling was done eight times from the flowering period, at, respectively, florescence (A), 9 d postflowering (B), 12 d (C), 18 d (D), 25 d (E), 30 d (F), 36 d (G), and 41 d (H). Sampling was done at 5 points in field cultivation and basically the same shape of grain ear is chosen at each point three times. The grains are frozen for 30 sec in liquid nitrogen and then freeze-dried. Some will be stored for endogenous hormone determination in a –60 °C refrigerator, and some are fixed in an oven at 105 °C for 30 min, and then shattered for storing in a desiccator for determination of phytic acid content after drying by baking to constant weight at 80 °C for 48 hours.

Methods

(1) Endogenous hormone content was determined by improved high-performance liquid chromatography (Wang et al 2002) and the equipment was an Agilent1100. (2) Phytic acid content was determined (Yuan et al 1997) by using a crushed sample weighing 1 g placed into a triangular flask with the addition of 10% Na_2SO_4-HCl 20 mL, and then oscillating for 1 hour before filtering; finally, filtrate is clarified with extract; the 001×7(732) strong acid cation exchange resin weight 3 g serves as

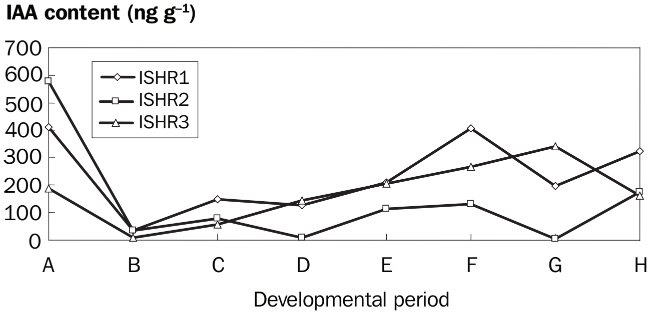

Fig. 1. Comparison of IAA contents among varieties at different developmental periods of grain.

pretreatment before the experiment (Tao 2000). Distilled water is used to soak it for 2 hours, and 0.5 mol L^{-1} NaOH solution with a volume of four times the amount of resin is soaked for 1 hour. Then, 1 mol L^{-1} HCl with the same volume is soaked for 0.5 hour. Finally, the pH of the eluent became neutral.The extract is infused into the ion exchange column (Φ1.6 × 8 cm) and the eluate is collected in a 25-mL volumetric flask. With suction, 5 mL of eluate is added to the reaction of $FeCl_3$-sulfosalicylic acid and centrifuged for 10 min at 3,000 rpm. With suction, the clarified liquid is used to determine the value of extinction with the blank (no eluate added) of distilled water by 500 nm (all the standard sample of phytic acid is from the Nation Medicine Group Limited Company of chemical reagents; the 001×7(732) strong acid cation exchange resin is from the Changsha Dayu Chemical Company).

Results and analysis

Changes in grain endogenous hormone content

Changes in IAA concentration of grains are shown in Figure 1. IAA concentration is high in the initial stage and then drops in the middle and later stages in the rice grain-filling process. Compared with varieties with different grain plumpness, the IAA of ISHR3 is slightly lower than that of the other two varieties (ISHR1 and ISHR2) in the initial stage, but in the later stage (18 days after flowering), ISHR3 shows an upward trend and is slightly higher than the other two.

Changes in Z concentration of grains appear in Figure 2. The Z concentration of ISHR3 is high in the initial stage, but the Z concentration of ISHR2 is as low as that of ISHR1, whose trend is from high to low as appears in ISHR3. Inversely, ISHR1 and ISHR2 show a uniform trend, which is from low to high in the initial stage. Compared to varieties with different grain plumpness, the Z concentration of ISHR1 and ISHR3 reaches a peak at around 18 and 30 days, respectively, but the peak of concentration around 25 days is postponed in ISHR2.

Changes in ZR concentration of grains are found in Figure 3. The ZR concentration of ISHR2 and ISHR3 is high in the initial stage while ZR concentration is low in ISHR1. ZR concentration within the three rice varieties shows a uniform

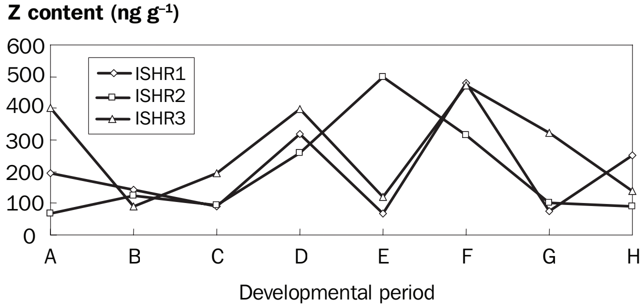

Fig. 2. Comparison of Z content among varieties at different developmental periods of grain.

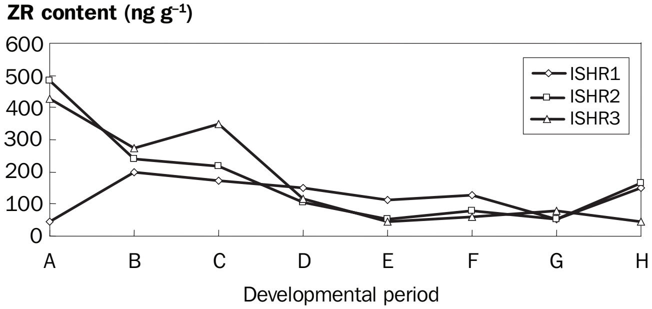

Fig. 3. Comparison of ZR content among varieties at different developmental periods of grain.

trend in that the content gradually declined with the rice grain-filling process from 9 days postflowering. However, from 41 days postflowering, the content in the grain increased slightly in ISHR1 and ISHR2, while it continued to decline in ISHR3.

Changes in ABA concentration of grains appear in Figure 4. The total trend performance in a single plot shows significant differences among species between some variables of the concentrations in the early stage after flowering. ISHR1 continued declining in the initial stage, achieved a minimum 18 days postflowering, and then rose slowly, reaching its peak 30 days postflowering. The content is relatively stable between 30 and 41 days. ISHR2 slows in grain filling early. ABA reached its peak ahead of schedule 12 days postflowering, then declined rapidly, reaching its lowest point 36 days postflowering. Finally, a small rise appeared 36 days postflowering. Grain filling increased rapidly early within ISHR3, the same as in ISHR2. ABA reached its peak at 12 days postflowering, then continued to drop. There was a small rise from 30 to 36 days postflowering, and then a rapid decline. Compared among the three varieties, ABA was maximum in ISHR3, intermediate in ISHR2, and minimum in ISHR3.

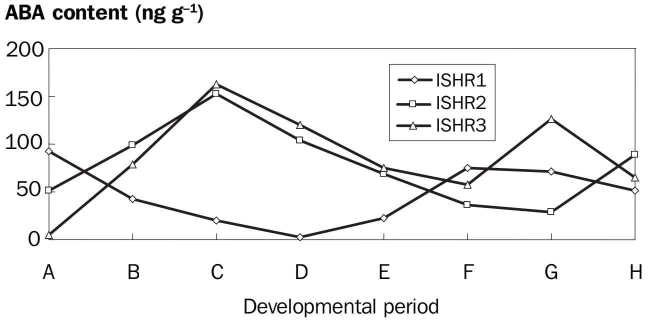

Fig. 4. Comparison of ABA content among varieties at different developmental periods of grain.

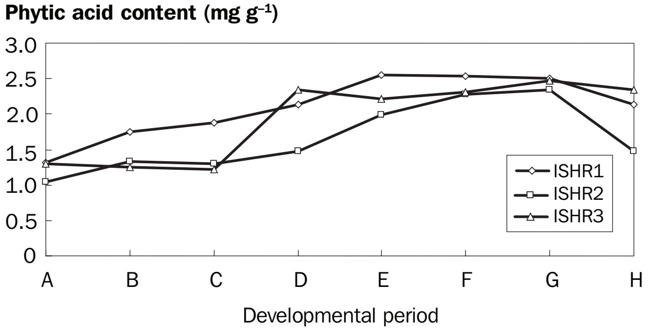

Fig. 5. Comparison of phytic acid content among varieties at different developmental periods of grain.

Changes in grain phytic acid content

Changes in phytic acid concentration of grains are found in Figure 5. The phytic acid content of different varieties shows a uniform trend, which gradually increased in grain development, especially with a rapid rise from 12 to 25 days postflowering. Phytic acid content increased slowly from 25 to 36 days postflowering, and declined from 36 days postflowering. Compared among different varieties, ISHR3 phytic acid content increased more significantly than in ISHR1 and ISHR2 in the entire process of grain filling.

The relationship of phytic acid and grain filling

Table 1 shows correlation analysis results of endogenous hormone and phytic acid content of grains. Both ABA content of ISHR1 and IAA content of ISHR2 are significantly negatively correlated with phytic acid from florescence to 15 days postflowering. From 15 to 30 days postflowering, the ZR content of ISHR1 and ISHR3 and ABA content in ISHR2 are all significantly negatively correlated with phytic acid. From 30

Table 1. Correlation coefficients of the contents of endogenous hormones at different developmental periods of grain with phytic acid content.

Developmental period	Endogenous hormone	ISHR1	ISHR2	ISHR3
A→C	IAA	–0.8680	–1.0000*	0.7532
	Z	–0.9560	0.8954	0.6881
	ZR	0.9231	–0.9850	0.5630
	ABA	–0.9980*	0.7853	–0.9960
C→F	IAA	0.6914	0.7346	0.8189
	Z	0.3169	0.7095	0.4844
	ZR	–0.9820*	–0.7950	–0.9530*
	ABA	0.5501	–0.9680*	–0.8000
F→H	IAA	–0.0450	–0.7460	0.7419
	Z	0.1504	0.4867	–0.0720
	ZR	–0.6450	–0.9880	0.8410
	ABA	0.9974*	–0.9990*	1.0000*

*Significant at 0.05 level.

to 41 days postflowering, the ABA content within ISHR1 and ISHR2 is significantly positively correlated with phytic acid, but ABA content within ISHR2 is negatively correlated with phytic acid content. In comparison with ISHR3, IAA content within ISHR1 shows a negative correlation with phytic acid from florescence to 15 days postflowering and from 30 to 41 days postflowering, but, in the same period, the IAA content of ISHR3 is positively correlated with phytic acid.

Interaction between endogenous hormones

The whole grain-filling process is often coordinated by a variety of hormone regulation and control, not by a single hormone. The results from this study (Table 2) can be seen from 12 to 30 days postflowering, in which IAA and ABA content within ISHR1 are positively correlated, IAA and ABA within ISHR3 have a very significant negative correlation, but the association in ISHR2 is not obvious. The important association of other hormones in the three varieties is not obvious in the entire process.

Discussion

Research indicates that the dynamic changes in hormone levels in different varieties of rice in the process of grain filling are significantly different. When analyzing phytic acid content in the process, we found that it gradually increased with development. This study also found that phytic acid content increased significantly in rice variet-

Table 2. Correlation coefficients of the contents of endogenous hormones during grain development.

Developmental period	Factor	ISHR1	ISHR2	ISHR3
A→C	IAA•Z	0.6829	–0.8830	0.9956
	IAA•ZR	–0.9920	0.9889	0.9677
	IAA•ABA	0.8831	0.8010	–0.6920
	Z•ZR	–0.7690	–0.8040	0.9871
	Z•ABA	0.9730	0.4274	–0.6210
	ZR•ABA	–0.8960	–0.8810	–0.4870
C→F	IAA•Z	0.6600	0.3866	0.4318
	IAA•ZR	–0.5440	–0.2730	–0.9060
	IAA•ABA	0.9796[a]	–0.5420	–0.9920[b]
	Z•ZR	–0.1720	–0.9230	–0.2910
	Z•ABA	0.6105	–0.7350	–0.3150
	ZR•ABA	–0.3880	0.8836	–0.4870
F→H	IAA•Z	0.9808	0.2191	0.6153
	IAA•ZR	0.7927	0.8406	0.9867
	IAA•ABA	0.0265	0.7806	0.7610
	Z•ZR	0.6587	–0.3440	0.4790
	Z•ABA	0.2207	–0.4390	–0.0440
	ZR•ABA	–0.5880	0.9947	0.8556

[a,b]Significant at 0.01 and 0.05 levels, respectively.

ies with high grain plumpness in the entire grain-filling period. This may mean that inorganic phosphorus is reduced by an increase in phytic acid content so that starch synthesis is speeded up by the action of the key enzyme ADP glucose pyrophosphorylase (Plaxton and Preiss 1987). Correlation analysis with the endogenous hormones and phytic acid content of grain shows that IAA, ABA, and phytic acid content relate significantly, and this may be an important role in the regulation of growth played by IAA-glucoside, which consists of the decomposition of inositol phytic acid and natural growth hormone (Stord and Karicanian 1978). In addition, ABA may be able to enhance starch synthesis by increasing the action of ADP glucose pyrophosphorylase and starch-branching enzyme (SBE) (Rook et al 2001), which need further experiments to verify. This study of endogenous hormone relations finds that IAA and ABA content in rice varieties with high grain plumpness are significantly negatively correlated in the intermediate stage of grain filling. In contrast, IAA and ABA content are positively correlated with poor grain plumpness. It seems that IAA and ABA content are closely related to grain development.

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Authors' address: Key Laboratory of Phytohormones, Hunan Agricultural University, Changsha 410128, China; e-mail: wangruoz@163.com.

Development of environment-sensitive genic male sterile lines and two-line hybrid rice

Mou Tongmin, Li Chunhai, Xu Junying, Wu Zhenyong, Tang Wendong, and Cheng Yun

Five environment-sensitive genic male sterile (EGMS) lines and six two-line hybrid rice varieties have been developed and released in the past six years. Hua102S, Hua103S, and Hua104S had very early growth duration, 77–81 days from seeding to heading in the spring season and 58–64 days in the summer season at Wuhan. They were used for developing first-crop two-line hybrids in central China. Hua888S had early growth duration, 83 and 63 days from seeding to heading in the spring and summer seasons, which was used for developing second two-line rice hybrids. Hua201S was a line with medium growth duration, 102 and 73 days from seeding to heading in the spring and summer seasons for developing a single-crop hybrid. Hua201S was photo-thermosensitive, and its critical temperature and photoperiod points of fertility alteration were 23.5 °C and 13.5 h, respectively. Other four-line hybrids were thermosensitive, and their critical temperature points of fertility alteration were 23.5 °C. Hua-liangyou103, Hua-liangyou105, and Hua-liangyou106 had very short growth duration, with 105–110 days from seeding to harvest, and 6.75 t ha^{-1} of yield for the first crop. Hua-liangyou8885 was used for a second crop, with 115 days from seeding to harvest and 7.0 t ha^{-1} of yield. Hua-liangyou1206 was a high-yielding hybrid for one crop and it had 8.6 t ha^{-1} of yield and 135 days of life cycle.

Keywords: two-line hybrid rice, environment-sensitive genic male sterile lines, breeding

A two-line hybrid rice breeding program has been used for more than 25 years since the discovery of photoperiod-sensitive genic male sterile rice reported by Shi (1981). Many environment-sensitive genic male sterile (EGMS) lines and two-line hybrids have been developed in China in the past two and a half decades (Mou et al 2003). The technology of two-line hybrid rice has improved rice quality, increased yield, and enriched the diversity of hybrid rice in China. The area under two-line hybrid rice increased steadily in the past two decades. About one-third of the hybrid rice planting area was occupied by two-line hybrid rice in China in 2007. The area covered by two-line hybrids surpassed the area planted to three-line hybrids in Hubei, Anhui, Henan, and Jiangsu provinces. The techniques for two-line hybrid rice were successful and accepted by more and more seed companies and farmers in China. This paper reports on the performance of five EGMS lines and six two-line hybrids developed by Huazhong Agricultural University in the past six years.

Materials and methods

Breeding EGMS lines

EGMS lines with desirable agronomic traits, which were developed in the past, were used for crossing sterile gene donors with conventional inbred breeding lines or crossing among EGMS lines. The F_1 generation was planted in normal environments. F_2 segregating populations were planted in environments with relatively lower temperature and long daylength, such as mountain areas with an elevation of about 1,000 meters in summer, where the daily mean air temperature in the sensitive stages (25 to 10 days before heading) was around 24 °C (minimum 19 °C, maximum 27 °C). Sterile plants with acceptable agronomic traits were selected at heading stage. The selected plants were ratooned and harvested for selfed seeds in the autumn season. Breeding lines of the F_3 generation were planted under induced fertile environments in the winter season in Hainan Province. Breeding lines of the F_4 generation were treated in the sensitive stage under lower temperature and long daylength conditions in a phytotron. Daily mean temperature was 23.5 °C (minimum 19 °C, maximum 27 °C), and daylength was 14 h. The pollens and spikelets of each plant were investigated under a microscope and bagged. Completely sterile plants were selected and ratooned and harvested for selfed seeds in the autumn season. The breeding lines in F_5–F_7 generations were planted in induced fertile environments.

Breeding lines with desirable and stable agronomic traits in the F_8 generation were sown at intervals of 10 days at different sites in the field. Dynamic changes in fertility/sterility were continuously observed once every two days in the growing season. Promising lines were identified in a phytotron with daily mean temperature of 23.5 °C and 14 h of daylength. The combining ability of EGMS breeding lines was analyzed through a crossing test with male parental lines.

Breeding two-line hybrids

EGMS lines developed with acceptable agronomic traits were used for testing with numerous male parents that included conventional varieties, inbred breeding lines, and other restorer lines of three-line hybrid rice. The heterosis of test crosses was identified in 30 plants from each cross. Promising combinations with heterotic yields and acceptable agronomic traits were selected and re-test crosses made in the next season. Observation yield trials of re-test crosses were made with three check hybrids representing a first, single, and second crop. Yield, growth duration, plant height, panicle number per plant, panicle length, grain number per panicle, seed-setting rate, and 1,000-grain weight were recorded. Rice quality was analyzed. Experimental seed production was made in an isolated area of 300 m^2 for the promising hybrids. Preliminary yield and multilocation trials were carried out in the same year. Promising hybrids with higher yield, better quality, and suitable growth duration went into provincial or national trials. Hybrids with yield, quality, disease resistance, and growth duration that reached the standards in two or three trials were approved by the national or provincial seed agencies and certified and released for commercial cultivation.

Results

Main characteristics, agronomic traits, and rice quality of five EGMS lines

Three of the five EGMS lines were derived from the offspring between EGMS lines and conventional inbred breeding lines, and the other two lines from offspring between EGMS lines (Table 1). Hua102S, Hua103S, Hua104S, and Hua888S have shorter growth duration. Days from seeding to heading were 77–81 in the spring season and 58–64 in the summer season. The main stem had 11.0–12.6 leaves. They can be used for developing first- or second-crop hybrids with growth duration shorter than 115 days. Hua201S has relatively longer growth duration, 102 and 82 days from seeding to heading in spring and summer seasons, respectively. It can be used for developing single-crop hybrids with growth duration longer than 130 days. All five EGMS lines have shorter plant height, 63–82 cm. Hua888S has stronger tillering ability, shorter panicle length, and more dense grains on panicles than other lines. Hua201S has more grains per panicle than other lines (Table 1).

The grain quality of five EGMS lines was analyzed according to the national standard method. Results showed that brown rice was 78.4–80.5%. Head-rice recovery in all five EGMS lines was above 50%. Hua104S had the highest head-rice recovery, reaching 69.4%. The chalkiness and degree of chalkiness (the area × chalky kernels) of Hua102S, Hua104S, and Hua201S were very low. But, Hua103S had a high proportion of chalky grain. The amylose content of Hua201S was low. The other four lines had intermediate amylose content. Hua102S, Hua104S, and Hua888S had medium gel consistency (GC), but Hua103S and Hua201S had soft GC. For grain size and shape, Hua102S and Hua201S had long and slender grain, and Hua103S, Hua104S, and Hua888S had medium grain (Table 2).

Performance of fertility alteration of five EGMS lines in phytotron

When EGMS lines developed in about 10 days after panicle initiation, rice plants were moved into a phytotron for treatment with different temperature and photoperiod. Some 15–18 days later, plants were moved out of the phytotron. The panicles headed 5–10 days after the end of the treatment and were used for investigating pollen fertility performance and bagged for a self-seed-setting test. The results showed that, when the daily mean temperature was higher than 23.5 °C, all five EGMS lines showed complete sterility for pollen and spikelets under different daylengths. This implied that these EGMS lines were mainly sensitive to temperature (Table 3). The critical temperature point (CTP) of fertility alteration was at 23.5 °C or less for daily mean temperature.

Table 1. Pedigree, growth characteristics, and main agronomic traits of five EGMS lines.

Line	Pedigree	Seeding time (day/ month)	50% heading time (day/ month)	Leaves on main stem (no.)	Plant height (cm)	Effective panicles per plant (no.)	Panicle length (cm)	Spikelets per panicle (no.)
Hua102S	810S/IR70	10-IV	27-VI	11.8	65.2	9.0	20.3	141.0
		30-VI	28-VIII	11.4	73.4	8.4	21.2	135.8
Hua103S	AnnongS-1/92153	10-IV	28-VI	12.6	63.6	8.6	20.8	122.3
		30-VI	27-VIII	11.0	70.7	9.4	20.0	127.9
Hua104S	I3S/314	10-IV	29-VI	13.0	73.8	6.8	22.6	126.2
		30-VI	1-IX	12.4	73.6	9.2	19.9	107.4
Hua888S	W9834S/Peiai64S	10-IV	1-VII	12.2	62.6	17.0	17.8	133.5
		30-VI	3-IX	11.6	63.9	10.6	19.1	159.2
Hua201S	W9593S/Peiai64S	10-IV	21-VII	15.0	81.7	8.4	23.8	175.9
		10-VI	1-IX	15.0	79.1	8.0	21.8	149.7

Performance of fertility alteration of five EGMS lines under natural conditions in central and southern China

Five EGMS lines were sown at intervals of 10 days in the rice growing season at Wuhan, located in central China, where rice can grow normally from early April to late September. Lines with short and medium growth duration headed in early June and early July, respectively. The fertility performance of pollen and spikelets was investigated from the beginning of heading to late September for each line. Results showed that all five EGMS lines were completely sterile before mid-September. They became partially fertile in late September, but seed setting was very low. Figure 1

Table 2. Rice quality of EGMS lines.

Item	Hua102S	Hua103S	Hua104S	Hua888S	Hua201S
Brown rice (%)	78.4	80.5	79.0	78.6	79.2
Head rice (%)	63.9	53.7	69.4	52.9	57.1
Chalky kernels (%)	0	89	4	26	6
Degree of chalkiness (%)	0.0	17.8	0.2	1.3	0.6
Amylose content (%)	23.2	19.8	25.5	21.6	12.1
Gel consistency (mm)	50	83	58	48	85
Grain length (mm)	7.0	7.0	6.2	6.1	7.0
Grain shape (length/width ratio)	3.5	2.6	2.8	2.9	3.2

Table 3. Fertility expression of EGMS lines in phytotron.

Line	Identifying conditions		Pollen sterility (%)	Self-seed set (%)
	Lighting time (h day^{-1})	Daily mean temperature (°C)		
Hua102S	14.5	23.5	99.42	0.00
	12.5	28.0	99.63	0.00
Hua104S	14.5	23.5	100.00	0.00
	12.5	28.0	100.00	0.00
Hua201S	14.5	23.5	99.75	0.00
	12.5	28.0	100.00	0.00
Hua888S	14.5	23.5	99.94	0.00
	12.5	23.5	99.91	0.00
		27.5	100.00	0.00
Hua103S	14.5	23.5	–	0.00
	13.5	28.0	–	0.00
		24.0	–	0.21
		23.0	–	0.18
	12.5	28.0	–	0.00
		24.0	–	0.00
		23.0	–	15.03
	11.5	28.0	–	0.00
		24.0	–	0.60
		23.0	–	7.30

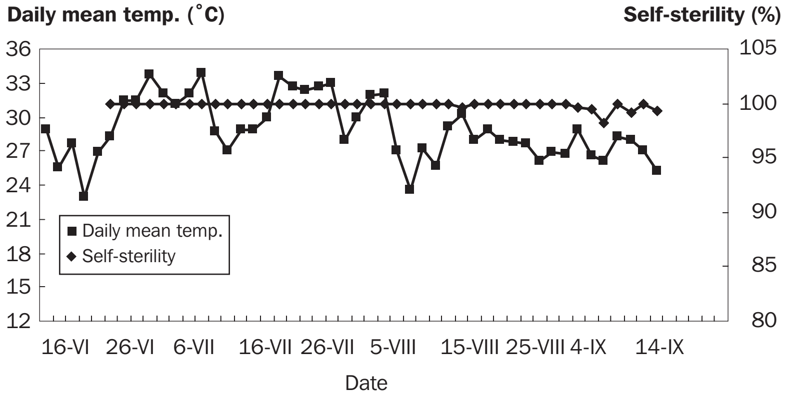

Fig. 1. The self-sterility curve of Hua103S at Wuhan in 2001.

showed the dynamic changes in self-seed setting of Hua103S and daily mean temperature at Wuhan in 2001. Other lines had a similar performance. Mid-August is a desirable period for hybrid seed production in central China because of rare rainfall and temperate climate. These five EGMS lines can be safely used for two-line hybrid seed production in central China. Seed production yields were 2.25–3.0 t ha^{-1} in the past three years. But the yield of seed multiplication was very low because the daily mean temperature was not stably lower than 23.5 °C in mid- to late September.

The plants of five EGMS lines were ratooned annually in Lingshui County of Hainan Island, southern China. Their pollen fertility was investigated under a microscope once every 3 days. The results showed that complete sterility periods of Hua102S, Hua103S, Hua104S, and Hua888S ranged from mid-April to mid-December. January and February were a desirable time for seed multiplication when the lines headed in that period. Pollen fertility surpassed 60%. Multiplication yield reached 3.0 t ha^{-1} on average. Hua201S had a fertility performance different from that of the other four lines. Its sterile period was shorter than that of others, ranging from late May to late October. It showed partial or complete fertility in other periods. The best time for seed multiplication was from mid-March to late April. Figure 2 shows the dynamic changes in pollen fertility alteration of Hua103S and Hua201S in Lingshui County of Hainan Province.

Development of two-line hybrid rice

Six two-line rice hybrids were developed by using five EGMS lines in the past six years. There are three types of rice cultivation system, double cropping (first or early and second or late) and single cropping or monocropping, in the central region of China. The hybrids used for the first crop should have very short growth duration, less than 110 days. The second crop of rice hybrids should mature early, less than 115 days from seeding. Hybrids with 135–150 days of growth can be used for single cropping.

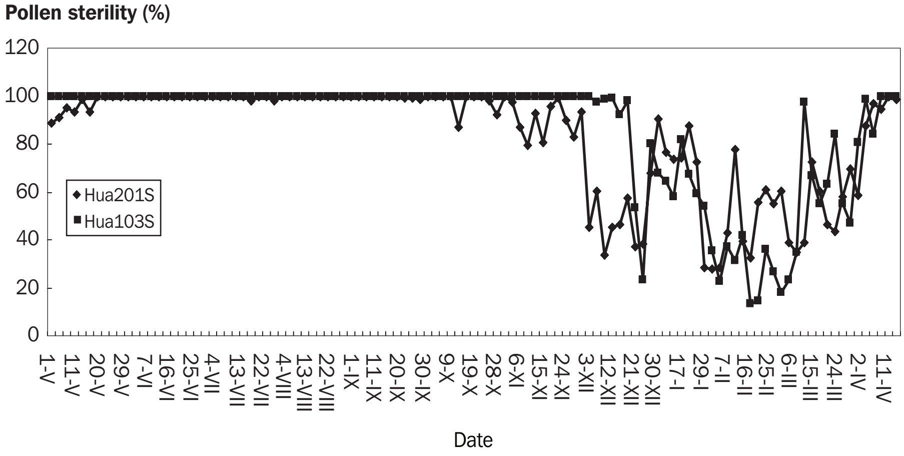

Fig. 2. Fertility performance of Hua103S and Hua201S in 2005-06 at Hainan.

Table 4. Developed two-line hybrids and their pedigree.

Name[a]	Pedigree	Growth duration (life cycle in days)	Released for commercial region and cropping	Certified by
HLY 103	Hua102S/T1007	108	First cropping in Hubei Prov.	Hubei Prov.
HLY 105	Hua103S/T1007	107	First cropping in Jiangxi Prov.	Jiangxi Prov.
HLY 106	Hua103S/T1005	107	First cropping in Jiangxi Prov.	Jiangxi Prov.
HLY 1206	Hua201S/T226	131	Single cropping in central China	Hubei Prov. and nation
HLY 407	Hua104S/Hua107	106	First cropping in Jiangxi Prov.	Being certified
HLY 8885	Hua888S/Hua2185	113	Second cropping in Jiangxi Prov.	Being certified

[a] HLY = Hua Liangyou.

Four of six two-line rice hybrids had very early maturity for first cropping (Table 4). Their growth duration was 106–108 days from seeding to harvest. Hua Liangyou8885 was an early-maturing hybrid for second cropping. Hua Liangyou1206 had long growth duration for single cropping.

The results of regional trials (provincial or national) showed that the average yield of very early hybrids was 6.59–7.30 t ha^{-1}, with an advantage over control

Table 5. Yield and agronomic traits of two-line hybrids.

Name[a]	Average yield (t ha^{-1}) in regional trial	Over check (%)	Plant height (cm)	Panicle number (10,000 ha^{-1})	Spikelets per panicle (no.)	Seed-setting rate (%)	1,000-grain weight (g)
HLY 103	6.59	0.20	91.2	330.0	108.4	83.2	25.6
HLY 105	7.15	4.75	87.8	348.8	100.8	78.3	27.7
HLY 106	6.92	2.40	91.3	354.0	108.6	73.4	27.1
HLY 1206	8.52	4.16	116.6	262.5	159.2	81.9	26.0
HLY 407	7.36	4.89	90.5	361.5	94.9	87.7	25.0
HLY 8885	7.30	5.85	110.0	321.0	120.5	80.2	26.3

[a]HLY = Hua Liangyou.

hybrids by 0.2–4.89%. The average yield of Hua Liangyou 1206 in national trials across six provinces in central China in two years was 8.52 t ha^{-1}, with an advantage over check hybrid II-You838 of 4.16%. The yield of Hua Liangyou 8885 in a second-cropping trial in Jiangxi Province was 7.30 t ha^{-1}, with an advantage over check hybrid Jinyou207 of 5.85%. The yields of first cropping were similar to those of second cropping, but single cropping outyielded first or second cropping (Table 5). Comparisons of agronomic traits among hybrids showed that panicle number per area of hybrids with shorter growth duration was higher than for hybrids with longer growth duration. But spikelets per panicle showed a contrast. Larger panicles were the main reason for higher yield in single-crop hybrids.

The results of rice quality analyses showed that brown rice percentage in different hybrids was similar. But other elements were different in different combinations. Hua Liangyou103 has the highest head-rice percentage. Hua Liangyou103 and Hua Liangyou8885 have lower chalky kernel percentages. Hua Liangyou105 and 106 have lower amylose content. The amylose contents of the other four hybrids were medium. The grain shape, except for Hua Liangyou1206, was slender (Table 6).

Discussion

Environment-sensitive genic male sterile rice, with air temperature and photoperiod during the booting stage controlling the performance of male fertility, is effective and practical for developing a two-line system of hybrid rice. Experience in the past two decades indicated that the critical temperature point of fertility alteration under natural conditions was an important parameter for EGMS lines and could be useful in a breeding program (Mou et al 1998). The daily mean air temperature in indica rice-planting areas worldwide is generally above 24 °C, the maximum is above 28 °C, and the minimum above 20 °C during the booting stage. Therefore, 24 °C of daily

Table 6. Rice quality of two-line hybrids.

Item	HLY103[a]	HLY 105	HLY 106	HLY 1206	HLY 407	HLY 8885
Brown rice (%)	80.5	80.3	79.0	81.7	81.1	79.7
Head rice (%)	65.8	52.9	61.2	60.2	58.8	59.0
Chalky kernels (%)	14	63.5	50.7	77.2	50	16
Degree of chalkiness (%)	2.2	8.0	7.8	15.2	5.0	1.6
Amylose content (%)	22.4	14.0	13.7	22.3	21.9	22.2
Gel consistency (mm)	80	77	86	63.7	68	56
Grain length (mm)	7.5	7.0	7.8	6.2	6.8	7.7
Grain shape (length/width ratio)	3.4	3.1	3.3	2.6	3.0	3.5

[a]HLY = Hua Liangyou.

mean temperature can be a CTP for a practical EGMS line. EGMS lines with 24 °C of CTP can be used for seed production safely in most indica planting areas and they can be easily multiplied in the autumn season in temperate regions or in the winter season in the tropics or by irrigated cold water in the booting stage in the summer season (Mou et al 2003).

The heterosis of rice hybrids with short growth duration used for first cropping in China was not as strong as that of hybrids with long growth duration in the three-line (CMS) hybrid rice in the past four decades in China. The rice quality of hybrids with short growth duration has not been improved. These two problems were solved by two-line hybrids through using EGMS lines with very early maturity and higher rice quality. The area planted to rice hybrids with short growth duration for first cropping increased rapidly in Hubei, Hunan, Jiangxi, and Anhui provinces in central China (Li and Mou 2005, Mou and Li 2005a,b, Zhou et al 2008).

EGMS rice does not need special restorer genes of male parents in hybrid rice breeding. Any normal fertile varieties, including conventional inbreds, local varieties, breeding lines, and CMS restorer lines, can be used as male parents. The frequency for screening heterotic combinations was higher than with CMS hybrids. The effectiveness will be more obvious in a Basmati and japonica hybrid rice breeding program using the EGMS system.

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Authors' address: National Key Laboratory of Crop Improvement, Huazhong Agricultural University, Wuhan 430070, China; email: tongmin58@mail.hzau.edu.cn.

Pyramiding of *xa5*, *xa13*, and *Xa21* genes for developing bacterial blight–resistant maintainers and restorers in hybrid rice

V.P. Khedikar, M.S. Phalak, G.T. Nikhade, S.B. Madse, H.P. Agrawal,
L.P. Aurangabadkar, and V.G. Kashikar

Two high-yielding rice genotypes, AR-9 and AR-15, having a maintainer background with good grain quality and two restorer lines, ARR-84 and ARR-513, with good combining ability were used to develop new maintainer and restorer lines with bacterial blight (BB) resistance. These parents and a donor, ARBN-137, were analyzed for BB resistance using DNA markers and artificial inoculation. The donor parent ARBN-137 was confirmed to possess *xa5, xa13,* and *Xa21* genes in homozygous condition, whereas selected parents were susceptible and did not possess targeted resistance genes.

The F_1s were confirmed to have BB resistance genes and segregating material, advanced through pedigree selection, was evaluated for the presence of BB resistance genes through marker-assisted selection. In each generation, plants with three resistance genes were selected. In the F_5 generation, 58 lines with good agronomic traits were stabilized and were homozygous for all three BB resistance genes. These pyramided lines were evaluated by artificial inoculation with eight diversified *Xanthomonas oryzae* (*Xoo*) isolates collected from different rice-growing regions of India. These lines with three genes in combination were found to provide high resistance against all the *Xoo* isolates tested.

Newly developed pyramided lines were evaluated for their restorer and maintainer status by crossing with a CMS line, IR58025A. Three lines—ARPB-1, ARPB-2, and ARPB-3—were identified as maintainers with more than 99% sterility and desirable grain quality and five lines—ARPR-1, ARPR-2, ARPR-3, ARPR-4, and ARPR-5—were identified as strong restorers with more than 88% fertility and good agronomic traits. These resistant maintainers and restorers with three resistance genes in combination are being used to develop broad-spectrum BB-resistant rice hybrids.

Rice (*Oryza sativa* L.) is a staple food crop of India, providing 43% of the calorie requirement for more than 70% of the Indian population. To meet the demands of increasing population and maintain self-sufficiency, current food grain production needs to be increased by at least 40% by 2020. In this context, hybrid rice is one of the readily adoptable genetic options to increase rice production (Viraktamath et al 2006), as hybrid rice varieties produce 20% higher yield than open-pollinated varieties.

The downside of hybrid rice is that it does not have the resistance required against many pests and diseases. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive diseases of rice worldwide (Ou 1982, Kameswara Rao et al 2003), and is especially destructive in Asian countries. Yield losses caused by *Xoo* are typically from 20% to 30% and can be as high as 50% in some areas of Asia (Ou 1985, Adhikari et al 1995). In India, it has been one of the major production constraints, ever since it first occurred in epidemic proportion in Bihar and other neighboring states of India during 1975. Now, the disease is prevalent in almost all states of the country (Sattar 1996) and in its severe form is known to cause yield losses of 74% to 81% in susceptible cultivars (Veena et al 1996).

Chemical control measures have very limited use in the management of this systemic disease (Devadath 1989). Host-plant resistance is the only safe, economical, and environment-friendly way for the absolute control of this devastating disease (Khush et al 1989). The incorporation of resistance genes into cultivars is the most economical and effective method for controlling this disease (Ogawa 1993). Currently, 30 major genes conferring host-plant resistance against various strains of *Xoo* have been identified and designated as a series from *Xa1* to *Xa29*. This includes 21 dominant resistance genes and 9 recessive genes (Chen et al 2002, Lee et al 2003, Tan et al 2004). Several of these resistance genes have been tagged by closely linked molecular markers (Sonti 1998, Rao et al 2002). Some of these genes have been incorporated into elite rice cultivars by using a traditional breeding approach (Khush et al 1989) and pyramided into breeding lines using marker-assisted selection (Huang et al 1997).

Although many genes for resistance have been genetically defined in rice germplasm (Ogawa and Khush 1989), the effectiveness of resistance genes varies over locations because of geographic structuring of the pathogen. DNA fingerprinting and pathotype analysis have indicated that there is a significant amount of diversity within the population of *Xoo* in India and other rice-growing countries (Adhikar et al 1995, Yashitola et al 1997, Shanti et al 2001, Gupta et al 2001, Singh et al 2003). Studies on pathogen variation revealed that breeding with single major genes for resistance may be ineffective because of a breakdown in resistance. Variability in the pathogen population (Devadath and Padmanabhan 1969, Rao et al 1971) and the prevalence of the most virulent races in tropical regions (Wakimoto 1967) caused further difficulties in developing resistant varieties. Therefore, the genes responsible for different races of *Xoo* need to be pyramided together to make a cultivar with multiple-race resistance, which would be more durable than single-race resistance.

In this investigation, marker-aided selection was employed to develop *xa5*, *xa13*, and *Xa21* pyramided maintainers and restorers in hybrid rice.

Materials and methods

Plant materials

Our study included the following rice lines: (1) AR-9, a short-slender, aromatic, mid-early-duration high-yielding genotype with a maintainer background; (2) AR-15, a short-slender, medium-duration high-yielding genotype with good cooking quality and a maintainer background; (3) ARR-84, a long-slender, medium-duration strong restorer having good combining ability; (4) ARR-513, a medium-slender, mid-early-duration strong restorer; and (5) ARBN-137, a long-slender medium-duration genotype with *xa5*, *xa13*, and *Xa21* genes. AR-9 and AR-15 were used to develop maintainers, ARR-84 and ARR-513 to develop restorers, and ARBN-137 was used as a donor for BB resistance genes *xa5*, *xa13*, and *Xa21*.

Crossing and selection scheme

Four genotypes—AR-9, AR-15, ARR-84, and ARR-513—were crossed with ARBN-137. Parents and F_1s underwent PCR analysis for the presence of *xa5*, *xa13*, and *Xa21* genes. Segregating generations from F_2 to F_5 advanced through pedigree selection underwent marker-aided selection for *xa5*, *xa13*, and *Xa21* genes. Homozygous-positive plants for three genes, in the F_5 generation, were crossed with IR58025A, a most commonly used CMS line with wild-abortive (WA) cytoplasm in order to assess their restorer and maintainer ability. A pollen fertility study was done under a microscope using 1% I-KI stain and seed fertility was studied by bagging of mother panicles.

Marker-assisted selection

Forty-day-old seedlings of the parent and segregating generations were used for DNA isolation and PCR analysis for foreground selection following the procedure of Zheng et al (1995). The three STS markers, RG556, RG136, and pTA248, closely linked to the BB resistance genes *xa5*, *xa13*, and *Xa21*, respectively (Huang et al 1997), were used to confirm the presence of the resistance allele of each gene in each segregating generation.

Screening for BB resistance

Eight *Xoo* isolates were collected from different geographical locations in India in the form of BB-infected leaf samples. The strains were collected from Sakoli and Ramtek (Maharashtra), Raipur (Chhattisgarh), Cuttack (Orissa), Mysore (Karnataka), Bansawara (Rajasthan), Muktasar (Punjab), and Hyderabad (Andhra Pradesh). These strains were designated as *Xoo-1* to *Xoo-8*, respectively. All strains of *Xoo* were isolated and maintained on Hayward's medium. To maintain the virulence, strains used for inoculation were passed through a susceptible host and re-isolated in the lab before inoculation. Forty-day-old plants were clip inoculated (Kaufmann et al 1973) with bacterial suspension of 10^9 cfu mL^{-1}, prepared from 48-h-old cultures. The top five leaves of at least ten randomly selected plants per entry were inoculated at maximum tillering stage. Observations were recorded 15 days after inoculation and mean lesion lengths were calculated. Plants were classified as resistant when the mean lesion length

Table 1. Evaluation of parents for BB resistance genes *xa5, xa13*, and *Xa21* using marker-assisted selection and artificial inoculation.

Breeding line	BB resistance genes[a]			Isolates/lesion length (cm)								Mean lesion length (cm)
	xa5	*xa13*	*Xa21*	*Xoo-1*	*Xoo-2*	*Xoo-3*	*Xoo-4*	*Xoo-5*	*Xoo-6*	*Xoo-7*	*Xoo-8*	
AR-9	A	A	A	18	14	29	26	27	15	28	18	21.8
AR-15	A	A	A	16	13	28	24	26	14	29	19	21.12
ARR-84	A	A	A	17	12	26	24	27	16	29	19	21.25
ARR-513	A	A	A	16	14	28	27	29	19	30	21	23.0
ARBN-137	P	P	P	0.5	0.5	4.0	3.0	3.5	1.0	4.0	1.5	2.25
TN-1 (SC)	A	A	A	24	23	35	33	32	25	36	25	29.12

[a]A = absent, P = present, SC = susceptible check.

ranged from 0 to 4 cm and plants with mean lesion length of >4 cm were considered as susceptible (Shanti et al 2001).

Evaluation of pyramided elite lines

Five competitive plants from each pyramided line were randomly selected to record observations for yield and yield-contributing traits. Grain quality of the pyramided lines was evaluated for several physico-chemical characteristics by following standard procedures (Little et al 1958, Beachell and Stansel 1963, Juliano 1971, IRRI 2002).

Results and discussion

PCR analysis revealed an absence of *xa5*, *xa13*, and *Xa21* genes in the parents, AR-9, AR-15, ARR-84, ARR-513, and susceptible check TN-1. All the parents showed a susceptible reaction with mean lesion length ranging from 21.12 to 23 cm, while TN-1 had mean lesion length of 29.12 cm. Donor parent ARBN-137 contained all three BB resistance genes in homozygous condition, with mean lesion length of 2.25 cm (Table 1).

F_1s were heterozygous for all three resistance genes. PCR analysis of four F_2 populations identified 55 plants with *xa5*, *xa13*, and *Xa21* genes. All these plants with a three-gene combination in homo- or heterozygous conditions were advanced to the F_5 generation through pedigree selection. In the F_5 generation, 58 homozygous plants with a three-gene combination (32 from the maintainer development program and 26 from the restorer development program) were selected. These homozygous-positive plants were crossed with IR58025A to assess their restorer and maintainer ability. Out of 32 crosses, three showed high sterility (>99%), whereas, out of 26 crosses, five showed more than 88% fertility. Thus, from a total of 58 pyramided plant progenies

Table 2. Evaluation of pyramided lines for BB resistance genes *xa5, xa13,* and *Xa21* using marker-assisted selection and artificial inoculation.

Pyramided line	BB resistance genes[a]			Isolates/lesion length (cm)								Mean lesion length (cm)
	xa5	*xa13*	*Xa21*	*Xoo-1*	*Xoo-2*	*Xoo-3*	*Xoo-4*	*Xoo-5*	*Xoo-6*	*Xoo-7*	*Xoo-8*	
ARPB-1	P	P	P	1.5	1.5	3.5	3.0	3.0	1.0	4.0	2.0	2.43
ARPB-2	P	P	P	1.0	2.0	4.0	3.0	3.0	1.5	3.0	1.0	2.31
ARPB-3	P	P	P	0.5	0.5	3.5	3.0	3.5	1.5	3.0	1.5	2.12
ARPR-1	P	P	P	1.5	1.0	3.0	3.0	3.0	2.0	3.5	2.0	2.37
ARPR-2	P	P	P	1.0	1.5	4.0	4.0	3.0	2.0	3.0	1.5	2.50
ARPR-3	P	P	P	1.0	2.0	4.0	3.5	3.0	1.5	4.0	2.0	2.62
ARPR-4	P	P	P	1.5	1.5	4.0	3.5	3.0	2.0	4.0	2.0	2.68
ARPR-5	P	P	P	1.5	1.5	3.0	3.5	3.5	1.5	3.0	2.0	2.43
ARBN-137	P	P	P	0.5	0.5	3.0	3.0	3.0	1.5	4.0	1.0	1.87
TN-1 (SC)	A	A	A	22	21	34	31	33	23	35	22	27.62

[a] A = absent, P = present, SC = susceptible check.

with a three-gene combination, three, ARPB-1, ARPB-2, and ARPB-3, were identified as good maintainers and five, ARPR-1, ARPR-2, ARPR-3, ARPR-4, and ARPR-5, were identified as restorers (Table 3).

These newly developed pyramided maintainers and restorers, when undergoing artificial inoculation with eight different *Xoo* isolates, showed a resistant reaction against all the isolates tested, exhibiting mean lesion length of less than 4 cm vis-à-vis susceptible check TN-1 (27.62 cm) (Table 2). The pyramided maintainers and restorers were evaluated for yield, agronomic traits, and quality traits (Tables 4 and 5). The most promising maintainer, ARPB-3, with desirable agronomic and quality traits, underwent CMS conversion. Restorers will be used as pollen parents for developing new bacterial blight–resistant hybrid combinations.

Hybrids obtained using these newly developed pyramided maintainers and restorers will provide durable and high resistance over different rice-growing areas in India. Since the resistance genes have been introgressed in a background for high yield and good quality, this will ensure hybrids with high yield and good grain quality. Sanchez et al (2000), in their study, observed that lines with *Xa21* generally had increased resistance when combined with either *xa5*, *xa13*, or both, compared with lines with *Xa21* alone. Huang et al (1997) also observed a similar reaction, wherein lines with multiple resistance genes in an IR24 background generally showed higher resistance than what was expected from a single resistance gene. Sundaram et al (2008) introgressed *xa5*, *xa13*, and *Xa21* in widely grown variety BPT 5204, popularly known

Table 3. Restoration confirmation of pyramided lines.

Pyramided lines	Pollen fertility % using I-KI stain	Seed set % using bagging method	Results
IR58025A × ARPB-1	0.18	0.50	Maintainer
IR58025A × ARPB-2	0.27	0.12	Maintainer
IR58025A × ARPB-3	0.11	0.00	Maintainer
IR58025A × ARPR-1	90.25	92.60	Restorer
IR58025A × ARPR-2	92.20	90.80	Restorer
IR58025A × ARPR-3	89.60	91.00	Restorer
IR58025A × ARPR-4	91.35	93.20	Restorer
IR58025A × ARPR-5	88.15	92.40	Restorer

Table 4. Agronomic traits of pyramided lines.

Pyramided line	Yield per plant (g)	Days to 50% flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Grains panicle^{-1}	No. of tillers plant^{-1}
ARPB-1	32.0	79	116	98	29.3	276	18
ARPB-2	30.5	76	115	100	28.5	270	15
ARPB-3	33.5	101	135	93	30.0	285	19
ARPR-1	33.8	95	130	108	29.5	250	17
ARPR-2	38.0	108	138	120	31.5	274	24
ARPR-3	36.5	92	122	110	29.4	298	20
ARPR-4	37.5	108	133	118	30.0	268	23
ARPR-5	34.0	88	120	112	28.5	265	28

as Samba Mahsuri, and observed broad-spectrum resistance against six different *Xoo* strains from India.

Our investigation demonstrated that marker-assisted selection can be efficiently used for the introgression of BB resistance genes into backgrounds with different yield and quality. In addition, pyramided lines with desirable yield and quality parameters could be used as prebreeding donor lines for the transfer of resistance in many rice-breeding programs. These newly developed maintainers and restorers will also produce bacterial blight–resistant, high-yielding, and good-quality hybrids.

Table 5. Quality traits of pyramided lines.[a]

Pyramided line	Test weight (g)	Milling (%)	Head rice recovery (%)	Kernel length (mm)	Kernel breadth (mm)	L/B ratio	Grain type	Grain chalkiness	ASV	Amylose content (%)	Gel consistency (%)
ARPB-1	14.8	72.0	65.5	5.64	1.69	3.34	SS	VOC	5	21.8	65
ARPB-2	15.1	71.6	66.3	5.29	1.58	3.35	SS	A	5	22.5	60
ARPB-3	14.2	73.0	68.4	5.73	1.61	3.56	SS	A	6	23.6	58
ARPR-1	18.7	69.6	65.0	6.25	1.85	3.38	LS	VOC	5	23.1	57.4
ARPR-2	20.7	72.3	66.4	6.87	2.07	3.32	LS	VOC	6	22.6	66
ARPR-3	19.9	74.3	64.6	5.59	2.21	2.53	MS	A	6	22.2	65.5
ARPR-4	20.5	71.5	70.8	5.58	2.16	2.58	MS	A	5	21.6	52
ARPR-5	19.8	73.5	69.2	5.90	2.13	2.77	MS	VOC	6	21.5	61

[a] SS = short slender, LS = long slender, MS = medium slender, L/B ratio = length and breadth ratio, ASV = alkali spreading value, VOC = very occasional chalkiness present, A = absent.

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Notes

Authors' addresses: V.P. Khedikar, M.S. Phalak, G.T. Nikhade, S.B. Madse, L.P. Aurangabadkar, and V.G. Kashikar, Research and Development, Ankur Seeds (P) Limited, 27, New Cotton Market Layout, Nagpur-440018, India. Email: vpkhedikar@ankurseeds.com, vinodkhe2002@rediffmail.com; H.P. Agrawal, senior scientist, T.B.C. College of Agriculture, Bilaspur, (C.G.), India.

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A study on population improvement and genetic risk control in thermo-sensitive genic male sterile rice

Wu Xiaojin and Yuan Longping

Thermo-sensitive genic male sterile (TGMS) rice lines are the key for breeding two-line hybrid rice. Improvement in general combining ability had been stagnant for a long time since the development of first-generation CMS lines. To overcome this stagnation, population improvement, or random poly-crossing, was used in our study. We concluded that population improvement can help comprehensively improve important characters such as combining ability, grain quality, resistance, and stigma exsertion and combine desirable characters together, thus enriching the variation in a breeding population and pyramiding the favorable genes controlling a single character. Especially, an elite TGMS line, ZhunS, had been developed and it performed very well. The stigma exsertion rate of ZhunS is 78.6%, 72.4% higher than that of V20A. Hybrids crossing ZhunS with R402, Minghui 77, and Minghui 63 yielded 3.8–5.8% higher and decreased chalkiness by 82.1–84.4% compared with those crossing V20A with the corresponding restorer lines.

There is a risk in exploiting hybrid rice in a TGMS system because sterility is controlled by temperature. However, this risk can be significantly minimized by using genes responsible for decreasing the sterility-inducing temperature.

Keywords: *Oryza sativa* L., male sterility, TGMS system, population improvement, risk control, heterosis

Important improvement in enhancing rice yield potential occurred twice in the late part of the 20th century: with the development of semidwarf rice varieties and the use of rice heterosis. However, yield potential had stagnated for a relatively long time since the release of the first hybrid rice variety, Nanyou No. 2, in 1976 in China. Therefore, how to break the yield ceiling of hybrid rice, especially how to enhance actual yield in commercial production, has been a strong concern of most hybrid rice breeders.

One reason for yield stagnation was that the relationship among maintainers was relatively close in the cytoplasmic male sterility system then used and the combining ability of the newly developed cytoplasmic male sterile (CMS) lines had not been improved compared with Zhenshan 97A or V20A, the representative CMS lines of the first generation released in 1974 (Wang and Xu 1996). To improve the combining ability of the maternal parents of hybrid rice, we tried thermo-sensitive gene–assisted population improvement and developing hybrid rice in a TGMS system. In this article, we present the results and progress in this research area.

Screening for a TGMS source suitable for population improvement

There are four major sources of photosensitive or thermo-sensitive male sterile rice in China: Nongken 58S, Annong S-1, Hengnong S-1, and 5460S. After a practical test for many years, Annong S-1 and its derivatives have been confirmed as suitable for developing usable TGMS lines (most of the usable TGMS lines released for commercial production are derived from Annong S-1) and therefore as genetic tools for population improvement. First, the thermo-sensitive male sterility of Annong S-1 is controlled by a single recessive gene (Wu and Yin 1992), and there are 25% sterile plants versus less than 5% sterile plants for Nongken 58S in F_2 segregating populations. Second, the critical fertility-sterility alteration–inducing temperature expresses differently when the TGMS gene is introduced into different genetic backgrounds or breeding materials, offering a broad genetic basis for developing usable TGMS lines (Wu and Yin 1997), but Hengnong S-1 and 5460S do not follow this pattern.

Procedures of TGMS line improvement

TGMS line improvement is done by crossing a TGMS line with different parental lines with desirable and complementary characters for a breeding program, then mixing and planting the F_2 seeds under sterile conditions, that is, at high temperature during the critical fertility-sterility alteration period, or from the pollen mother cell to meiosis stage. A small amount of GA_3 can be sprayed and artificial pollination carried out during the flowering period of the majority of the plants in the TGMS line to promote random poly-crossing. In the mature period, seeds of both desirable sterile and fertile plants should be harvested and mixed for the next cycle, but many more sterile plants should be selected than fertile plants. Practically, there are two procedures of TGMS line improvement: continuous random poly-crossing (procedure 1) and random poly-crossing followed by mass selection (procedure 2) (Fig. 1).

Using continuous random poly-crossing to develop TGMS lines

With the help of wide compatibility genes, we selected seven indica and seven japonica varieties or lines as parents to develop TGMS lines through continuous random poly-crossing with Annong S-1 as a genetic tool. A TGMS line with good combining ability, 133S has been developed. In a multilocation yield trial organized by the agricultural department of the government, its hybrid, 133S/Minghui 63, significantly outyielded the check hybrid Zhenshan 97A/Minghui 63 by 8.8% in total yield and by 13.1% in daily yield (Table 1), thus overcoming the stagnation in combining ability of male sterile lines with Minghui 63 as the same tester in multilocation yield trials (Wang

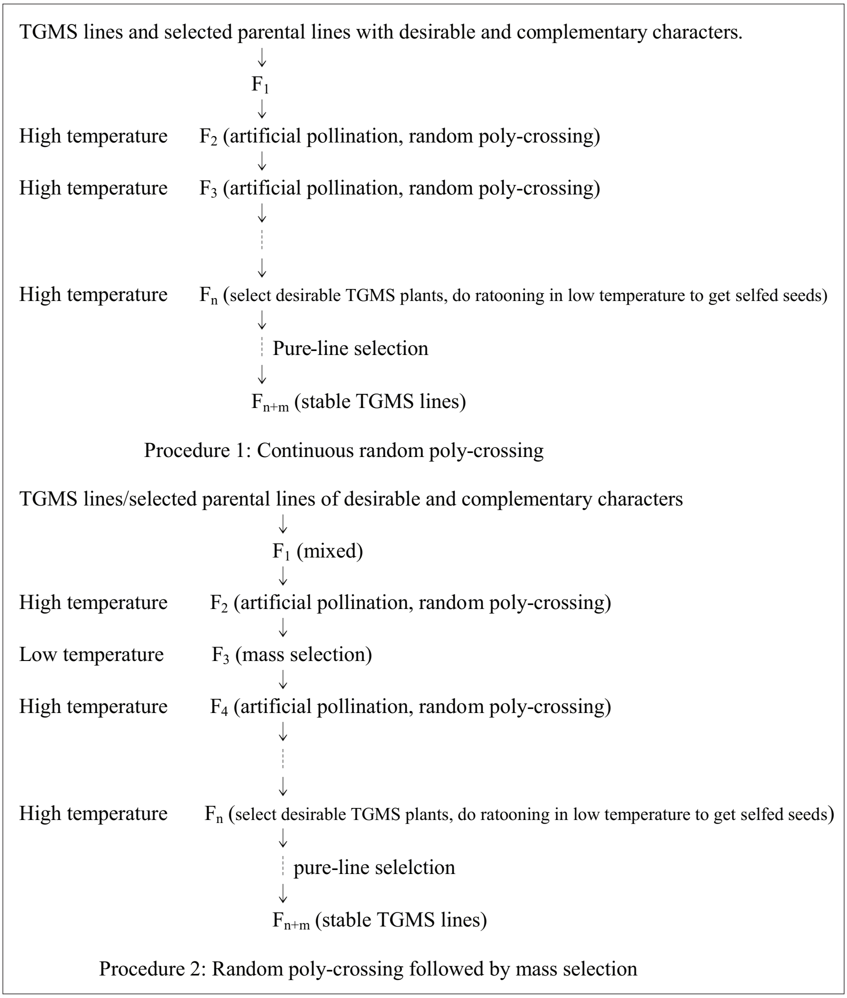

Fig. 1. Procedures of TGMS line improvement.

and Xu 1996). It also performed much better than the check in resistance to the two major diseases, blast and bacterial leaf blight, and in chalkiness and head-rice recovery (Table 2). The reason for the improved combining ability of 133S is that some genes or characters of japonica rice have been successfully introgressed into the S line, which is basically an indica type, but it possesses some typical characters of japonica. The introgressed characters or genes have made the genetic diversity of the two parents wider in 133S/Minghui 63 than in Zhenshan 97A/Minghui 63 and the hybrids of other CMS lines crossing with Minghui 63, therefore resulting in better heterosis.

Table 1. Performance of hybrids when crossing various male sterile lines with the same tester (Minghui 63) in multilocation yield trials organized by the Agricultural Department.

Male sterile line	Hybrids	Checks	Performance advantage (%)
Maxie A	Maxie A/Minghui 63	Zhenshan97A/Minghui 63	0
D shan A	D shan A/Minghui 63	Zhenshan97A/Minghui 63	0
Aihong 9 A	Aihong 9 A/Minghui 63	Zhenshan97A/Minghui 63	0
Hong I A	Hong I A/Minghui 63	Zhenshan97A/Minghui 63	0
Simiao A	Simiao A/Minghui 63	Zhenshan97A/Minghui 63	+1.80
133S	133S/Minghui 63	Zhenshan97A/Minghui 63	+8.83

Source: Wang and Xu (1996).

Table 2. Comparison between 133S/Minghui 63 and Zhenshan97A/Minghui 63 in daily yield in multilocation yield trials, resistance to blast and bacterial leaf blight (BLB), head rice recovery, and chalkiness.

Character	133S/Minghui 63	Zhenshan97A/ Minghui 63	Advantage (%)
Daily yield (kg ha^{-1})	66.00	58.35	+13.11
Blast (grade)	3 (moderately resistant)	7 (susceptible)	
BLB (grade)	3 (moderately resistant)	7 (susceptible)	
Head-rice recovery	56.67%	48.45%	+16.97
Chalkiness	7.8%	24.6%	–68.29

Comparing random poly-crossing followed by mass selection and absolute mass selection

In 1991, we used Huaizao 4 (having good combining ability and resistance), Xiang-II-B (having good combining ability and eating quality), and Zaoyou-1 (good quality) as parents with N-8S (derived from Annong S-1 and possessing better combining ability and quality than Annong S-1) as a genetic tool to practice population improvement and compare it with mass selection. Three kinds of breeding populations were obtained: S_1 (absolute mass selection), C_1S_1 (one cycle of random poly-crossing followed by mass selection), and C_2S_1 (two cycles of random poly-crossing followed by mass selection). The objective for selection was good outcrossing and agronomic traits, apparent quality, plant type, and other characters relative to combining ability on the basis of breeding experience. It was found that population improvement was superior to mass selection in improving these characters (Wu and Yuan 1998). To improve the percentage of exserted stigma, one of the most important outcrossing characters, population

Table 3. Mean and variation of percentage of exserted stigma in different populations (1994, Changsha).

Population	Mean		Range of variation		Variation	Coefficient of variation
	Sin$^{-1}\sqrt{□}$	□%	Sin$^{-1}\sqrt{□}$	□%		
S_1	47.07	53.6	37.01–56.62	36.2–70.0	993.25	66.96
C_1S_1	48.85	56.7	38.20–59.56	38.2–74.3	1,134.10	68.94
C_2S_1	49.65	58.1	36.59–61.09	35.5–76.6	1,157.67	68.53

improvement was significantly better than mass selection. On average, the percentage of exserted stigma was 8.4% higher in C_2S_1 than in S_1 (Table 3), and also more plants had better stigma exsertion than the check V20A in C_2S_1 than in S_1. The best plants had 76.6% stigma exsertion in C_2S_1 versus 70% in S_1 (Table 4). Population improvement was also superior to mass selection in improving comprehensive outcrossing characters (Table 5). The chance to obtain a desirable percentage of exserted stigma (PES$\geq$ 65%), percentage of spikelets enclosed in the sheath (PE$\leq$30%), and percentage of cleistogamous spikelets (PC$\leq$5%) was higher in C_2S_1 than in S_1.

From 1994 on, we selected promising TGMS lines in S_1, C_1S_1, and C_2S_1 by the pure-line method to obtain good outcrossing and agronomic traits, apparent quality, plant type, and other characters relative to combining ability on the basis of breeding experience. The 20 best TGMS lines were selected in each population to test-cross Minghui 63, Minghui 77, and R402 using V20A as a check male sterile line. It was found that the lines showed better combining ability for yield in C_2S_1 than in S_1, on average (Wu and Yuan 2004). The hybrids derived from C_2S_1 lines crossed with testers were better than those hybrids derived from C_1S_1 lines crossed with testers; the latter were also better than those with S_1 lines crossed with testers (Table 6). For average yield, hybrids from C_2S_1 lines yielded 4.85–10.14% higher than those hybrids from C_1S_1 hybrids, 10.09–13.11% higher than the hybrids from S_1 lines, and 0.1–1.95% higher than check hybrids derived from V20A. For the highest yield, C_2S_1 hybrids yielded 2.84–6.15% higher than the C_1S_1 hybrids, 5.54–10.17% higher than the S_1 hybrids, and 4.46–5.55% higher than the V20A hybrids. The same effect was found for improving chalkiness (Table 7). For average chalkiness, C_2S_1 hybrids had 10.49–16.28% less chalk than the C_1S_1 hybrids, 18.64–35.40% less than the S_1 hybrids, and 49.83–69.75% less than the V20A hybrids. For the least chalkiness, C_2S_1 hybrids had 14.29–32.81% less than the C_1S_1 hybrids, 30.43–43.42% less than the S_1 hybrids, and 79.49–85.12% less than the V20A hybrids. For breeding purposes, when we set criteria for TGMS lines that their percentages of exserted stigma should be higher than 75%, at least one tester yields 5% higher than V20A hybrids with the same tester. For chalkiness, at least one hybrid with a tester was less than 5%, and only one line in C_2S_1 met these criteria. This line was designated as Zhun-S. It had 78.6% exserted stigma and the hybrid crosses with testers R402, Minghui 77, and Minghui

Table 4. Frequency of plants with exserted stigma (equal to or more than V20A) in different populations (1994, Changsha).

Population	Percentage of exserted stigma (%)												Total plants observed
	40.0–49.9		50.0–59.9		60.0–64.9		65.0–69.9		70.0–74.9		75.0–79.9		
	No. of plants	Frequency (%)	No. of plants	Frequency (%)	No. of plants	Frequency (%)	No. of plants	Frequency (%)	No. of plants	Frequency (%)	No. of plants	Frequency (%)	
S_1	35	29.17	56	46.67	13	10.83	11	9.17	1	0.83	–	–	120
C_1S_1	19	15.83	36	30.00	28	23.33	28	23.33	6	5.00	–	–	120
C_2S_2	25	20.83	40	33.33	21	17.50	25	20.83	5	4.17	3	1.67	120
V20A	15	100											15

Table 5. **Number of plants with three desired outcrossing characters in various populations (1994, Changsha).**

Population	No. of plants				
	PE[a]≤30%	PC≤5%	PE≤30% PES≥65%	PC≤5% PES≥65%	PC≤5% PE≤30% PES≥65%
S_1	97	102	9	11	7
C_1S_1	99	83	29	26	20
C_2S_2	107	71	27	26	23

[a]PES = percentage of exserted stigma, PE = percentage of spikelets enclosed in the sheath, PC = percentage of cleistogamous spikelets.

Table 6. Yield performance of hybrids in crossing of three testers with lines from different population improvement methods (1997, Changsha).

Male	Female						Hybrids of V20A (kg m^{-2})
	Average (kg m^{-2})			Highest (kg m^{-2})			
	S_1	C_1S_1	C_2S_1	S_1	C_1S_1	C_2S_1	
R402	0.813	0.842	0.895	0.846	0.878	0.932	0.883
Minghui 77	0.804	0.845	0.886	0.849	0.882	0.927	0.885
Minghui 63	0.963	0.996	1.097	1.065	1.093	1.124	1.076

Table 7. Performance for chalkiness of hybrids in crossing of three testers with lines from different population improvement methods (1997, Changsha).

Male	Female						Hybrids of V20A (%)
	Average (%)			Minimum (%)			
	S_1	C_1S_1	C_2S_1	S_1	C_1S_1	C_2S_1	
R402	17.8	16.2	14.5	7.6	6.4	4.3	28.9
Minghui 77	10.2	8.6	7.2	6.9	5.6	4.8	23.4
Minghui 63	11.3	8.7	7.3	6.6	5.7	4.2	23.8

Table 8. Yield performance of Zhunliangyou 527 (Zh527, Changsha).

Year	Yield (t ha^{-1})			Yield advantage (%)	
	Zhunliangyou 527	V5 27	Shanyou 63	V5 27	Shanyou 63
2000	10.09	8.77	8.51	15.1	18.5
2001	9.87	8.86	8.92	11.4	10.7
2002	9.96	8.45	8.83	17.9	12.8

Table 9. Resistance and quality performance of Zhunliangyou 527 (2001).

Combination	Blast (grade)[a]	Bacterial leaf blight[b]	Percentage of panicles damaged by stem borer[c]	Head rice (%)[d]	Chalkiness (%)[d]	Amylose content (%)[d]
Zh527	2	5	3.6	58.3	7.4	23.9
V527	2	–	28.7	47.1	28.6	22.8
Shanyou 63	5	7	27.9	49.4	21.5	23.3

[a] Induced result at Chenxi, Hunan. [b] Induced result at Anren, Hunan. [c] Natural result with 3 replications in Changsha, Hunan. [d] Sample of single season in Changsha, Hunan, in 2001.

63 had 4.5%, 4.8%, and 4.2% chalkiness and yielded 5.8%, 4.2%, and 3.8% higher than crosses of V20A with R402, Minghui 77, and Minghui 63, respectively.

Zhun-S was used for testcrossing with nearly 300 parental lines to screen the most promising hybrids for commercial production. The testcross Zhun-S/R527, named as Zhunliangyou 527, performed very well. It outyielded V-you-527 (V20A/R527) by 11.4–17.9% and the commercial check hybrid Shanyou 63 (Zhenshan-97A/Minghui 63) by 10.7–18.5% (Table 8), with the same growth duration as the two check hybrids. Its resistances to blast, bacterial leaf blight, and stem borer; head-rice recovery; and chalkiness were also improved (Table 9). In addition, it yielded more than 12 t ha^{-1} at four different locations in Hunan and Guizhou provinces on areas of more than 6.7 ha, meeting the criterion for the second phase of development of super rice in the research program launched by the Chinese Ministry of Agriculture in 1996.

Male sterility–assisted population improvement was practiced in self-pollinating crops such as soybean and wheat and suggested for rice by Khush in 1984. In our research, we concluded that TGMS–assisted population improvement has three advantages. First, it can help pyramid the favorable genes controlling a single character. For example, both the average and highest percentage of exserted stigma were higher in poly-crossing followed by mass selection than in an absolute mass selection population under the same selection pressure, and the more cycles of poly-crossing, the better the effect of improvement. Second, it can help comprehensively improve important characters such as combining ability, grain quality, resistance, stigma exsertion, etc., and combine the desirable characters together. As was shown in the above results, the chance was greater to obtain good plants with comprehensively improved outcrossing characters or good lines with improved yield-combining ability, resistance, and grain quality, which are higher in poly-crossing followed by mass selection than in an absolute mass selection population under the same selection pressure. Third, it can help to increase variation in the breeding population, as Table 3 shows.

Population improvement or recurrent selection was suggested to improve a single character controlled by micro-genes, but this would result in worsening of other important characters (Misevic and Alexander 1989). In our research, TGMS line improvement was exploited to improve comprehensively important characters. However, depending on the breeding objective, population improvement should be used for improving a single character or comprehensively important characters. If the objective is to create excellent germplasm with a single character, population improvement should target a single character and the basic population should be composed of crosses among parental lines excellent in this character. If the objective is to develop a commercially usable variety or parental line of hybrid rice, population improvement should target comprehensively important characters and the basic population should be composed of crosses among parental lines excellent and complementary in the targeted characters.

Genetic control of risk in using TGMS rice

Compared with the cytoplasmic male sterility system now commercially used in hybrid rice production, the TGMS system has four advantages: (1) no need for a maintainer, (2) freedom of pollen parent choice, (3) higher yield of S-line seed production, and (4) reduced genetic vulnerability due to a single cytoplasmic source (Yuan 1987). However, the fertility of TGMS source lines is influenced by temperature and its minimum sterility-inducing temperature is relatively high, with the result that there is a risk of obtaining selfed seeds of the S line if the system is used for hybrid seed production in regions with daily mean temperatures below 26 °C (Yuan 1992). Finding genetic strategies to reduce this risk is a priority for hybrid rice breeding programs deploying TGMS systems. The use of marker genes has been suggested to demonstrate purity, and TGMS lines with recessive genes controlling leaf color were developed (Dong et al 1995, Shu et al 1995). Using this strategy, TGMS line N8S with colorless sheath was developed, and another line, Xiang 2B with purple sheath, was used as the pollen parent. The hybrid N8S/Xiang2B showed a purple sheath. This strategy was practiced in 1992 and 1993, but proved to be labor-intensive and hence difficult to implement. The following strategy was proposed to reduce the risk of contamination of hybrid seed with selfed seed of the female parent.

Annong S-1 contains a recessive gene that causes male sterility if the mean daily temperature is above 25 °C during the 11–15 days before heading, but at temperatures below 22 °C pollen fertility is high (Yin and Wu 1990, Wu and Yin 1992). Because the *ts* gene is stable, it has been widely used as a donor for developing new TGMS lines (Yuan 1992, Wu et al 1992). It was found in 1990 that plants randomly selected in

F_2 populations showed different fertility alterations at the critical temperature range, which indicated that their genetic backgrounds might influence the expression of the *ts* allele. This was confirmed in studies on two TGMS lines from the same cross (Annong S-1/Bi8) that had the same growth duration, 90336S and 90332S. 90336S had a lower sterility-inducing temperature than 90332S, which in turn had a sterility-inducing temperature similar to that of Annong S-1 (Wu and Yin 1997).

In 1994, Annong S-1, 90336S, 90332S, 90336S/90332S, 0336S//90336S/90332S, 90336S/90332S//90332S, and the F_2 population from 90336S/90332S were planted in Sanya. In all experiments, a single seedling was transplanted per hill using wide spacing (26 by 33 cm) in order to obtain more tillers per plant with a relatively longer duration of flowering. The critical stage sensitive to temperature was arranged within the period of 20 February and 10 March, when the possibility of wide temperature fluctuations is highest. The percentage of stainable pollen grains of the parents, F_1, F_2, and BC_1s was determined microscopically in samples taken on 8, 12, 18, and 22 March.

Table 10 shows that (1) the sterility-inducing temperature was lower for 90336S than for 90332S, (2) low sterility-inducing temperature was dominant, and (3) plants in the F_2 and backcross 90336S/90332S//90332S segregated into two male fertility classes at three times of sampling, but the boundary between the classes varied with time. On 8 March, the fertility classes were 0–30% and 51–80%, on 12 March the classes were 11–40% and 61–90%, and on 18 March the groups were 0–20% and 31–90%. The parents also showed different fertility on 12 and 18 March (Table 10). The segregation ratios for fertility in the F_2 and 90336S/90332S//90332S backcross suggested that the induction of sterility at a lower temperature was controlled by two dominant genes, L_1 and L_2, which decrease the sterility-inducing temperature that is effective in the TGMS genotype (ts ts) in AnnongS-1. The genotypes of 90336S and 90332S then are $L_1L_1L_2L_2$ and $l_1l_1l_2l_2$, respectively. Table 11 shows that the observed segregations fit the ratios expected on the two-locus model. The reason for the wide segregation, 31–90%, on 18 March may be that the phenotypes of the plants with the genotype L_1—l_2l_2 and $l_1l_1L_2$—were altered from fertile to sterile, but plants with the genotype $l_1l_1l_2l_2$ remained fertile. Some minor genes may also influence the expression of the TGMS gene.

The frequency of occurrence of temperature lower than a given critical temperature (Tc) increases as Tc increases. For example, the number of days with temperatures lower than 26 °C was four times greater than those with temperatures lower than 24 °C in Changsha during the putative critical period for hybrid seed production, 15 July to 15 August. It can be inferred that, if the sterility-inducing temperature of TGMS lines decreases from 26 to 24 °C, the risk of selfed seed contamination incurred by using TGMS lines would sharply decrease by about 75%. Therefore, we can conclude that the use of TGMS lines with the $L_1L_1L_2L_2$ genotype will markedly reduce the risk relative to those with the $l_1l_1l_2l_2$ genotype.

Table 10. Segregation into male sterility classes of plants in 90336S/90332S-F_2 and in two backcrosses under different inducing temperature ranges during the critical developmental stage at Sanya in 1994. Lines 90332S and 90336S are coded A and B, respectively.

Date	Average temperature (°C)	Cross and generations	Percentage of stained pollen grains								
			0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90
8 March	24–25	B/A-F_2	50	5				4	1		
		B//B/A	15								
		B/A//A	9	3	2			4	2	1	
		B/A-F_1	5								
		B	5								
		A						3	2		
		AnnongS-1						4	1		
12 March	21–22	B/A-F_2		13	21	1	1	1	2	2	19
		B//B/A		8	6	1					
		B/A//A		2	4	1	1	1	2	3	7
		B/A-F_1		1	2	2					
		B		3	2						
		A									5
		AnnongS-1									5

Continued on next page.

Table 10 continued.

Date	Average temperature (°C)	Cross and generations	Percentage of stained pollen grains								
			0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90
18 March	22–24	B/A-F_2	24	8		2	6	5	6	3	6
		B//B/A	14	1							
		B/A//A	3	1		1	3	3	2	2	6
		B/A-F_1	4	1							
		B	5								
		A									5
		AnnongS-1								1	4
22 March	25–27	B/A-F_2	20								
		B//B/A	10								
		B/A//A	11								
		B/A-F_1	5								
		B	5								
		A	5								
		AnnongS-1	5								

Table 11. X^2 goodness of fit tests of the observed segregations to the ratios expected from the two-locus model controlling a lower sterility-inducing temperature.[a]

Date	Filial and backcross generations	Observed frequencies	Theoretical ratio	X^2	Probability
8 March	90336S/90332S-F_2	55:5	15:1	0.160	0.75–0.50
	90336S//90336S/90332S	15:0	1:0		
	90336S/90332S//90332S	14:7	3:1	0.397	0.75–0.50
	90336S/90332S-F_2	36:24	9:7	0.321	0.75–0.50
12 March	90336S//90336S/90332S	15:0	1:0		
	90336S/90332S//90332S	8:13	1:3	1.286	0.50–0.25
	90336S/90332S-F_2	32:28	9:7	0.191	0.75–0.50
18 March	90336S//90336S/90332S	15:0	1:0		
	90336S/90332S//90332S	4:17	1:3	0.143	0.75–0.50
	90336S/90332S-F_2	20:0	1:0		
22 March	90336S//90336S/90332S	10:0	1:0		
	90336S/90332S//90332S	11:0	1:0		

[a] Percentages of stainable pollen grains delimiting the male sterile and fertile class boundaries were <30% and >50% on 8 March, <50% and >50% on 12 March, and <20% and >30% on 18 March.

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Notes

Authors' address: Tsinghua Institute, China National Hybrid Rice Research & Development Center, Shenzhen, Guangdong 518172, China.

Modified single-cross for hybrid rice breeding

Xinqi Li, Jinhua Xiao, Fangming Xie, and Longping Yuan

L129A is a cytoplasmic male sterile (CMS) line of the WA type. L129-4-8, L129-4-9, L129-6-2, and L129-12-7 are sister lines of L129B with partial maintainers for male sterility. It was observed that high outcrossing was associated with low male sterility in the population of V20A/6*L129-12-7 BC_6F_1. When L129A was crossed with the four sister lines of L129B, it produced male sterile F_1s with much improved outcrossing habit. The results showed that the F_1 of A/partial maintainer induced less negative impact of male sterility on outcrossing. In this paper, a modified single-cross approach to develop hybrid rice was suggested. A method of using regular CMS A, B, and R lines and a partial maintainer line (B′) was proposed to increase the seed yield of seed production of hybrid rice and make CMS lines with poor outcrossing useful. The breeding method for B′ was also studied.

Keywords: cytoplasmic male sterility, modified single-cross, hybrid rice

Hybrid rice is considered as a major, readily available approach to increase food production in developing countries. Many rice scientists have focused on hybrid rice breeding since the 1980s. However, commercial hybrid rice production outside China is being practiced in only a few countries, mainly in South and Southeast Asia. Even in China, though hybrid rice is a major crop in rice production, it still covers only about 58% of the rice area. The absence of available maintainers and their corresponding cytoplasmic male sterile (CMS) lines is a major constraint to the development of hybrid rice. Due to the genetic complication of male sterility and the low efficiency of selection, it is difficult to develop an elite maintainer and its corresponding CMS line (Li et al 2006). As a result, in the past 30 years, only a few elite CMS parents, such as Zhenshan 97A, V20A, and II-32A, have long been used as the major female parents in hybrid rice production. Seeking new approaches for breeding hybrid rice could solve this problem.

Materials and methods

Materials

Indica wild-abortive (WA) CMS A and B lines were selected for the experiment: H50A/H50B—a CMS line with complete male sterility but with poor flowering traits; L129A/L129B—a CMS line derived from progeny of H50B/Bt39; V20A—a common CMS line used extensively in hybrid rice; You-1A—a CMS line with incomplete male sterility but good outcrossing; 161-5A/161-5B—a CMS line with complete male sterility and high combining ability but poor outcrossing. Indica partially sterile maintainer lines were also selected: Bt39—a partially male sterile maintainer with wide compatibility (WC); and L129-4-8, L129-4-9, L129-6-2, and L129-12-7—all sister lines with similar agronomic traits derived from the progeny of H50B/Bt39. Ce64 (selected from IR9761-19-1), an indica WA-CMS restorer line, was used as the pollen parent.

Hybrid seed production for V20A/6*L129-12-7 BC_6F_1//Ce64 and modified single-crosses

Hybrid seeds from BC_6F_1 of V20A/6*L129-12-7/Ce64 (designated as a modified single-cross) were produced. All seedlings were 25 days old when transplanted. Spacing was 13.3 cm × 20 cm within male and female populations, and 20 cm between male and female parents. The row ratio in seed production plots was 2:6:2 of male:female: male. When 10% of the panicles were at heading stage, 150 g ha^{-1} of gibberellic acid (GA_3) was sprayed on plants after clipping flag leaves (12–15 cm). Supplementary pollination was carried out two times a day during the peak blooming period from 0900. The female plants had two random replications with 120 plants per plot. The plant populations grew well in the seed production field. The number of spikelets per m^2 ranged from 24,000 to 31,000. The heading stage of male and female parents was synchronized.

Inspection of outcrossing traits in F_1s of L129A/sister lines of L129B

Male sterility, selfed-seeding set, flowering rate before noon, bloomed spikelet rate, panicle exsertion, and stigma viability were inspected in 60-plant populations for each F_1 progeny of the crosses among L129A, L129A/L129B sister maintainer lines, V20A, and You-1A.

Trait and yield comparison between modified single-cross and corresponding single-crosses

The hybrids derived from the crosses between experimental CMS lines with Ce64 were laid out in a field using a randomized complete block design with three replications. Seedlings were transplanted as one seedling per hill in plots of 12 (row) × 15 (hill) at 16.7 × 20-cm spacing. All 180 plants of each plot were harvested to determine grain yield. Yield components and other traits were measured from samples of five successive plants in the center of the fourth or ninth row in each plot.

Breeding B′ of 161-5A

Fifty-eight BC_3F_1 plants from the cross of 161-5B*3/Bt39 were testcrossed with 161-5A to screen promising progeny as 161-5B′. Thirty plants of each F_1 (161-5A/161-5B′) and 161-5A (check) were used as female parents for seed production with Ce64 as the male parent to test outcrossing ability. The major technique used for seed production was the same as that used for V20A/6*L129-12-7//Ce64. Twenty F_1s with high outcrossing evaluated preliminarily were selected for further precise outcrossing examination. The seeding set of all 30 plants from each F_1 was used to determine F_1 outcrossing habit.

Trait inspection

Two spikelets from the upper, middle, and lower parts of each panicle were collected. Two to three anthers from each spikelet were squashed in 1% I_2-KI solution and checked for sterility/fertility. Pollen grains intensely stained, round, and fully developed were scored as fertile, and other types of pollen scored as sterile. Thirty panicles were bagged before flowering. Pollen sterility was calculated as the mean of sterile pollen rate from 30 randomly selected panicles from 30 plants (for a population) or of three panicles (for a plant). Selfed seed rate was calculated as the average seeding set rate of 30 randomly bagged panicles. Selfed-seeding rate on ratooning was calculated as the average seeding set rate of three randomly bagged ratooning panicles per plant. A sterility pollen rate greater than 99.5% was considered as the standard for male sterility; even complete male sterility requires a 100% sterile pollen rate. Spikelets with stigmas exserted were marked and pollinated on the second day after flowering. The mean seeding set rate was used to determine stigma viability. For a plot, outcrossing rate was calculated as the average fertilized spikelet rate of 15 random seed parent plants minus the selfed-seeding rate; for one plant, it was the fertilized spikelet rate subtracted by the selfed-seeding rate on ratooning. Panicle exsertion rate was calculated as the mean rate of exserted spikelets over all spikelets of 30 random panicles out of the sheath. Bloomed spikelet rate was calculated as the mean rate of bloomed spikelets over all spikelets exserted from the sheaths of 30 random panicles. Flowering rate before noon was the mean of bloomed spikelet numbers before noon over bloomed spikelets during the whole day from 10 random panicles before 1500. Flag-leaf length or flag-leaf width was the mean length or width of flag leaves on 10 main stems.

Results

Differences in outcrossing rates among V20A/6*L129-12-7 B C_6 F_1s

H50B/Bt39 was used to breed CMS B-lines with WC genes and ideal outcrossing habits. Plants with elite agronomic traits were selected in the F_2 and F_3 generations. F_4 plants were crossed with V20A to test their ability to maintain male sterility. L129 was one of those F_4 plants.

Of the 24 F_5 L129 plants, 18 were selected and backcrossed with the F_1 (V20A/L129). After continuous backcrossing, one male sterile line from the 18 backcrosses (BC_4F_1) showed good performance and was named as L129A. However, backcross

Table 1. Relationship between male sterility and outcrossing of V20A/6*L129-12-7 BC_6F_1.

CMS line	V20A/6*L129-12-7 BC_6F_1					Checks		
Sample	1	2	3	4	5	L129A	V20A	You-1A
Sterile pollen rate (%)	99.99	99.9	99.5	99.0	97.9	100	100	99.62
Plants (no.)	7	12	18	44	15	30	30	30
Self-seed rate on ratoon (%)	0.0	0.0	0.01	0.2	1.3	0.0	0.0	0.01
Outcrossing rate (%)	33.9	40.3	37.8	61.2	72.4	27.6	35.6	51.3

progenies of 16 F_5 plants showed segregation in male sterility from BC_1F_1-BC_3F_1 and all of them were partially male sterile in the BC_4F_1 progenies, meaning those 16 F_5 plants were partial sterility maintainers. L129-4-8, L129-4-9, L129-6-2, and L129-12-7 were four lines from those 16 L129 F_5 plants. The backcross progenies with L129-12-7 always showed segregation in male sterility and partial sterility. An interesting phenomenon was observed in the BC_5F_1 of V20A/5*L129-12-7: although uniform in performance phenotypically among those BC_5F_1 plants, the natural outcrossing rates (no supplementary pollination) were greatly different and varied with sterility, that is, high outcrossing was associated with lower sterility. To further test the outcrossing of the different sterile plants, one male sterile BC_5F_1 plant was selected to be backcrossed with L129-12-7, and the BC_6F_1 plant was used as the female parent to cross with Ce64 to produce hybrid seeds. The BC_6F_1 still showed segregation in male sterility and outcrossing (Table 1).

Since the BC_6F_1 plants have similar genetic backgrounds, the differences in outcrossing rates can be considered to be associated with male sterility, that is, male sterility could be one factor affecting the outcrossing ability and it could be possible to improve outcrossing with a lower degree of male sterility.

Outcrossing ability of L129A crosses with L129B sister lines

How do we apply partial sterility in hybrid rice development? It was designed that a CMS line with improved outcrossing could be produced from a CMS line crossed with a partial sterility maintainer (B′). To further study the male sterility and outcrossing ability of L129A, the CMS line (L129A) was crossed with four L129B sister lines. The F_1s were used as female parents for seed production with Ce64. Table 2 lists the results of male sterility and outcrossing habit of those F_1s.

Compared with the regular L129A, the outcrossing traits of bloomed spikelet rate, flowering rate before noon, spikelet exsertion rate, and stigma vitality of the F_1s derived from the sister lines showed increases of 13.9–30.4%, 45.5–73.8%, 5.4–25.1%, and 66.7–85.8%, respectively. The pollen sterilities of all F_1s remained over 99.5%. In a seed production trial, outcrossing rate and seed production yield of all hybrids of L129A with its sister lines significantly increased by 32.6–55.4% and 27.6–40.8%, respectively (Table 2).

Table 2. Outcrossing and male sterility of hybrids of L129A with sister lines of L129B.

Trait	L129/ L129-4-8	L129/ L129-4-9	L129/ L129-6-2	L129/ L129-1-2-7	Checks		
					L129A	V20A	You1A
Pollen fertility (%)	99.80	99.74	99.84	0.0	0.0	0.0	99.86
Self-seed rate (%)	0.0	0.02	0.01	0.0	0.0	0.0	0.01
Bloomed spikelet rate (%)	72.7	81.6	75.3	71.3	62.6	76.8	88.4
Early flowering rate (%)	49.2	56.5	50.8	47.3	32.5	43.7	63.1
Spikelet exsertion rate (%)	79.2	74.4	78.5	66.7	63.3	62.1	73.8
Stigma vitality (%)	46.2	46.7	43.5	48.5	26.1	47.5	60.2
Outcrossing rate (%)	40.4*	46.3**	39.5**	44.1**	29.8		
Hybrid seed yield (m^2 g^{-1})	316.9**	331.1**	324.7**	300.1**	235.1		

**$P<0.01$.

Trait comparison between modified single-crosses and their corresponding single-cross

Results of variance analysis indicated that there were no significant differences in eight agronomic traits between hybrids of modified single-crosses (L129A/B//Ce64) and hybrids of their corresponding single-cross (L129A/Ce64) (Table 3).

To further determine the uniformity of each population, variance coefficients (CV) were calculated (Table 4). The CVs of the modified single-crosses were similar to those of the corresponding single-crosses.

Method to obtain B′ of 161-5A

A breeding strategy was proposed to obtain B′ of some current CMS lines as in the following examples of developing 161-5B′.

Table 3. Variance analysis for eight traits of hybrids.

Trait	Variance		
	Replications	Combinations	Error
Grain yield	56.85	532.91	231.32
Plant height	6.49	4.58	8.96
Days to heading	1.40	1.26	1.14
Days to maturity	2.26	2.15	3.23
Spikelets per plant	549.45	2,937.35	5,873.57
Percent seed set	1.18	6.9	6.61
Flag-leaf length	0.10	1.82	1.74
Flag-leaf width	0.02	0.1	0.04

Table 4. Agronomic traits and their CVs (in parentheses) in modified single-crosses.

Material	Grain yield	Plant height	Days to maturity	Days to heading	Spikelets per plant	Percent seed set	Flag-leaf length	Flag-leaf width
L129A/ L129-4-8//Ce64	661.3	106.5	133.6	94.8	1,446.3	58.8	46.1	1.85
		(3.41)	(2.69)	(2.62)	(3.42)	(3.12)	(2.43)	(2.78)
L129A/L129-6-2//Ce64	649.7	105.3	134.4	95.7	1,374.7	61.2	45.5	1.82
		(2.61)	(2.57)	(2.34)	(3.76)	(3.36)	(2.15)	(3.05)
L129A/L129-12-7//Ce64	681.3	103.6	132.7	96.3	1,390.5	62.1	47.2	1.93
		(1.78)	(1.14)	(2.22)	(2.82)	(3.43)	(2.62)	(1.94)
L129A/Ce64 (CK)	669.5	105.8	132.6	95.2	1,415.8	61.9	45.6	1.82
		(2.25)	(2.17)	(2.34)	(2.91)	(3.05)	(1.96)	(2.52)

Table 5. Outcrossing habits and male sterility of crosses between 161-5A and 161-5B′s.

Material	161-5A/ 161-5B′-1	161-5A/ 161-5B′-2	161-5A/ 161-5B′-3	161-5A	Zhenshan 97A
Pollen fertility (%)	0.00	0.00	0.00	0.00	0.00
Self-seed rate (%)	0.00	0.00	0.00	0.00	0.00
Bloomed spikelet rate (%)	76.7	74.3	56.8	38.1	68.8
Early flowering rate (%)	56.4	60.3	67.4	28.3	63.7
Spikelet exsertion rate (%)	76.9	82.3	81.7	64.7	72.1
Stigma vitality (%)	68.4	73.5	60.1	62.4	67.5
Outcrossing rate (%)	43.7**	38.4**	35.2**	15.3**	39.3**
Hybrid seed yield (m^2 g^{-1})	321.8**	267.2**	285.4**	146.6**	290.6**

**P<0.01.

161-5A/Bt39
↓
161-5A/F_1
↓
161-5A/BC_1 (fertile plants selected as male parents)
↓
161-5A/BC_2 (fertile plants selected as male parents)
↓
BC_3F_1 (self-crossed, stable plants similar to 161-5B selected as candidate 161-5B′)
↓
161-5A/161-5B′ candidates
↓
F_1s /Ce64 (male sterile F_1s screened for hybrid seed production trial)

Forty-nine out of the 58 F_1s of 161-5A//161-5*3/Bt39 were found to be male sterile. Six male sterile F_1s were still found to have more than 30% outcrossing rate compared with the 17.6% outcrossing rate of 161-5A (an increase of 85.2–117.6%). Table 5 shows the outcrossing habits and male sterility values of crosses between 161-5A and 161-5B′-1, -2, and -3.

Discussion

The male sterility and outcrossing habit of WA-type CMS lines indicates a co-existing relationship between cytoplasm and nuclear donor. Most of these donor parents (B lines) in China belong to the early-maturing indica rice group, and late- or medium-maturing indica varieties are usually unable to maintain the male sterility of WA-CMS even if their nuclear donors show good flowering habits. The WA-CMS system has less germplasm diversity in maintaining male sterility; only less than 0.1% of indica germplasm showed the ability to keep WA cytoplasm sterile. In CMS line breeding, breeders are very unsure of a CMS line if good flowering habit could be obtained from a nuclear donor parent until almost all genetic components from the donor parent are backcrossed into the sterile cytoplasm. The segregation of early generations in breeding B lines even reduces breeding effiency.

Heterozygous sterility produced from the F_1 of a CMS line crossed with a partial maintainer was often found in maintainer screening. Many nuclear donors were partial maintainers, even if their F_1s showed male sterility when crossed with a CMS line. Partially fertile plants are usually found in backcross progenies when a complete CMS line X-A (maintainer X-B) is crossed and backcrossed with a complete sterility maintainer Y-B (X and Y refer to different CMS B-lines). Our breeding experience shows that many progenies of hybrids derived from two complete maintainers were unable to maintain male sterility. These phenomena can be considered as the result of segregation, recombination, or epistasis of polygenic fertility genes. In addition, completely male sterile CMS lines can become partially fertile under high temperature at the young panicle stage (Xu et al 1991). Polygenic fertility genes also exist in complete male sterile CMS lines and may not show phenotypically.

Male sterility in crops often results in poor flowering habit. In rice, the flowering time of WA-CMS lines is usually later than that of the male parent (conventional rice) with other poor flowering habits such as unbloomed spikelets, spikelets blooming diversely, enclosed panicle in sheath, and decreased stigma viability (Wang and Gao 1998). Observation indicated that the flowering habit of CMS lines was related to the degree of male sterility. The BT or HL type showed better flowering habit than the WA type, but a few of the BT- or HL-CMS lines are completely male sterile, although CMS lines of the WA type usually have a lower outcrossing rate but higher male sterility. Breeders have successfully obtained CMS lines of the WA type with good flowering habit (e.g., You-1A, BoA, Xie A, Jin23A), but the male sterilities of these CMS lines were less than that of V20A and Zhenshan 97 A (Wang and Xu 1996). Polygenic fertility genes seem to be an important genetic element that affects flowering habit. Some CMS lines show poor flowering, probably because of the absence of polygenic fertility genes. Too many polygenic fertility genes may result in partial sterility, but too few may give rise to bad flowering habit. The improvement of the outcrossing habit of A/B′ in the modified single-cross can be considered the result of the increase in polygenic fertility genes and the decrease in the effect of male sterility on flowering habit.

Floral structure is the second (flowering habit being the first) most important factor influencing outcrossing rate (Xu 1994). The components of floral structure that affect outcrossing are length, width, size, exsertion rate, hairiness of stigma, and angle of glume opening. The exserted stigma could be pollinated and fertilized on days following the bloom stage. A large stigma size is helpful in increasing the chance to capture pollen. Size, length, and exsertion rate of stigma are dominant or incompletely dominant traits. Therefore, B′ with its high stigma exsertion rate and suitably large stigma can improve the floral traits of A/B. The elite floral traits of B′ may not be difficult to obtain since stigma size and exsertion rate showed high heritability.

Heterosis can be enhanced to widen the genetic diversity between parents. Some CMS lines with partial japonica genetic pedigree (such as 161-5A) showed high combining ability. However, seed production yield was too low, which limits its practical usage. A modified single-cross offers a possible solution to that problem, as it may enhance the seed production yield of current rice hybrids by obtaining elite B′ lines. Furthermore, the modified single-cross method in hybrid rice may also be applied to other crops to decrease the disadvantageous effect of male sterility on outcrossing. Polygenic fertility genes in A × B′ could enhance male fertility and stability of F_1 of A/B′ × R and safeguard the female parent by controlling the B line in A × B multiplication.

Spikelet fertility is not necessarily parallel with pollen fertility in CMS lines. A high rate of fertile pollens may not necessarily result in a high selfed-seeding set because other factors, such as pollen germination, also affect seed formation. However, partial male sterility in a CMS line will reduce the uniformity in seed production and hybrid rice grain production. Plants with high fertile pollens can reproduce more seeds due to good flowering habit with increased individual plants in the population. Within a few generations, plants with high pollen fertility will increase greatly, even achieving normal seeding levels (Zhou et al 1992, Chen et al 1995), which results in a high percentage of impurity of hybrid seeds. In the proposed “modified single-cross” here, male sterility of a CMS A line is generally complete and very stable because it uses an original B line for parental seed production, and the B′ line is only used once in the last generation of female reproduction. Seed purity can be controlled and ensured. Therefore, male sterility of A/B′ could be controlled with impurity, but with much higher outcrossing for hybrid seed production in the next generation.

The genetic difference between hybrids of 161-5A/Ce64 and 161-5A//161-5*3/Bt39///Ce64 can be controlled within 1.6%, assuming that 50% of the nuclear genome is introduced to a CMS line in each crossing and backcrossing generation. The experience of modified single-cross in maize hybrids requires some genetic differences between two sister lines to produce heterosis in the F_1 of A/B′. But, the difference between B and B′ in the modified single-cross can be limited to the genes controlling male fertility and floral traits. So, the population uniformity from the modified single-cross should not be greatly affected in comparison with the original A line. If agronomic traits of yield, quality, and maturity are not affected in the modified single-cross, some genetic difference between B and B′ should be permitted. Restorer lines with dominant genes controlling those agronomic traits will show heterosis without a difference between hybrids produced from A/B//R and A/B′//R.

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Notes

Authors' addresses: Xinqi Li, Jinhua Xiao, and Longping Yuan, China National Hybrid Rice Research & Development Center, Changsha 410125, China; Fangming Xie, International Rice Research Institute, Los Baños, Philippines.

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Breeding of herbicide-resistant hybrid rice in China

Guoying Xiao

Besides direct seeding and weed control in rice fields, Chinese scientists used herbicide resistance genes to increase the purity of hybrid seed and to implement mechanization of hybrid seed production. Elite restorer lines such as Minghui 63, R752, T461, R402, D68, and E32 were transformed directly using herbicide resistance genes. D68 and E32 are restorer lines under the two-line system and the others are used for the three-line system. Because almost all restorer lines come from indica varieties, which were recalcitrant in transformation, many herbicide-resistant near-isogenic restorer lines were developed by sexual hybridization between indica and japonica varieties and then later backcrossed with indica restorer lines, such as Ce64, Minghui 63, Teqing, Miyang46, R402, and 9311. The elite photoperiod-sensitive/thermo-sensitive genic male sterile lines, such as Pei'ai64S, P88S, 4008S, and 7001S, were transformed using herbicide resistance genes. A few herbicide-resistant male sterile lines—Bar1259S, Bar2172S, 05Z221A, and 05Z227A—were developed through sexual hybridization and subsequent systematic selection. With the use of herbicide-resistant male sterile lines or herbicide-resistant restorer lines, a few herbicide-resistant hybrid rice varieties were developed: Xiang125S/Bar68-1 and Pei'ai64S/Bar9311. Based on herbicide resistance, further research will be done to incorporate insect resistance, drought tolerance, etc., in parental lines of hybrid rice.

Rice is the most important staple food and hybrid rice developed by Chinese scientists has played a crucial role in increasing cereal supply in China. From the 1990s, the area planted to hybrid rice has occupied half of the total rice area, with output accounting for 58%. Hybrid rice, developed in the 1970s, was based on the three-line system. There were some limitations in using this system: it is hard to find restorer lines, the sterile cytoplasm has negative effects, and multiplication of male sterile lines is complicated. In the 1980s, Chinese scientists developed a new hybrid rice system, the two-line system. The core technique involves the use of photoperiod-sensitive and/or thermo-sensitive genic male sterile (P/TGMS) lines. It has some advantages over the three-line hybrid rice system (Yuan 1990), especially in resolving the conflict between early maturation and high yield, early maturation and good grain quality, and good grain quality and high yield (Chen et al 1996, Yin et al 1998, Lu et al 2000). It had become an effective approach in super hybrid rice breeding (Yuan 1996, 1997, Lu et al 2000). But the two-line system was not perfect—the sterility of P/TGMS lines was unstable at abnormally low temperatures. To minimize the loss caused by sterility fluctuation of P/TGMS lines in hybrid seed production, a strategy using herbicide resistance genes in hybrid rice was put forward in 1996 (Xiao 1997, Xiao et al 2007a,b). Herbicide resistance genes, such as *Bar*, *Epsps*, and *Als*, can be transferred to restorer lines of two-line hybrid rice; the restorer line and its hybrid then acquire the herbicide resistance gene but the sterile line does not, which enables the herbicide to kill the selfed P/TGMS plants and other off-type plants in the seedling bed of the F_1 hybrid, thus ensuring the purity of F_1 hybrids in the field. The herbicide resistance gene could also be helpful in mechanizing hybrid rice seed production by mixed planting of male and female plants, spraying herbicide to kill the pollen plant after pollination, and harvesting the hybrid seed on the surviving female plants if the herbicide resistance gene was transferred to the female sterile line only.

Herbicide resistance of the crop is getting more attention in China. To minimize labor cost and to lessen drudgery, fine tillage and careful rice cultivation are being replaced by zero tillage and direct seeding or throw-transplanting, which results in weed overrun. For weed control in the rice field, the best way is to develop herbicide-resistant hybrid rice.

Breeding of herbicide-resistant restorer lines

Research on two-line hybrid rice started in the late 1980s and some remarkable pioneer combinations were achieved in different years—e.g., Pei'ai64S/Teqing (Luo et al 1994) at the beginning of the 1990s, and Xiang125S/D68 (Yin et al 1998) and Pei'ai64S/9311 (Lu et al 2000) at the end of the 1990s. The transgenic herbicide-resistant restorer lines of the two-line system were successfully developed from R187 and Xiushui04 (Hu et al 2000) by *Agrobacterium*-mediated transformation of the *Bar* gene, and from D68 (Xiao et al 2007a,b) and E32 (Wang et al 2004) by transformation of the *Bar* gene with particle bombardment and the pollen-tube pathway method, respectively (R187 and Xiushui04 were from japonica varieties and the others were from indica varieties).

Besides the P/TGMS lines, some cytoplasmic male sterile (CMS) lines such as II-32A and LongtepuA also have unstable sterility under abnormally high temperature, which also results in a decrease in hybrid seed purity. Some restorer lines for CMS were also transformed by the *Bar* gene and herbicide-resistant restorer lines were obtained such as Minghui 63 (Xue et al 1998), 752 (Zhang et al 2000, Rao et al 2003), T461 and R402 (Rao et al 2003), Jingyin119 (Zhu et al 1996), Jingdao162 and Jingdao18 (Shi et al 2004), and H84 (Wang et al 2007). (Jingyin119, Jingdao162, Jingdao18, and H84 were from japonica varieties and the others were from indica varieties.)

As the transformation efficiency of indica varieties was much lower than that of japonica varieties, herbicide resistance was transferred to many elite indica restorer lines through sexual hybridization with japonica transgenic plants and then backcrossing with elite indica restorer lines. For example, near-isogenic herbicide-resistant restorer lines were obtained from Ce64, Minghui 63 and Teqing (Li et al 2000), Milyang 46 (Xue et al 2001), R402 (Zhong et al 2000), and 9311 (Wang et al 2002); 9311 was a restorer line of two-line hybrid rice and the others were restorers of the three-line system.

The cultivation of transgenic hybrid rice Xiang125S/Bar68-1 showed that the purity of hybrid rice in paddy could be increased remarkably by spraying herbicide Basta on the seedbed, which resulted in a yield increase (Xiong et al 2004). This demonstrated the feasibility of using a herbicide-resistant restorer line to reduce the risk of P/TGMS lines mixing with hybrid seeds.

Breeding of herbicide-resistant male sterile lines

At present, male and female plants are transplanted in rows separate from each other in the hybrid seed production field. The pollen plant should be harvested by hand before the female plant, thus making hybrid seed production complex and costly. The herbicide-resistant male sterile line can facilitate mechanical harvesting of hybrid seed by mixed planting of male and female plants, then spraying herbicide to kill the pollen plants after pollination, and harvesting the hybrid seed from the surviving female plant. This was made possible by the transfer of the herbicide resistance gene to the female sterile line only (Xiao 1997). The herbicide resistance *Bar* gene was first introduced into Pei'ai64S, a popular indica P/TGMS line used as the female parent of many two-line hybrid rice combinations, such as Pei'ai64S/Teqing, Pei'ai64S/9311, and Pei'ai64S/E32; mechanical harvesting of hybrid seed was carried out in a small area and it proved to be a success (Fu et al 2001). Other functional genes such as stress tolerance genes and insect resistance genes were co-transferred along with the herbicide resistance gene in one transformation, which opened the door for future genetic modification in crops. *Bar* and *DREB1A* genes were transferred to P88S, a recently developed indica P/TGMS line noted because of its super hybrid combination P88S/0293. These genes conferred herbicide resistance and drought tolerance to P88S and its hybrids (Chen 2008). Another japonica P/TGMS line, 4008S, was transformed at the same time using *Bar* and *DREB1A* genes and it performed well in terms of drought tolerance and herbicide resistance (Chen 2008, Chen et al 2008). Three insect resistance genes—*Bt*, *GNA*, and *Pin*II—were co-transferred with another herbicide resistance gene, *Epsps*, into japonica P/TGMS line 7001S; its herbicide resistance and insect resistance were manifested in the T_1-T_3 generations (Deng 2008, Deng et al 2008).

To enlarge the genetic background and retain the combining ability of herbicide-resistant male sterile lines, the previously developed herbicide-resistant variety Bar68-1 (Xiao et al 2007a,b), a restorer line of two-line hybrid rice as well as a maintainer line of three-line hybrid rice, was used as a donor of a herbicide resistance gene and hybridized with other P/TGMS lines and maintainer lines. Two P/TGMS lines, Bar1259S and Bar2172S, were developed from the cross of Pei'ai64S/Bar68-1 and Xiang125S/Bar68-1, respectively, and their sterilities were evaluated every year at Changsha. The sterile critical temperature was established in 2005 in the phytotron (unpublished data). Two elite CMS lines, 05Z221A and 05Z227A, were also obtained from successive backcrossing of corresponding maintainer lines derived from FengyuanB/Bar68-1. The sterility of CMS lines was evaluated in every backcross generation. They were found to be completely sterile. The grain quality of the maintainer lines was also assayed and it met the national standard of high-quality rice as their characters remained stable in 2006 (unpublished data). The four male sterile lines described above were used to develop herbicide-resistant hybrid rice.

Breeding of herbicide-resistant hybrid rice

Under a strictly controlled field trial of genetically modified rice in China, only a few herbicide-resistant combinations were evaluated (Li et al 2000, Xue et al 2001, Xiong et al 2004). Only the herbicide-resistant hybrid Xiang125S/Bar68-1 participated in the regional trial held during the early rice season in Hunan Province in 2004. The results of the field trial showed that yield, grain quality, and disease resistance of Xiang125S/Bar68-1 were better than those of the control; consequently, this transgenic hybrid rice successfully passed the first-year regional trial (Xiao et al 2007b). The following regional trials in 2006 and 2007 were likewise successful. The report mentioned the variety's strong vigor, effective panicles, good grain quality, and yield equal to that of the check (unpublished data). This new hybrid rice could be put into commercial use if permitted by the Chinese government.

An elite restorer line, Bar9311, was developed from Bar68-1/9311 by pedigree selection and the heterosis and grain quality of combinations Pei'ai64S/Bar9311, 244S/Bar9311, and 81S/Bar9311 were evaluated in 2004 and 2006. Pei'ai64S/Bar9311 performed well, giving a yield of 11.96 t ha^{-1}. Grown on 0.1 ha in 2006 in an isolated plot of our institute in Changsha, it achieved the standard of super hybrid rice in China (unpublished data).

Prospects

The global area of transgenic crops in 2006 reached 102 million ha. An increase of 12 million ha was seen from 2005 to 2006, equivalent to an annual growth rate of 13% in 2006, 60 times over that of 1996. Herbicide-resistant crops—soybean, maize, canola, cotton, and alfalfa—have become the most widely planted genetically modified (GM) organisms in the world, accounting for 68% of the total area (69.9 million ha) in 2006. At present, some GM crops, including cotton, tomato, papaya, sweet capsicum, poplar, and petunia, have been authorized by the Chinese government for commercial production, but only *Bt* insect-resistant cotton was planted on a large scale. It covered 3.5 million ha in 2006, which accounted for 66% of the total area planted to cotton in China (James 2006). The transgenic crops have shown good prospects for minimizing cost and protecting the environment and will become dominant crops in the future, despite the conservative views of some governments and environmentalists. Because of its importance as a staple food and the rise of transgenic rice technology, rice will have the most area of GM crops in China in 3 to 5 years. Once transgenic rice is allowed by the government, the development trend of transgenic rice in China will be similar to that of GM crops abroad in the past decade. Herbicide-resistant GM rice will be dominant in terms of area and output as zero tillage and direct seeding become prevalent in rice cultivation. At the same time, other important traits such as insect resistance and drought tolerance will be integrated into herbicide-resistant transgenic rice. Herbicide-resistant GM rice with other stacked traits will usher in a new era in Chinese agriculture.

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Notes

Author's address: Institute of Subtropical Agriculture, Chinese Academy of Sciences, Mapoling, Changsha 410125, Hunan Province, China.

Accelerating the development of japonica hybrid rice in China

Li Chengquan

This paper reviews the detailed process of developing japonica hybrid rice in China and discusses breeding achievements with cytoplasmic male sterile (CMS) lines, photoperiod-sensitive genetic male sterile (PGMS) lines, as well as three-line and two-line japonica hybrid rice. Evidence shows the periodic breakthroughs of japonica hybrid rice breeding that have occurred since 2000.

To overcome the weaknesses in developing japonica hybrid rice, we suggest the following plans to accelerate development. (1) Strengthen standard heterosis with the main aims of increasing panicles and seed set, improving grain quality by reducing chalky rate and chalkiness and increasing resistance to stripe leaf blight and false smut. (2) Because japonica hybrid rice has a relatively stronger photosensitivity to growth and narrower adaptability than indica hybrid rice, several types of varieties should be bred to satisfy the needs of different ecological regions, including early-season and middle-season japonica hybrid rice suitable for northern China; middle-season, late-maturing middle-season, and late-season japonica hybrid rice suitable for southern China; and single-season japonica hybrid rice suitable for the southwest plateau of China. Varieties with drought or salinity and alkalinity tolerance are also needed. (3) New types of male sterile lines with better early flowering, high outcrossing, and stable sterility should be bred. Parent multiplication and hybrid seed production must be strictly carried out to increase yield and secure the purity. (4) Cultivation technologies for new hybrid rice combinations should be developed simultaneously for different regions to achieve high yield potential, improve grain quality, and save cost. (5) Nationwide cooperation should be organized, research investment should be increased, and annual regular meetings should be held.

Keywords: accelerating development, japonica hybrid rice, China

The great success of hybrid rice breeding and development in China is well known. It has helped the country to secure its food supply and at the same time increased rice farmers' income greatly. Recently, Robert S. Zeigler, the director general of the International Rice Research Institute (IRRI), said: "Certainly, the success of hybrid rice in China is well known, and the potential for hybrid rice to have an impact across the rest

Table 1. The growing area of japonica inbred rice and hybrid rice in China.

Region	Province or municipality	Ecological type	The growing area of japonica rice (10^4 ha)	The growing area of japonica hybrid rice (10^4 ha)
Central China and East China	Jiangsu, Shanghai, Zhejiang, Jiangxi, Anhui, Hubei, Hunan	Medium and late japonica	244	14
North China	Beijing, Tianjin, Hebei, Shandong, Henan	Medium japonica	190	8
North east China	Heilongjiang, Jilin, Liaoning, Inner Mongolia	Early and medium japonica	300	1
Southwest China	Yunnan, Guizhou , Chongqing, Sichuan, Tibet	Medium japonica	40	2
Northwest China	Xinjiang, Gansu, Ningxia, Shanxi	Early and medium japonica	20	0
South China	Taiwan, Fujian	Medium japonica	34	0
Total			828	25

Source: Deng Huafeng et al (2006).

of the rice-growing world is something that we all believe is real." He added, "There is no question that we need technologies that will improve the productivity of rice and certainly hybrid rice is at or near the top of the list of technologies." Currently, the area planted to hybrid rice in China is about 17.58 million ha annually, which is 57.3% of the total rice-growing area (30.67 million ha). Hybrid rice in other rice-growing countries has been increasing annually. Indica hybrid rice is a major contributor to rice production in China, which is planted on about 17.33 million ha (80% of the national area of indica rice) annually. The rice grains increased by hybrid rice can provide food for an extra 70 million people every year. However, in contrast, annual planting area for japonica hybrid rice was only 0.25 million ha in 2004, accounting for just 3% of the total japonica area of 8.28 million ha in China (Table 1). If the area of japonica hybrid rice could be increased from 3% to 30% with about 2.5 million ha annually, it would add a minimum of 30 million tons of rice and provide food for 15 million people in a year. It is important to do our best to explore this huge potential area. Furthermore, the world has about 12.54 million ha of japonica rice. People in some countries, such as Japan, South Korea, North Korea, Egypt, and Australia, grow and consume only japonica rice. Therefore, accelerating the development of japonica hybrid rice is important.

Breeding achievements with japonica hybrid rice

Achievements with japonica hybrid rice outside China

A study on using hybrid heterosis in rice globally began with japonica rice. During 1958-68, Japanese breeders successfully developed Fujisaka 5A that carried cytoplasmic male sterility (CMS) from a Chinese wild rice with red awn. Another CMS line, Taizhong 65A, which carried CMS from Chinsurah Boro II, an early indica rice from India, was also developed. Original varieties Fujisaka 5 and Taizhong 65 were used as maintainers (B lines) for male sterility (MS). When an MS restorer line (R line) was screened or developed, three-line japonicas (CMS A line, maintainer line, restorer line) formed a complete set for a hybrid rice system for seed production. Fujisaka 5 was also developed from another CMS line carrying CMS cytoplasm from indica rice variety Lead from Myanmar in 1968. However, japonica hybrid rice derived from all the above CMS A lines did not have strong enough heterosis to be commercially exploited because of the close genetic relationship among the parents.

In past decades, scientists in Japan were also trying to develop an efficient hybrid rice production system. Mitsui Chemicals Inc. developed new japonica MS lines MH2003A and MH2005A by cell fusion technology. Through collaboration with China Seed Company and some agricultural research institutes and universities in Anhui, Liaoning, Jiangsu, and Fujian, these MS lines were applied in three-line hybrid production for japonica rice. Three combinations, III You 98 (MH2003), MH2005, and MH3001, all with mid-season maturity, were bred and released commercially in both countries. Two varieties released and demonstrated in five provinces of China and nine counties in Japan showed a 20% yield advantage over check variety Koshihikari, a japonica inbred rice with excellent grain quality. Scientists in the United States, the Philippines, North Korea, and South Korea were also involved in japonica hybrid rice. Japonica MS lines with cytoplasm of Birco and *Oryza glaberrima* were developed at the University of California. Another japonica hybrid rice, Donghael, was bred by North Korean scientists.

Achievements with three-line japonica hybrid rice in China

Breeding for three-line japonica hybrid rice in China began with the discovery of a natural MS line at Yunnan Agricultural University in 1965, almost at the same time as the beginning of indica hybrid rice breeding. A Hongmaoying japonica MS line, Dian 1, was bred in 1969. Subsequently, a series of Dian-type MS lines was developed. However, they were not used for commercial production until recent years as new MS lines and japonica varieties with specific characteristics for plateau regions were developed. The BT CMS line Taizhong 65A was introduced into China in 1972 and transferred into a CMS line, Liming, successfully by scientists in Hunan and Liaoning. Only a few sources of restorer lines are available in japonica rice. So, Professor Yang at the Liaoning Academy of Agricultural Sciences pioneered the technology of restorer line breeding by "bridging between indica and japonica." In 1975, japonica restorer line C57, which contains 25% of indica pedigree, was developed from the cross of IR8/Keqing 3//Jingyin 35. The first commercial japonica rice hybrid, Li You

57, which had high heterosis, derived from Liming A × C57, was bred and released in a large area. Thereafter, three-line japonicas (A, B, and R lines) completed a system of hybrid seed production. After that, a lot of japonica rice hybrids using BT CMS lines and restorer lines derived from C57 were developed for southern and northern rice-growing regions in China. These combinations were planted in a large area in the 1980s, with accumulated area of 1.78 million ha. However, japonica hybrid rice production decreased in subsequent years due to insufficient seed purity and yield heterosis. It was difficult for japonica hybrids to compete with elite japonica inbred varieties that usually have high yield and good grain quality. In the late 1990s, wide compatibility (WC) genes and other beneficial genes were introduced to broaden the gene pool of japonica hybrid rice. Good restorers, such as C418 with a specific wide compatibility, and new japonica CMS lines with a partial indica background were developed by using the bridging technology. This technology broadened the genetic background of japonica rice and improved its outcrossing. New japonica rice hybrids with high yield, good grain quality, and stress tolerance were developed and released commercially. Incomplete data showed that 60 CMS lines and more than 150 rice hybrids (Tables 2, 3) have been bred and used in production nationwide. Provinces or municipalities such as Liaoning, Jiangsu, Zhejiang, Anhui, Shanghai, and Tianjin, the major areas of japonica rice or japonica and indica mixed growing regions, showed strength in japonica hybrid rice breeding (Table 1).

By collaborating with Japanese scientists, several japonica rice hybrids were developed at the Anhui Academy of Agricultural Sciences (AAAS). These hybrids were evaluated and demonstrated at multiple locations in Japan for years. Results showed that seven hybrids were suitable for production in Japan (Tables 4, 5). Corresponding technology for seed production is being developed simultaneously.

Achievements with two-line japonica hybrid rice in China

Two-line hybrid rice usually means that a hybrid is derived from a T(P)GMS (thermo or photo-sensitive genic male sterile) line and a pollen parent that is a conventional inbred variety without a requirement of specific restorer genes. The sterile line can be multiplied by selfing under specific conditions of photoperiod and temperature combinations without a maintainer.

The discovery of a Hubei photoperiod-sensitive genic male sterile (PGMS) line, Nongken 58S, led to the breakthrough of study for two-line japonica hybrid rice in 1973. Later, in 1985, Nongken 58S was officially named as Hubei photoperiod-sensitive genic male sterile rice (HPGMR). In 1987, studies on PGMS lines and two-line hybrid rice breeding were undertaken in the project of the “863” National High-Tech Plan, which included a series of studies on the mechanism of fertility transformation, evaluation for fertility, sterile line and hybrid breeding, sterile line multiplication, and seed production and industrialization. Two-line hybrid rice was commercially applied to production in 1995. Up to now, 27 T(P)GMS lines have passed technical evaluation and 15 two-line japonica rice hybrids have been released commercially (Tables 6 and 7). The accumulated growing area of two-line hybrid rice is now about 1.55 million ha. Some two-line hybrids have yielded a record of more than 13 t ha^{-1}.

Table 2. Information on major three-line japonica MS lines.

Name	Breeding institute	Release year	Type of cytoplasm	Ecotype	Source
LiMing A	Liaoning and Hunan Academy of Agricultural Sciences	1977	BT	Medium-maturing early-season japonica	Taizhong 65A/LiMing
Xiuling A	Liaoning Academy of Agricultural sciences (LAAS)		BT	Medium-maturing early-season japonica	JujinA/Xiuling
Fengjin A	LAAS		Dian I	Medium-maturing early-season japonica	Dian Type A/Fengjin
Liao 30 A	LAAS	2002	BT	Medium-maturing early-season japonica	LiMing A/LS2/Zhenzhujing//326
Liao 02 A	LAAS	2002	BT	Medium-maturing japonica	
Liao 105 A	LAAS	2004	BT	Medium-maturing early japonica	XiulingA/LS2/Zhenzhujing/Liaojing 294
Liao 5216 A	LAAS	2005	BT	Medium-maturing japonica	Qiuling A/Liao5216, and TiJin A, Liao40A, Liao60A, Liao846A, Liao326 A, 151A, 99A
Liuqianxin A	Jiangsu Academy of Agricultural Sciences (JAAS)	1978	BT	Mid-season japonica	AiganhuangA/Liuqianxin (691/ Qianchonglang //Zenith)
Zhuziqing A	JAAS	1981	BT	Mid-season japonica	LiuqianxinA/Zhuziqing
863A	JAAS	2000	BT	Late-maturing mid-season japonica	BT-type A/863 (Nanjing 36 siblings)
Shidao 8 A	JAAS		BT	Mid-season japonica	
9703A	Taihu Institute of Agricultural Sciences (TIAS), Jiangsu Province	2002	BT	Early-maturing late-season japonica	Hanfeng A/Wujin 9703
8006A	TIAS	2006	BT	Early-maturing late-season japonica	863A/8006

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Table 2 continued.

Name	Breeding institute	Release year	Type of cytoplasm	Ecotype	Source
Wuyunjing 7 A	Changshu Institute of Agricultural Sciences, Jiangsu Province	2002	BT	Early-maturing late-season japonica	Ai A/Wuyunjing 7
Lingfeng A (original name SAI)	Agricultural College of Yangzhou University (ACYU)	2006	BT	Medium-maturing mid-season japonica	Sidao 8 A/Kuifeng
Lingxiang A	ACYU		BT	Mid-season japonica	
YanJing 902A	Xuzhou Institute of Agricultural Sciences (XIAS), Jiangsu	1980	Dian I	Mid-season japonica	
Xu 9201A	XIAS	1996	BT	Medium-maturing mid-season japonica	Liming A/9201
Xu 91068A	XIAS	2003	BT	Medium-maturing mid-season japonica	The second grade of rice (national standard)
Xu 9320A	XIAS	2005	BT	Medium-maturing mid-season japonica	Xudao 2 A/Medium japonica Xu 9320
Xu 364A	XIAS	2006	BT	Medium-maturing mid-season japonica	9201A/Xu 364
Yanjing 5 A	Yandu Institute of Agricultural Sciences (YIAS), Jiangsu Province				
Yan 93538A	YIAS	2003	BT	Medium-maturing mid-season japonica	Sidao 8 A/Yanjing 93538
Aizhixiang A	Fenghe Rice Research Institute, Suqian County, Jiangsu	2003	BT	Mid-season japonica	LiuqianxinA/Aizhixiang
Nonghu 6 A	Wu County Institute of Agricultural Sciences, Jiangsu	1980	India wild	Late japonica	India wild rice/Nonghu 6

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Table 2 continued.

Name	Breeding institute	Release year	Type of cytoplasm	Ecotype	Source
Dangxuanwan 2 A	Anhui Academy of Agricultural Sciences (AAAS)	1978	BT	Early-maturing late japonica	Liming A/Dangxuanwan 2
80-4A	AAAS	1988	BT	Late-maturing mid-season japonica	Dangxuanwan 2 A/Huijing 80-4
Double 9A	AAAS	2002	BT	Early-maturing late japonica	80-4A/Double9 (LiuqianxinB/ Guandong 136
910A	AAAS	2005	BT	Mid-season japonica	23A/910
915A	AAAS	2005	BT	Late-maturing mid-season japonica	23A/915
Zhen 5A	Guangde Institute of Agricultural Sciences, Anhui	2002	WA	Early-maturing late japonica	Nonghu 26A/Zhenzhu 115
Pin 3A	Shanghai Academy of Agricultural Sciences (SAAS)	2000	BT	Late japonica	Hanfeng A/Pin 3
Hanfeng A	SAAS	1987	BT	Late japonica	Nongjin2A/Hanfeng (Kengui
/Keqing3//Liming)					
8204A	SAAS	1990	BT	Late japonica	Hanfeng A/Xiushui 04
5016A	SAAS	1999	BT	Late japonica	Hanfeng A/5016
Shen 6A	SAAS	2007	BT	Late japonica	8204A/Shen 6
Shen 6A	SAAS	2007	BT	Late japonica	8204A/Shen 6
Nongjin 2 A	Huazhong Agri-university	1980	BT	Early-maturing late japonica	Taizhong65A/Nongjin2
Nong 6-209A	Zhejiang Academy of Agricultural Sciences (ZAAS)	1980	BT	Early-maturing late japonica	
76-27A	Taizhou Institute of Agricultural Sciences, Zhejiang	1980	Dian I	Early-maturing late japonica	

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Table 2 continued.

Name	Breeding institute	Release year	Type of cytoplasm	Ecotype	Source
Zhe 04A	ZAAS	2007	BT	Early-maturing late japonica	Wuyunjing 7 A/Zhe 04 Xiushui 110/8204B tolerance of high temperature
Nonghu 26A	ZAAS			Medium japonica	
Ning 67A	Ningbo Institute of Agricultural Sciences (NIAS), and Ningbo Seed Co. (NSC), Zhejiang	1997	Dian I	Late japonica	
Yongjing 2 A	NIAS and NSC	2000	Dian I	Medium-maturing late japonica	
Hongmaoying A	Yunnan Agricultural University (YAU)	1969	Dian I		MS plant of Taibei 8/Hongmaoying
DianYu 1 A	YAU		Dian I		Hongmaoying A/Dianyu 1
Yumi 15A	YAU	2003	Dian I		Dianyu 1 A/Umi15
Yunjing 109A	Yunnan Academy of Agricultural Sciences		Dian I		Hongmaoying A/Dianyu 1
Jing 6A	Beijing Academy of Agricultural Sciences (BAAS)		BT	Medium japonica	
Zhongzuo 59A	BAAS	1997	BT	Medium japonica	
Zhao Hua 2 A	Tianjin Academy of Agricultural Sciences				
D Jie 71-1A	Jilin Academy of Agricultural Sciences	1974	Dian I	Early japonica	Hongmaoying/Ji 71-1
4788A	Rice Research Institute of Jiangxi Academy of Agricultural Sciences	2003	WT	Medium japonica	Dongxiang wild rice G4A/Jing 02982//0298; tolerance of cold, it can survive through winter
Chunjiang12 A	China National Rice Research Institute				

Table 3. Released three-line japonica hybrid rice combinations.

Province	No.	Year	Mid-season japonica	Late-season japonica
Liaoning	15	Before 2000 (4)	Liyou 57, Xiuyou 57, Tiyou 418, Fengyou 57 (cumulative extension area 1.50 million ha)	
		This century (11)	Liaoyou5218, Liaoyou0201, Liaoyou2006, Liaoyou1518, Liaoyou3225, Liaoyou1052, Liaoyou14 (aerobic rice), Liaoyou16, Liaoyou20, Liaoyou441, Liaoyou34188	
Beijing	4	Before 2000 (2)	Qiuyou 20, Zhongzha 1	
		This century (2)	Jingyou13 (lowland or upland rice), Jingyou14 (resistance to drought)	
Jilin	1	Before 2000 (1)	Jiyou 1	
Ningxia	2	This century (2)	Ningyou 1, Ningyou 3	
Tianjin	9	This century (9)	Jingjingzha 2, Jingjingzha 3, Jingjingzha 4, Jingjingzha 5, 3 You 18, 10 You 18, Zhongjingyou 1, Jingyou 29, Jingyou 2003	
Jiangsu	43	Before 2000 (17)	Yanyou 57, Yanyouke 57, 15 You C104-2, Liuyou 1, Liuyou 3-2, Liuyou 21, Xuyou3-2, Xuyou 8628, Siyou 522, Siyou 9083, Siyou 9028, Siyou 9092, Xuyou 3, 9 You 138	Siyou 422, Liuyouxuanfei, Liuyou 2730
		This century (26)	8 You 682, 69 You 8, 9 You 418, Liuyou 8, Liuyou 3, Siyou 523, Siyou 418, Siyou 88, Si You 9022, Siyou 12, Yanyou 1, Yanyou2, Xuyou201, Xuyou202, Xuyou301, Xuyou631, Lingxiangyou 18	Changyou 1, Changyou 2, Changyou 3, Changyou 4, 86 You 242, Suyou 22, Sujingyou 2, Sujingyou 3, 86 You 8

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Table 3 combinations.

Province	No.	Year	Mid-season japonica	Late-season japonica
Anhui	24	Before 2000 (8)	Liuyou 121, 80 You 121	Dangyou C Bao, Liuyou C Bao, Dangyou 82022, Liuyou 82022, 80 You 9, Dangyou 9
		This century (16)	You 98, 80 You 1, Jingfeng 19, Aiyou 18, 36 You 959, 9201A/R-8, T You 5, T You 6312, Aiyou 39, Tianxie 1, Tianxie 13	80 You 1027, 80 You 98, Shuangyou 3402, Shuangyou 4183, Shuangyou 3404
Zhejiang (including China National Rice Research Institute)	30	Before 2000 (6)		Taizha 1, Taizha 2, Huyou 1, Qiyou 2, Qiyou 6, Qiyou 7
		This century (24)		Jiayou 1, Jiayou 2, Jiayou 04-1, Jiayou 229, Jiayou 169, 8 You 8, 8 You 052, Yongyou 1, Yongyou 2, Yongyou 3, Yongyou 4, Yongyou 5, Yongyou 6, Yongyou 8, Yongyou 10, A1-6211, 02-E54, 02-E08, Zhayou 2146, Xiuyou 5, Xiuyou 169, Jialeyou 2, Chunyou 2, Chunyou 58
Shanghai	13	Before 2000 (5)		Hanyouxiangqing, 8 You Xiangqing, Hanyou 1027, 8 You 161, Mingyou 128
		This century (8)		Puyou 801, Shenyou 1, Shenyou 4, Shenyou 8, Shenyou 254, Shenyou 693, Qiuyou Jingfeng, Hua you 14
Hubei	3	Before 2000 (1)		Zhu you 5-2
Hubei		This century (2)		E You8, E You 9
Hunan	1	Before 2000 (1)		Huyou115
Yunnan	6	Before 2000 (2)	Yuzha29, Xunzha 29	
		This century (4)	Dianzha31, Dianzha32, Dianzha35, Dianzha36	
Total	151	Before 2000 (47)		
		This centuryy (104)		

Table 4. Selection standards and results of trials on Anhui three-line japonica hybrid rice in Japan (Mitsui Chemicals Inc., Tokyo, Japan, January 2008).

Selection standard	Check varieties	MH2003	MH2005	MH3001
Yield	Brown rice yield	23A/XH022 23A/XH05	23A/XH02 23A/XH043	25A/Liaohui8 25A/XH04 23A/XH019
Quality, taste	Quality analysis and total taste value	23A/XH022 23A/XH05	23A/XH043	25A/XHliaohui8 23A/XH019 45A/XH04
Characteristics of cultivation	Heading, tolerance of disease, and lodging	23A/XH022 23A/XH05	23A/XH02 23A/XH043	25A/XHliaohui8 45A/XH022 23A/XH019

Table 5. Performance of Anhui three-line japonica hybrid rice in Japan at Tsukuba Agri-center (2007).

Variety	Heading M/D	Brown rice yield (kg/0.1 ha)	Compared with CK1 (%)	Compared with CK2 (%)
23A/XH022	8/26	700	114	127
23A/XH04	8/26	624	102	123
23A/XH05	8/26	675	110	123
25A/XH02	8/26	566	92	103
25A/XH04	8/21	657	107	119
23A/XH019	8/24	633	103	115
23A/XH02	8/30	696	113	127
23A/XH043	8/28	723	118	101
23A/XH053	8/26	619	101	113
25A/XH019	8/20	511	83	93
25A/XH022	8/11	659	107	120
25A/Liaohui 8	8/24	881	143	160
21A/XH019	8/10	655	107	119
21A/XH019	8/11	674	110	123
45A/XH019	8/20	598	97	109
45A/XH022	8/21	607	99	109
01A/XH04	8/29	630	103	115
WA/XH04	8/27	654	107	119
MH2003 (check 1)	9/2	614	100	112
MH2005 (check 2)	8/30	550	90	100
Koshihikari	8/17	482	79	88

Table 6. Brief introduction on japonica PTGMS lines passing provincial technical evaluation.

Source	Breeding institute	Year of evaluation	Ecotype	Critical temperature and photoperiod for inducing sterility		Origin
				(°C)	(h)	
Nongken 58S(NK58S)	An area of original seeds in Shahu, Xiantao, Hubei	1985	Late japonica	>26	13.75–14	Natural MS plant from Nongken 58
N5047S	HAAS	1988	Late japonica	24	14.0–14.25	NK58S/5047
N5088S	HAAS	1992	Late japonica	24	13.5–14.0	NK58S/Nonghu26
N9643S	HAAS	1998	Late japonica	24	>14.0	NK58S/9643
N95076S	HAAS	1998	Late japonica	24		5088S/7001S
31111S	Huazhong Agricultural University	1988	Late japonica	24	14.0–14.75	NK58S/31111
6334S	Huazhong Normal University	1988	Late japonica			
1541S	Yichang Institute of Agricultual Sciences, Hubei	1989	Late japonica		13.75–14.0	
M105S	Wuhan University					^{60}Coγ radiating 105
WD1S	Wuhan University	1988	Late japonica		14–14.5	NK58S/WD1
Double 8-2S	Wuhan University	1988	Late japonica		14–14.25	NK58S/Double 8-2
7001S	AAAS	1989	Early-maturing late japonica	22	13.75–14.0	NK58S/917 (HuXuan19/IR661//C57)
8087S	AAAS	1993	Early-maturing late japonica	23	14.0	7001S/Zhao 107
3502S	AAAS	1993	Early-maturing late japonica	22.6	14.0	7001S/Pecos

Continued on next page

Table 6 continued.

Source	Breeding institute	Year of evaluation	Ecotype	Critical temperature and photoperiod for inducing sterility		Origin
				(°C)	(h)	
3516S	AAAS	1997	Early-maturing late japonica	23.5	14.0	N5047S/(7001S/Zhao107)
4008S	AAAS	1999	WC late japonica	24.0	14.0	7001S/Reyan 2
C407S	CAAS	1989	Japonica			Eyi MR
Zhenong 1S	ZAAS	1991	Late japonica			NK58S/
3008S	Hubei Agricultural College	1992	Japonica			NK58S/
N422S	Hunan Hybrid Rice Research Center, CAU	1994				7001S/lun hui 422
108S	NAAS					NK58S/9022
02428S	JAAS	1990				NK58S/
Liuqianxin S	JAAS					NK58S/
J-3S	JAAS					NK58S/
916S	JAAS					NK58S/
5021S	NAU and JAAS	2007				Short daylength of MS type. Natural mutant from Wu Yu 5021.
1647S	BAAS	2006				

Table 7. Major two-line japonica rice released.

Name	Type	Release year	Institute	Source	Accumulated area (000 ha)
Ejingzha 1	Late japonica	1995	HAAS	N5088S/R187	700
Huajingzha1	Medium-maturing late japonica	1995	HAU	7001S/1514	–
Huajingzha 2		2001	HAU	N5088S/41678	–
Ejingzha 2	Medium-maturing late japonica	2003	HAAS	N5088S/R183	16
Ejingzha 3	Medium-maturing late japonica	2004	HAAS	N5088S/Minghui128	25.4
70 You 9	Early-maturing late japonica	1994 in Anhui 2001 in China	AAAS	7001S/Wanhui 9	500
70 You 04	Early-maturing late japonica	1994	AAAS	7001S/Xiushui 04	190
70 You Double 9	Early-maturing late japonica	1997	AAAS	7001S/Shuang 9	25
40 You 04	Late japonica	1999		4008S/Xiushui 04	–
WanHanYou 1	Early-maturing medium japonica	2004 in China	AAAS and CAU	N422S/R8272	–
Yunguang 8	Plateau japonica	2000	YAAS	N5088S/Yunhui 11	21
Yunguang 9	Plateau japonica	2002	YAAS	7001S/Yunhui 124	15
Yunguang12	Plateau japonica	2003	YAAS	95076S/Yunhui 124	24
XinZhaJing1		2003	Xinyang Agricultural Institute, Henan	Peiai 64S/Yujing 3	32
LiangYouPeiJing		2003 in China	Xinyang Agricultural Institute, Henan	Peiai 64S/94205	–
Total					1,548.4

The combination of 70 You 04 yielded 15.5 t ha^{-1} in Midu County, Dali, of Yunnan; Ejingzha 1 yielded 13.02 t ha^{-1} in Yongping County, Yunnan; Yunguang 101, growing at a location at 1,900 m altitude, yielded 15.3 t ha^{-1}; Yunguang 12 had 15.18 t ha^{-1} of estimated yield on 66.7 ha of demonstration fields in Luliang County, Yunnan, in 2003 and 12.99 t ha^{-1} on 3.3 ha of demonstration fields at 1,901 m in Chongming County.

Because of the advantages of sterile stability, easy multiplication, high purity of hybrid seeds, freedom of hybridization, and nonrestrictions in the relationship between maintainer and restorer lines compared with three-line japonica hybrid rice, we can see a quick application of two-line japonica hybrid rice in production.

Constraints to developing japonica hybrid rice

Although great progress has been made in developing japonica hybrid rice, and the growing area of japonica hybrid rice has increased significantly in many provinces or municipalities since the 1980s, such as 31% of total japonica rice in Shanghai, 21.8% in Anhui, 10% in both Jiangsu and Hubei, and 6% in northern rice regions, the growing area of japonica hybrid rice has been declining gradually in recent years. The main constraints to japonica hybrid rice production are discussed below.

Yield increases with reduced standard heterosis

Standard heterosis means the yield advantage of a hybrid over a standard inbred check variety. Although yield of japonica hybrid rice has been increasing, however, it could not outpace the development of japonica inbred varieties because of the narrow genetic backgrounds in japonica rice using heterosis application. In addition, the grain quality of most japonica rice hybrids is inferior to that of japonica inbreds. In 1996, a research project for developing super inbred rice in northern China was started with a great number of resources and financial support. Many inbred varieties with erect and large panicles were developed. These varieties yielded more than 10.5 t ha^{-1} when grown in a large area (approx. 667 ha) and with a record of 12 t ha^{-1} on small production areas. Data from Liaoning showed that japonica inbred rice covered about 95.4% of the total growing area, whereas japonica hybrid rice covered only 4.6% of the growing area during 1974-97. It was also difficult for japonica hybrid rice to be successful because of its unstable yield. For example, in the regional yield trials of the southern rice regions, the average yield of five elite japonica hybrids surpassed that of inbred check varieties by only 4.3%, 7.8%, and 8.0% in Jiangsu, Zhejiang, and Shanghai in 2004, respectively. In 2005, the average yield advantages were only 0.1%, 2.9%, and 2.2%, respectively, at these locations. The yield gap between inbreds and hybrids declined significantly. Again, in 2006, the average yield advantage of japonica hybrid rice was –3.9% in Jiangsu and 1.5% in Shanghai because of a slightly higher (2–3 °C) temperature than usual during mid- and late August (Table 8).

The results showed that low seed set was the main reason for low yield heterosis in japonica hybrid rice (Table 8). Seed set was strongly influenced by environmental factors. For example, in the yield trials of the southern regions in 2006, the highest and lowest seed sets were 94.7% and 87.1% for JiaYou 04-1 and 78.0% and 52.7%

Table 8. Comparisons between japonica hybrid rice and inbred rice for average yield and seed setting of five elite varieties in Jiangsu, Zhejiang, and Shanghai regional trials during 2004-06.

Year	Region	Yield			Seed setting (%)		
		Hybrid (t ha^{-1})	Inbred (t ha^{-1})	(±%)	Hybrid	Inbred	(±%)
2004	Jiangsu	9.56	9.16	4.3	88.2	92.0	3.8
	Zhajing	8.69	8.06	7.8	83.5	90.2	6.7
	Shanghai	9.48	8.78	8.0	89.1	92.0	2.9
2005	Jiangsu	8.49	8.48	0.12	79.7	89.7	10.0
	Zhajing	8.73	8.49	2.9	81.6	90.9	9.3
	Shanghai	8.53	8.35	2.2	87.6	92.5	4.9
2006	Jiangsu	9.26	9.63	–3.9	82.4	93.0	10.6
	Shanghai	9.58	9.44	1.5	86.9	88.8	1.9

for Shengyou 1, respectively. The difference between the highest and lowest seed sets ranged from 7.6% to 25.3%. A study at Yangzhou University using 229 japonica rice combinations derived from a 16 × 16 diallel crossing, with inbreds of Wuyunjing 7 and Wuyunjing 3 as checks, found that 80% of the hybrid rice had high vegetative heterosis and high yield potential, but only minor or negative heterosis in seed set. However, 43% of the combinations had seed set higher than that in checks, indicating low seed set as the main constraint to yield heterosis of japonica hybrid rice, but with potential to be improved.

The second cause of the low standard heterosis of japonica hybrids is their large panicle size but insufficient panicle number. But this has been improved recently. Data obtained from trials of the southern rice region and Anhui in 1990 and 2003 showed that the difference in panicle number in japonica inbreds and hybrids decreased. For example, the average panicle numbers of japonica hybrids were –18.4% and –19.4% less than those of japonica inbreds, respectively, in 1990, whereas panicle numbers of hybrids increased to –1.7% and +3.6% of those of japonica inbreds in 2003. The data also showed not much improvement in seed set for both japonica and indica hybrids. However, a difference in seed set related to yield between indica and japonica hybrids was that yield of indica hybrid rice could be compensated for by heterosis of panicle size (Table 9).

In recent years, some new japonica rice hybrids with high standard heterosis have been developed. Two mid-season japonica hybrids, 9 You 138 and 9 You 418, developed by the Xuzhou Institute of Agricultural Science, yielded 8.9 and 9.4 t ha^{-1} in regional yield trials, respectively, and outyieded inbred check variety Yujing 6 by 9.5% and 11.4%. The total growing area for 9 You 138 was 330,000 ha in the 1990s. 9 You 418 performed better than 9 You 138, with a yield increase of 7.8% in the regional trials. The yield components of 9 You 418 are about 0.26–0.27 million panicles ha^{-1},

Table 9. Comparison of yield components between hybrid rice and inbred varieties.

Region trial	Variety type	Effective panicles ha^{-1}		Total grains per panicle		Filled grains per panicle		Seed setting	
		ha	±%	Grains	±%	Grains	±%	%	±%
Single-season late japonica regional trial in southern rice region, 1990	JHR	327.0	–18.4	141.5	+51.0	111.1	+39.6	78.5	–6.4
	Inbred variety Xiu Shui 04	400.5		93.7		79.6		84.9	
Single-season late japonica regional trial in Anhui, 1990	JHR	310.5	–19.4	127.7	+42.2	97.7	+33.1	76.5	–5.2
	Inbred variety Xiu Shui 04	385.5		89.8		73.4		81.7	
Indica mid-season regional trial in Anhui, 2003	Indica hybrid rice	259.5	+22.7	170.6	+2.7	127.1	–2.4	74.5	–3.9
	Indica inbred rice	211.5		166.1		130.2		78.4	
Japonica mid-season regional trial in Anhui, 2003	Japonica hybrid rice	261.0	–1.7	150.5	+9.0	111.4	+1.5	75.3	–5.2
	Japonica inbred rice	265.5		138.1		109.8		80.5	
Late double-season japonica trial in Anhui, 2003	Japonica hybrid rice	303.0	+3.6	121.7	+34.5	83.4	+12.1	70.2	–13
	Japonica inbred rice	292.5		90.5		74.4		83.2	

with 180–200 spikelets per panicle and 80–85% seed set. Its 1,000-grain weight is 27–28 g. The hybrid has a yield potential of 12 t ha^{-1}. In addition, the hybrid has good resistance to stripe leaf blight and excellent appearance at the maturity stage. It will gradually replace 9 You 138 in production. The annual growing area for this hybrid is about 100,000 ha and cumulative growing area has been about 700,000 ha. Other hybrids, such as Xuyou 201, Xuyou 631, and Xuyou 303, could yield more than 12 t ha^{-1} with high resistance to diseases and "grade 2" rice quality as scored by national quality standards. This indicates that breeding a variety with outstanding traits should be the key in developing japonica hybrid rice and no doubt this will come true.

Improved rice quality and disease resistance

Increasing parents' diversity could enhance the yield heterosis of F_1 hybrids, but also promote wide segregation for F_2 grain traits. Therefore, the quality of high-yielding japonica hybrids is generally inferior to that of japonica inbreds. Hua Zetian and Hao Xian Bin (2007) studied the quality traits of 36 japonica hybrids and 78 japonica inbred varieties released in China during 2000-06. Their analyses revealed that 39% and 49% of japonica hybrids did not have "grade 3" as classified by the national quality standards for chalky rate and chalkiness degree. Some 12% of the combinations did not attain grade 3 for head rice recovery and 6% did not attain grade 3 for gel consistency and amylose content. Chalkiness between hybrids and inbreds was the biggest difference among all of the quality traits. Ji Jianin et al (2006) analyzed the qualities of hybrids and inbreds sampled from the mid-season regional trials of Jiangsu and found that all quality traits of japonica hybrids were inferior to those of japonica inbreds. The chalky rates were 28.7% and 22.7% in two years, respectively, and 13.7% and 8.2% higher than those of inbred rice. Comprehensive quality scores of hybrids were 9.0–24.7% lower than those of inbreds. A genetic study on quality traits of japonica hybrids by Li et al (2006) showed that chalky rate and chalkiness degree were the main traits that directly influenced food taste, but had no effect on yield. So, a reduction in chalk should be a key for the simultaneous improvement in yield and quality of japonica hybrid rice.

Rice stripe disease, caused by SPBH (*Laodelphax striatellus* Fallen), has become more and more serious in the past few years. It threatens japonica rice, whether hybrid or inbred. Successful experience with resistance breeding for rice stripe disease in Japan and Korea indicated that breeding resistant varieties is the most effective method for controlling the disease. Chen Tao et al (2007) reported that the *Stvb-1* gene in variety Modan from Pakistan is one of the most deeply investigated genes and the most widely used resistance gene. By using molecular marker-assisted selection (MAS), an SSR marker, RM11-8, showed polymorphism between resistant parent Guandong 136 and susceptible parent Wujing 13. The progenies derived from these two parents were selected for resistance with a 90.7% accuracy by MAS. Thirty-nine lines with high yield and good grain quality were developed by 2007, and four of them went through provincial regional yield trials. Among all the lines, 37 lines that have a resistance band in molecular analysis showed high resistance in field identification. Tang et al

(2008) reported their studies on the improvement of resistance to japonica rice by using MAS, such as Wuyujing 3, Wuyujing 7, Wuyujing 8, and Guanglingxiangjing. The resistance of BC_5F_1 progenies was clearly enhanced. This indicated that not only MAS could be used as a tool for improving resistance, but it could also be used for improving other traits.

Low yield, low purity, and high cost in multiplication and seed production limit the extension of japonica hybrid rice

An elite hybrid in production should include its parents possessing good flowering traits and high yield in seed production besides other elite traits. This is crucial for production or a good hybrid could be eliminated from production because of low yield of seed production and low purity. Hybrids of 80 You 121 in Anhui, Yanyou 57 and Xu3-2 in Jiangsu, and Li You 57 in Liaoning have provided typical examples. A BT CMS line, 80-4A, which could be self-crossed or form an iso-cytoplasmic restorer under high temperature, caused very low hybrid seed purity in 80 You 121. The female parent for hybrids of Yanyou 57 flowers 1–1.5 hours behind the male parent, with up to 50 days of seeding interval between the two parents, and insufficient pollen load in the male parent caused a yield of less than 450 kg ha^{-1} in multiplication and 750 kg ha^{-1} in hybrid seed production. BT and Dian types of male sterile lines are usually both self-crossed and form an iso-cytoplasmic restorer. Tang et al (2008) investigated a seed production field of Liuyou 3 at Xiangshui, Jiangsu, in 1997 and found that the iso-cytoplasmic restorer plants of Liuqianxing A accounted for more than 10%. In a study of 2002, Liuqianxing A was bagged for selfing and results showed that 64.3% of the panicles were selfed, with seed set of 3.7% to 11.9%. PGMS lines could also be self-crossed under lower than critical temperature point at the sensitive stage of panicle formation during the male sterility stage. Furthermore, some male sterile lines, whether two-line or three-line, have a small angle of glume opening, late flowering time, and lower pistil extension rate. Their flowering traits and outcrossing rate are not as good as current major commercial indica male sterile lines. All these problems mentioned have restricted high yield in seed production and discouraged seed enterprises from taking production risks, and made them hesitant to advance.

Narrow adaptability of japonica hybrid rice limits its growing area

Mid- and late-season japonica rice hybrids are photosensitive. Most of the parents of japonica hybrid rice, especially restorers, have been integrated with partial genetic components of indica rice. So, all of this resulted in japonica hybrid rice being more sensitive to environmental conditions, as well as the high or low temperature at heading stage that caused an unstable seed set and restricted its growing regions. This is different from indica hybrid rice, which can be grown widely. For example, Shanyou 63 has shown wide adaptability and its annual growing area reached a high of 6.7 million ha. In addition, less attention and investment also caused slow development of japonica hybrid rice.

Suggestions for accelerating the development of japonica hybrid rice

Stable seed set and more panicles should be the main targets to increase standard heterosis

The lack of strong standard heterosis in japonica hybrid rice results from its narrow genetic base. This is indicated by unstable seed set and insufficient panicles, as mentioned above. Considering the difference in genetic bases between parents, a high seed set as in indica hybrid rice is not expected in japonica hybrid rice; however, parents can be selected for appropriate genetic difference, as well as moderate proportions of indica and japonica backgrounds. Wide compatibility (WC) genes can be introduced in breeding with high yield and high and stable seed set. Three-line japonica hybrid rice was based on a narrow genetic background because of the lack of restorer genes in japonica germplasm, but two-line hybrid rice is free from the restorer constraint. So, Yuan Longping (2006) suggested that developing two-line japonica hybrid rice should be the major target of japonica hybrid rice breeding in China, or at least in southern China.

Improving chalk quality and increasing multiple resistance abilities

Research showed that chalky rate and chalkiness degree are mainly controlled by additive or dominant genetic effects. So, parents have to be selected with a low chalky rate and degree of chalkiness to minimize F_2 segregation. Some combinations with high yield and that scored grade 2 in quality traits have been developed and released in southern and northern rice regions, such as Shenyou 4, Changyou 1, Changyou 2, Xuyou 201, Xuyou 631, Yongyou 3, III You 98 in the south; Liaoyou 2006 and Liaoyou 0201 in the north; Jingyou 29 in Tianjing; and Dianzhe 31 and Yunguan 101 in Yunnan. These combinations should be used extensively.

Stripe leaf blight and false smut have become more serious in China. Molecular technology-based breeding, including MAS, is the most effective method for introducing disease-resistance genes. It can also be used in pest-resistance breeding. Liu Hao Jie et al (2007) reported success in improving pest resistance for N5088. Marker-free transgenic plants carrying *Crylc* have been developed by using the co-transformation system of double agro-bacterial/double plasmid. The transgenic plants showed very good pest resistance and stable sterility. The new lines were named Hua 122S, Hua 125S, and Hua 218S. This research should be strengthened to accelerate its application in production as soon as possible.

Breeding new types of male sterile lines to overcome difficulties in seed production

BT and Dian-1 types of japonica male sterile lines can be easily self-crossed under high temperature. PGMS lines can also be self-crossed under lower than critical temperature. Therefore, breeding new types of male sterile lines with early flowering time, high stigma exsertion, and stable sterility will become the keys for overcoming the difficulties in japonica hybrid rice application. The following observations are noted:

1. Japonica male sterile lines with a partial indica genetic background should be bred by introgressing good indica traits such as early anthesis and high outcrossing.
2. Among multiple cytoplasmic male sterile lines, HL-type japonica male sterile lines performed better than others. They are more stable in male sterility and more easily restored for fertility in F_1 than the wild-abortive type. HL male sterile lines also have no obvious difference from the BT type in flowering traits. So, HL-type CMS lines might be used to replace part of the BT-type CMS in the future.
3. Protocols for MS seed production and purity examination should be strictly implemented. The method of 1 year of multiplication for 2 or 3 years' use should be promoted to prevent a reduction in male sterility as generations increase.
4. In recent years, Wang et al (2006) have developed and patented in China a new type of MS line called an SA line. The keys of this method are to use a BT-type sterile line as the donor of sterile cytoplasm (A line) and a PGMS line (S line) with CMS maintainer genes as a maintainer to develop a new type of male sterile lines through crossing and backcrossing. 2308SA and 2310SA have been developed by this method. The sterility of SA lines is controlled by two independent gene systems. Under high temperature and long daylength, a PGMS gene controls sterility to avoid self-fertilization of BT-type sterile lines. Under an appropriate temperature and long daylength, both genes control sterility. Under low temperature and short daylength, a CMS gene controls sterility, avoiding PGMS selfing. This innovative technique can guarantee the safety of japonica hybrid seed production. The yield of SA lines in multiplication approached 2.24 t ha^{-1} in spring 2008 in Hainan. Both hybrid seed production and hybrids performed very well and were expected to meet production requirements.

Breeding new combinations for different ecological regions by tackling key problems together

Mid- and late-season japonica rice has strong photosensitivity. Usually, different varieties have to be grown in different ecological regions to satisfy local requirements. This trait is controlled by specific genetics of japonica subspecies, and it is difficult for breeders to make any changes. But, parents with relatively weak photosensitivity can be selected and crossed with each other. Different ecotype combinations fitting different regions can be developed nationwide by breeders together. They should include early- and mid-season japonica hybrid rice suitable for northern China; mid- and late-maturing mid-season and late-season japonica hybrid rice suitable for southern China; and single-season japonica hybrid rice suitable for the southwest plateau of China. Varieties with drought tolerance, such as Wanhanyou 1, Liaoyou 3225, Tiyou418, and Jingyou 13, or with salinity and alkalinity tolerance, are also needed. In the 1980s, a group of hybrid rice combinations was developed by using germplasm from different provinces, and these hybrids were released by different provinces through a cooperative group for japonica hybrid rice in eastern China. These combinations included Huyou 115 (A lines from Zhejiang) in Hunan, Liuyou 1 in Jiangsu, Hanyou Xiangqing (R lines from Zhejiang) in Shanghai, and Liuyou C Bao (A lines from Jiangsu) in Anhui. It is a very good example that the breeders in Anhui, by cooperating with Japanese breeders and using male sterile lines from Japan, successfully developed new hybrids and released them in both countries.

Developing cultivation technology for high yield, high quality, and cost-saving

The experiments carried out in Jiangsu and Anhui in the 1990s showed that japonica hybrid rice differed significantly from indica hybrid rice in grain filling. In terms of dry matter formation in panicles of japonica hybrids, the ratio of the dry matter contributed from culm and sheath to the total dry matter of the panicle ranged from 9.5% to 13.8% and the ratio of dry matter synthesized after heading to the total dry matter of the panicle was 86.2% to 90.5%. However, in indica hybrids, the two ratios were 25.4–28.1% and 71.9–75.6%, respectively. This significant difference indicated that, in the late growth stage, the dry matter transferred from culm and sheath and grain-filling rate in indica hybrids were higher than those in japonica hybrids, which was favorable for seed set and high yield. In japonica hybrids, the net dry matter synthesized in the late growth stage contributed much more to yield than in indica hybrids, but the transferring rate from culm and sheath to panicles was lower with a long grain-filling duration. For root vigor, japonica hybrids had a more vigorous root system and more active canopy and higher biological function of leaves in the late growth stage than those in indica hybrids. The nitrogen content of leaves at 20–30 days after heading decreases by 15% in japonica hybrids but by about 25% in indica hybrids and with the same trend for chlorophyll content. Therefore, for japonica hybrid cultivation, assimilates of the transferring materials in the late growth stage should be strengthened through the application of fertilizer, especially by topdressing of potassium at the grain-filling stage. Irrigation should also be practiced in the late growth stage to obtain full advantage of japonica hybrids in biological functions of leaf and root vigor, so that seed set and yield could be improved.

In addition, simple cultivation techniques for saving costs and increasing efficiency should be developed. These include direct seeding, seedling throwing, machine transplanting, and machine harvesting to overcome scarce labor and lower costs in rural areas.

Strengthening leadership, enlarging investment, and speeding up the development of japonica hybrids

Starting in 2004 and managed by academician Yuan Longping, regular annual meetings on national japonica hybrid rice technology innovation have been held in China. In 2005, the Tianjin Hybrid Rice Research Center was established. Research for japonica hybrid rice was listed in the Premier Foundation Project in 2006, and subsequently listed in the National Science and Technology Projects in 2007. Then, development of japonica hybrid rice appeared on the national agenda. A national coordination mechanism was formed and an innovation platform was also preliminarily established. All of this encouraged researchers to advance this opportunity to re-double our efforts in developing japonica hybrid rice. At the same time, we also hope that government agencies at all levels and Prof. Yuan Longping persistently pay attention to japonica hybrid rice, increase research investment, strengthen management, and promote more enterprises to join japonica hybrid rice research. All of this will promote the rapid development of japonica hybrid rice. The target of covering 30% of the japonica rice-growing area with japonica hybrid rice should be just around the corner.

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Notes

Author's address: Rice Research Institute, Anhui Academy of Agricultural Sciences, Hefei, Anhui 230031, China. Email: lichqu@mail.hf.ah.cn.

Applications of biotechnology in hybrid rice breeding

Developing highly insect-resistant transgenic super hybrid rice and the effects of transgenic rice on nontarget arthropod population density

Song-Biao Chen, Jun Su, Zhao-Yang Deng, Qiang Fu, Hong-lin Xu, Jian-Min Chen, Hong-Li Zhai, Zai-Jie Chen, Xiao-Li Wei, Chang-Quan Hu, Jing-Wan Yan, Yue-Ren Li, Wan-Kui Gong, Xiang Liu, Guifang Xiao, Hua-An Xie, Feng Wang, and Zhen Zhu

Transgenic technology provides a new approach to breed insect-resistant crops for controlling pests. Here, we report on the development of a transgenic line of a super hybrid indica rice restorer line, Minghui86, which carries *sck*, a modified cowpea trypsin inhibitor gene, and a *Bacillus thuringiensis* δ-endotoxin gene, *crylAc*. The transgenic Minghui86 (MSB), and its derived hybrid combinations II-A/MSB and SE21s/MSB, exhibited high protection against rice lepidopteran pests, and still possessed super high-yielding characteristics. The arthropod population density in rice fields was also investigated, and the results showed that the biodiversity of arthropods in transgenic rice paddies was relatively higher than in nontransgenic rice paddies. The two transgenic super hybrid rice combinations were approved by the Ministry of Agriculture of China for production trials and national rice regional trials in 2005.

Rice is one of the world's most important food crops, but is susceptible to attack by a wide range of herbivorous insects. The most destructive insects for rice are the lepidopteran pests, which cause estimated losses of 10–30% of total yield (Herdt 1991). Conventional control of lepidopteran pests in rice cultivation still depends mainly on the use of chemical insecticides, but the long-term and massive use of insecticides has caused public concern about food safety and environmental pollution (Xu et al 1996). The control of lepidopteran pests through breeding of insect-resistant rice could be a sustainable approach, but this cannot be achieved by conventional breeding because no known rice germplasm has enough insect resistance. Since the early 1990s (Fujimoto et al 1993, Nayak et al 1997, Cheng et al 1998, Maqbool et al 1998, Tu et al 2000, Khanna and Raina 2002, Breitler et al 2004), advances in rice biotechnology have brought success in producing insect-resistant rice by introducing insecticidal genes into rice. This transgenic breeding technology now offers an effective approach to control insect predation of rice.

The super hybrid rice breeding program began in China in the late 1990s. China has now succeeded in developing super hybrid rice with a 10–15% increase in yield over traditional hybrid rice. However, the super high-yielding attributes of super hybrid rice are closely associated with vulnerability to insect outbreaks. To develop insect-resistant super hybrid rice, the *sck* gene, a modified cowpea trypsin inhibitor gene that contains a signal sequence to a subcellular target that can guide the cowpea trypsin inhibitor (CpTI) into endoplasmic reticulum (Deng et al 2003), was used to produce transgenic rice. The parental rice material, Minghui86 (MH86), is a super hybrid indica rice restorer line bred in China in the late 1990s and one of its derived hybrid combinations, II-3A/MH86, achieved a new world output record, reaching 17.95 tons ha^{-1} in Yongsheng County, Yunnan Province, China, in 2001 (http://english.people.com.cn/english/200109/12/ eng20010912_80001.html). The *sck* gene has been proven to express an increased CpTI accumulation and result in higher insect resistance than the wild *cpti* gene in transgenic tobacco plants (Deng et al 2003). In transgenic rice, assays based on the inhibitory activity of leaf protein extracts (Nayak et al 1997) showed that the average CpTI accumulation level of 20 independently derived T_0 *sck*-transformed rice plants was 2.3 times higher than that of 16 independently derived T_0 *cpti*-transformed rice plants. Field tests at Fuzhou, Fujian Province, China, in 1999, showed that *sck*-transformed rice lines were significantly more resistant to rice stem borers than nontransgenic control plants. The percentage of tillers with whiteheads and percentage of dead spikes of 19 T_1 transgenic lines were 5.7% and 1.2%, while those of control plants were 67.4% and 25.5%, respectively. The *sck*-transformed rice lines also showed a relatively higher insect resistance than the *cryIAc*-transformed rice lines. Only 6.8% *sck*-transformed lines were attacked seriously by insects, with a pest severity index of >60%, whereas 32.2% *cryIAc*-transformed lines had a pest severity index of >60%.

Materials and methods

Plasmid construct and plant transformation

The *sck* gene was obtained by modifying the *cpti* gene in our laboratory. The *sck* gene controlled by the rice *actin1* promoter (McElroy et al 1990) and the *cryIAc* gene (Cheng et al 1998) controlled by the maize *ubiquitin* promoter (Christensen and Quail 1996) were inserted into the plasmid pCAMBIA1300 (www.cambia.org/main/r_et_1300_1301_c.htm) to generate plant expression vector pCUBAC-Hyg. The pCUBAC-Hyg was electroporated into *Agrobacterium tumefaciens* LBA4404. The calli from immature embryos of indica rice restorer line Minghui86 were used for *Agrobacterium*-mediated transformation (Hiei et al 1994). Stably transformed plants were selected for hygromycin resistance.

Molecular analysis

Total genomic DNA was extracted from leaves, as described by Murray and Thompson (1980). PCR was carried out under standard conditions with oligonucleotide primers for *hpt*, *sck,* and *cryIAc* genes, resulting in fragments of 0.85 kb, 0.42 kb, and 0.74 kb, respectively. Primer sequences were 5′-TACACAGCCATCGGTCCAGA-3′ and 5′-TAGGAGGGCGTGGATATGTC-3′ for *hpt*, 5′-AAAATGAAGAGCACCATCTTC-3′ and 5′-TCTAGAGTTCATCTTTCTCATC-3′ for *sck*, and 5′-TGCAGAGAGCTTCAGAGAGTG-3′ and 5′-ACACCCTGACCTAGTTGAGC-3′

for *cryIAc*. For Southern blot analysis, about 20 μg of genomic DNA digested with restriction endonuclease (NEB) was separated through electrophoresis in 1.0% agarose gel and blotted onto Hybond-N$^+$ nylon membranes (Amersham Pharmacia). Blots were hybridized with *sck*, *cryIAc*, *hpt,* and *cre* using the PCR-generated fragments of *sck*, *cryIAc*, *hpt,* and *cre* (primers and conditions as described above) labeled with [α-^{32}P] dCTP as probes.

ELISAs

CryIAc and CpTI protein levels in the transgenic rice leaves were analyzed by the double antibody sandwich double method (Clark and Adams 1977) and were indicated as the percentage of total soluble protein. Total soluble protein content in the transgenic rice leaves was quantified by Bradford's method (Bradford 1976).

Plant development

Transgenic plants were maintained in the greenhouse to produce selfed seeds. T_1 seeds collected from primary T_0 transgenic rice plants were germinated aseptically on MS medium containing 50 mg L^{-1} hygromycin for about 10 days to assay hygromicin resistance. Based on a hygromycin resistance assay and PCR analysis, the seeds from T_1 transgenic plants with homozygous *cryIAc* and *sck* genes were selected and subsequently used for developing homozygous offspring. A transgenic restorer line, MSB, in its sixth generation was then screened. The nontransgenic MH86, the nontransgenic hybrid combinations produced from the cross II-A × MH86 (IIyouming86) and SE21s × MH86 (Lingyouming86), the transgenic MSB line, and the transgenic hybrid combinations produced from the cross II-A × MSB and SE21s × MSB were used for our study. II-A is an elite indica cytoplasmic male sterile (CMS) line and SE21s is an elite indica genic male sterile (TGMS) line.

Insect bioassays

Insect bioassays were conducted using the tube or petri dish testing method. For the striped stem borer (*Chilo suppressalis* Walker), yellow stem borer (*Scirpophaga incertulas* Walker), and pink stem borer (*Sesamia inferens* Noctuidae), about 3 stems (approx. 10 cm) were placed on a moistened cotton pad in a tube (25 mm diameter, 200 mm length) and infested with 11 first-instar larvae. About 9–10 replications were carried out. For the leaffolder (*Cnaphalocrocis medinalis* Guenee) and rice swift (*Borbo cinnara* Wallace), about 3 leaves (approx. 10 cm) were placed on a moistened cotton pad in a petri dish (100 mm diameter) and infested with 11 first-instar larvae. About 5–10 replications were carried out.

Field evaluations

Field evaluations were conducted at the experimental farm of the China National Rice Research Institute, Hangzhou, and the experimental station of the Fujian Academy of Agricultural Sciences, Fuzhou.

At Hangzhou, the field trial had three treatments: the transgenic line MSB and the nontransgenic control line MH86 untreated and treated with chemical insecticides, arranged in a randomized block design, with three replications. The plots were about 60–80 m^2 and rice plants were spaced at 20 cm × 20 cm (one plant per hill). The seeds were sown on 11 May and the plants were transplanted on 17 June. Plants were cultivated under normal agronomic practices and no chemical treatment was applied, except in the control treated plots. For MH86 treated with chemical insecticides, buprofezin (750 g ha^{-1}) and fipronil (600 mL ha^{-1}) were sprayed on 17 July, and buprofezin (750 g ha^{-1}), fipronil (600 mL ha^{-1}), and bisultap (3,750 g ha^{-1}) were sprayed on 1 August. The damage symptoms were checked weekly from 16 July to 10 September. Sixteen randomly chosen sites (2 plants per site) were investigated per plot. The parameters used for measuring the severity of plants were the percentage of hills with deadheads and percentage of tillers with deadheads for damage by SB, and the percentage of hills with folded leaves and damage index for damage by LF. The damage index was scored by [(scraped leaves with Grade 1 × 1 + scraped leaves with Grade 2 × 2 + scraped leaves with Grade 3 × 3)/(total leaves × 3)] ×100. The grade of damage to the leaves scraped by LF was scored with Heinrich's scale (Heinrichs et al 1985). The population densities of whitebacked planthopper, green rice leafhopper, and spiders were investigated on 23 July and 6 August. Five randomly chosen sites (10 plants per site) per plot were sampled using an improved insect collector.

At Fuzhou, the transgenic line MSB, the transgenic hybrid combinations II-A/MSB and SE21s/MSB, and their nontransgenic control MH86, IIyouming86 (II-A/MH86), and Liangyouming86 (SE21s/MH86) were planted like a chessboard to evaluate the insect resistance of transgenic rice from comparative field testing. The area consisted of 72 elementary plots and each plot was about 3.0 m × 3.0 m. Rice plants were spaced at 20 cm × 20 cm (one plant per hill). Plants were cultivated under normal agronomic practices except that no chemical treatment was applied.

For the hybrid's yield evaluation, the plots of the transgenic hybrid combinations II-A/MSB and SE21s/MSB, and their nontransgenic controls, IIyouming86 (II-A/MH86) and Liangyouming86 (SE21s/MH86), and an elite hybrid combination, Shanyou63, were arranged in a randomized block design, with three replications. The elementary plots were 22.2 m × 6 m and rice plants were spaced at 20 cm × 20 cm (one plant per hill). The seeds were sown on 8 May and the plants were transplanted on 1 June. Plants were cultivated under normal agronomic practices and a chemical treatment was applied to allow the evaluation of the actual yield of nontransgenic controls.

For a community-level evaluation of arthropod population density, the plot design was similar to that for the hybrid's yield evaluation. Plants were cultivated under normal agronomic practices except that no chemical treatment was applied. The population densities of arthropods were investigated at the tillering and booting stages. Five randomly chosen sites (0.25 m^2 per site) per plot were sampled using an improved insect collector.

The field trials were authorized by the Ministry of Agriculture of China (references of the corresponding application files: environmental release of insect-resistant rice, transformed with double genes, RABt(c)SCKH in Beijing and Fujiang Province, 2002-T053).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by a multiple comparison test.

To broaden the resistance range against different pests and prevent the resistance evolution of insects (Zhao et al 2003), the *sck* and *Bacillus thuringiensis* δ-endotoxin gene *cryIAc* (Maqbool et al 1998) were simultaneously used to develop insect-resistant super hybrid rice. The *sck* and *cryIAc* were under the control of the rice *actin1* promoter (McElroy et al 1990) and the maize ubiqutin promoter (Christensen and Quail 1996), respectively. A total of 45 independently transformed plants were obtained through *Agrobacterium*-mediated transformation (Hiei et al 1994). Transgenic plants were transferred to soil for assay and producing selfed seeds. Homozygous lines at the T_2 and successive generations were then screened as confirmed by a hygromycin resistance assay and molecular analysis. Two homozygous lines, designated as MSA and MSB, with stable insecticidal gene expression, were selected at the T_3 generation. The MSA and MSB lines were selfed for up to six generations (T_8) in 2004, and the stable co-inheritance and co-expression of *cryIAc* and *sck* were examined at every generation. At the T_6 generation, the average levels of CpTI and CryIAc of MSA plants estimated by ELISA were about 0.17% and 0.08% of total soluble protein, and those of MSB were about 0.22% and 0.07% of total soluble protein, respectively. The MSA and MSB plants had high toxicity to major kinds of lepidopteran pests of rice. In the bioassay in 2002, the corrected mortalities of striped stem borer (*Chilo suppressalis* Walker) (SSB) and leaffolder (*Cnaphalocrocis medinalis* Guenee) (LF) fed with the cut tissues of plants at the tillering stage were 98.9–100% (Table 1). The corrected mortalities of two pests fed with the cutting tissues of plants at the grain-filling stage declined a little, but still remained very high (Table 1). In the bioassay in 2003 (Table 2), the corrected mortalities of yellow stem borer (*Scirpophaga incertulas* Walker) (YSB), LF, and rice swift (*Borbo cinnara* Wallace) (RS) were 100% for both MSA and MSB in 48 h, and the corrected mortalities of SSB reached 91.2% and 97.1%, respectively, for MSA and MSB in 120 h. For the pink stem borer (*Sesamia inferens* Noctuidae) (PSB), the corrected mortalities were 63.4% and 58.5%, respectively, for MSA and MSB after feeding for 120 h. However, although nearly half of the PSB survived after the feeding bioassay, the development of the surviving pests was notably retarded.

The MSB line, with better agronomic performance, was used to produce hybrid combinations II-A/MSB and SE21s/MSB. II-A is an elite indica cytoplasmic male sterile (CMS) line and SE21s is an elite indica genic male sterile (TGMS) line. Field investigation at Fuzhou in 2002 showed that the major agronomic traits of the two transgenic hybrid rice combinations were similar to those of the two nontransgenic control combinations, II-A/MH86 and SE21s/MH86 (Table 3). This suggested that the insertion and expression of the two foreign insecticidal *cryIAc* and *sck* genes did not influence the high yield potential of MSB. The two transgenic hybrid rice combinations were also compared with Shanyou 63, the most widely used hybrid combination in rice production in China over the past 20 years. The observed yields of II-youming 86 and Liangyouming 86 were about 15.5% and 6.3% higher than that of the control

Table 1. Mortality of larvae of striped stem borer (SSB) and leaffolder (LF) on transgenic rice lines at different stages.

Stage of rice	Rice material	Striped stem borer		Leaffolder	
		Survival rate (%)	Corrected mortality (%)	Survival rate (%)	Corrected mortality (%)
Tillering	MSA	0.0 ± 0.0 C[a]	100.0	0.0 ± 0.0 B	100.0
	MSB	0.9 ± 0.9 C	98.9	0.0 ± 0.0 B	100.0
	MH86 (CK)	85.5 ± 3.4 A	0.0	89.1 ± 5.3 A	0.0
Grain filling	MSA	6.4 ± 3.0 BC	92.8	1.8 ± 1.2 B	98.1
	MSB	18.2 ± 7.1 B	79.3	5.5 ± 5.5 B	94.3
	MH86 (CK)	87.9 ± 3.4 A	0.0	95.5 ± 3.0 A	0.0

[a] Means within the same column followed by the same letter are not significantly different at the 0.01 level.

Table 2. Insecticidal activity of transgenic rice lines for major rice lepidopteran pests.

Pest	Corrected mortality (%) in 48 h		Corrected mortality (%) in 120 h	
	MSA	MSB	MSA	MSB
Yellow stem borer	100.0	100.0	–	–
Rice leaffolder	100.0	96.1	–	100.0
Rice swift	100.0	91.7	–	100.0
Striped stem borer	17.1	14.6	91.2	97.1
Pink stem borer	8.1	11.3	63.4	58.5

Shanyou 63, respectively (Table 3). The super high-yielding characteristics therefore ensured the high commercial value of the transgenic hybrid rice.

The MSB and its hybrid combinations showed significantly high resistance to SB and LF under natural field conditions. In the field test at Fuzhou in 2002, while the nontransgenic MH86 and its hybrid combinations II-A/MH86 and SE21s/MH86 were attacked seriously by LF and SB, the MSB, II-A/MSB, and SE21s/MSB showed very high resistance (Fig. 1). In the field test at Hangzhou, Zhejiang Province, in 2002, the percentage of hills with whiteheads, percentage of tillers with whiteheads, and percentage of hills with folded leaves on MSB were less than 7.3%, 1.5%, and 16.7% during the entire investigation period from 16 July (tillering stage) to 10 September (grain-filling stage), in contrast to 45.8%, 8.4%, and 100.0% at maximum hills with

Table 3. Field performance of transgenic super hybrid rice combinations under field conditions (Fuzhou, China, 2002).

Hybrid combinations	Panicles per plant	Total grains per panicle	Filled grains per panicle	Seed setting rate (%)	1,000-seed weight (g)	Yield performance		
						Observed yield (t ha^{-1})^{a}	Compared with check 1 (%)	Compared with check 2 (%)^{b}
II-A/MSB	11.8	150.2	132.4	88.2	28.5	9.51	15.5	0.8
Ilyouming 86^{c} (check 2)	11.9	152.9	137.8	90.1	28.0	9.43	14.6	–
SE21s/MSB	12.9	139.7	120.6	86.3	27.5	8.76	6.3	−0.8
Liangyouming 86^{d} (check 2)	12.3	141.0	126.6	89.8	27.5	8.83	7.2	–
Shanyou 63 (check 1)	11.5	136.1	120.9	88.8	28.2	8.23	–	–

[a] The observed yield was measured by converting the production of the three subplots into tons per hectare. [b] For II-A/MSB, check 2 was the combination Ilyouming 86 and, for SE21s/MSB, check 2 was Liangyouming 86. [c] Combination Ilyouming 86 was made from sterile line II-A and restorer line nontransgenic Minghui 86. [d] Combination Liangyouming 86 was made from sterile line SE21s and restorer line nontransgenic Minghui 86.

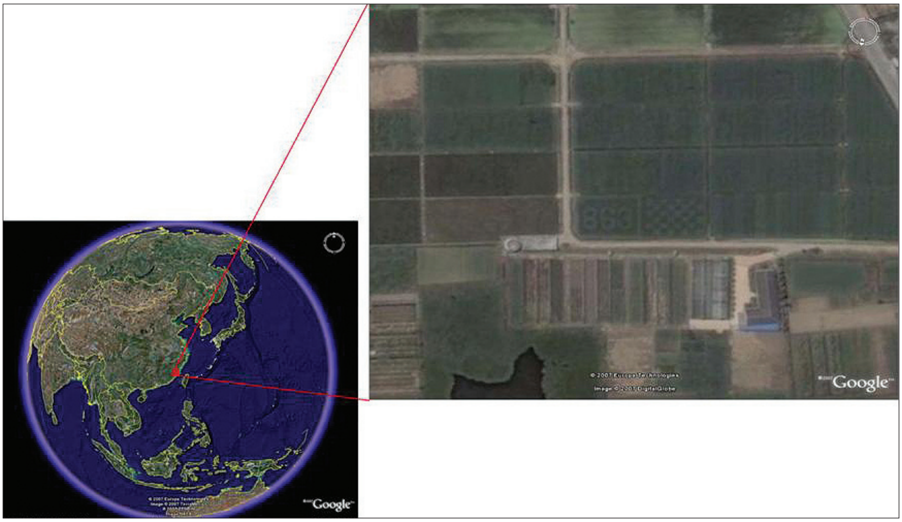

Fig. 1. Field performance of the transgenic restorer line MSB and its hybrid combinations II-A/MSB and SE21s/MSB under heavy outbreaks of leaffolder (Fuzhou, China, 2002). To the left of the yellow line are transgenic restorer line MSB and nontransgenic control Minghui86. To the right of the yellow line are transgenic combinations II-A/MSB and SE21s/MSB and nontransgenic control combinations IIyouming86 (II-A/MH86) and Liangyouming86 (SE21s/MH86). The transgenic rice and nontransgenic rice were planted like a chessboard for comparative testing; II-A/MSB and SE21s/MSB were arranged in a randomized block design. All lines and combinations were sown on 8 May and transplanted on 1 June.

folded leaves on nontransgenic control MH86, respectively (Fig. 2A,B). Compared with the nontransgenic MH86 with chemical insecticide treatment, the MSB also showed slightly better controlled effects on SB and LF (Fig. 2A,B).

Meanwhile, the population densities of arthropod organisms in transgenic and nontransgenic rice fields were investigated. The individual number of phytophagous insects in transgenic rice fields was significantly lower than that in nontransgenic rice fields at both the tillering and booting stages and the individual number of parasitic insects in transgenic rice fields was a little lower (Fig. 3A,B). The decrease in parasitic insect densities might be closely related to the great decrease in moth-pest populations in transgenic rice fields. For neutral insects and predatory natural enemies, although the individual numbers in transgenic rice fields and nontransgenic rice fields were similar at the tillering stage, the individual numbers in transgenic rice fields were significantly higher than those in nontransgenic rice fields at the booting stage (Fig. 3A,B), suggesting that the nonpesticide appropriate milieu in transgenic rice fields might provide neutral insects with a fitting habitat, leading to a quantitative proliferation of neutral insects and predatory natural enemies. Differences in the arthropod population density between the transgenic and nontransgenic fields were found in transgenic cotton (Sims 1995), maize (Orr and Landis 1997, Pilcher et al 1997, Jasinski et al 2003), and potatoes (Riddick and Barbosa 1998). Our results coincided

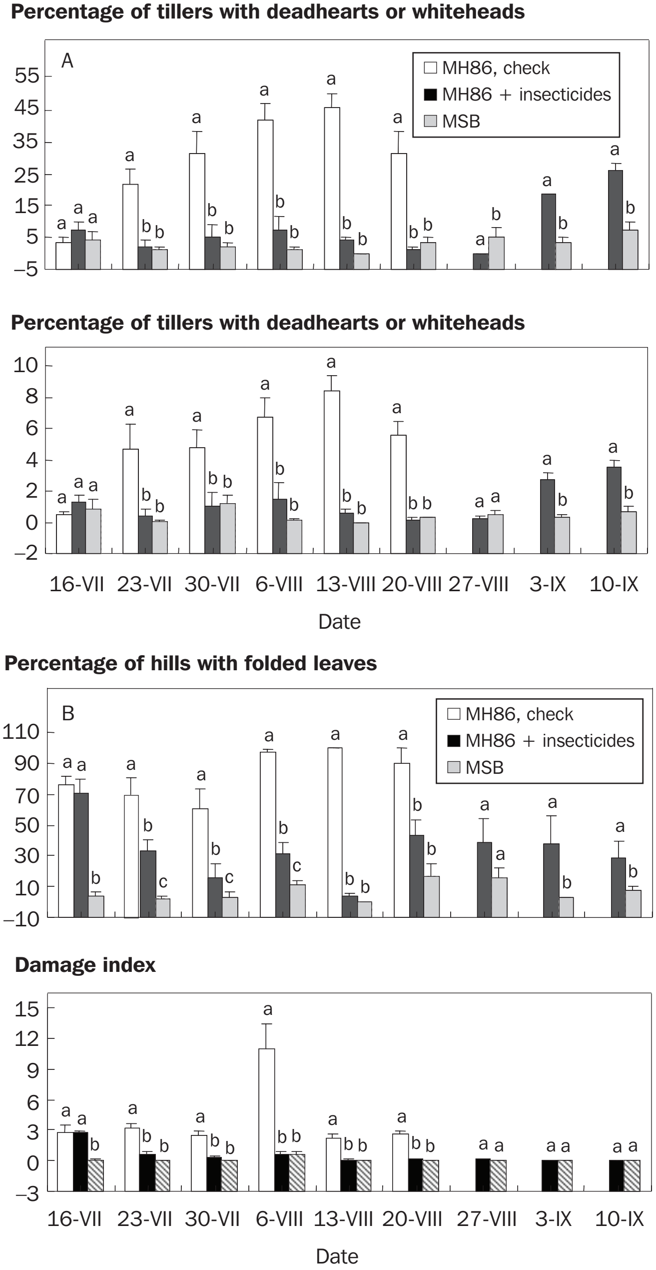

Fig. 2. Resistance reaction of the transgenic restorer line MSB against stem borers and leaffolder under field conditions (Hangzhou, China, 2002). (A) Damage from stem borers to different rice materials. (B) Damage from leaffolder under field conditions. The corresponding values followed by the same letter are not significant at the 0.05 level on the same sampling date (Duncan's multiple range test). Insecticides applied were fipronil, bisultap, and buprofezin.

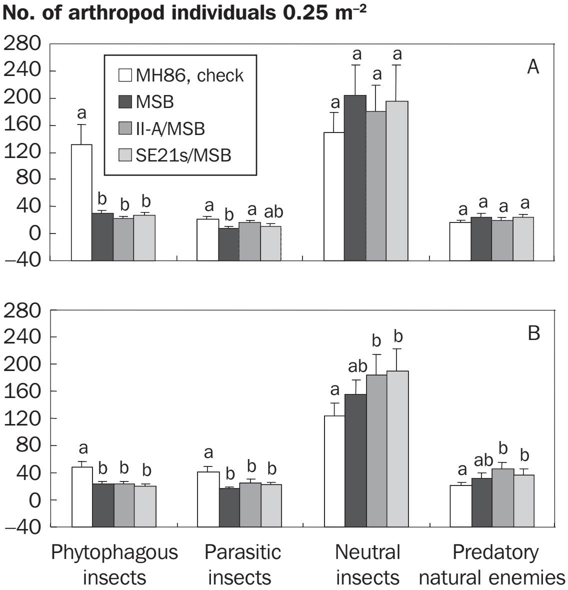

Fig. 3. Population densities of arthropod community in transgenic and nontransgenic rice fields at tillering (A) and booting stages (B). The corresponding values followed by the same letter are not significant at the 0.05 level on the same sampling date (Duncan's multiple range test).

with those results, and showed that the biodiversity of arthropods in transgenic rice paddies was relatively higher.

In addition to field tests of transgenic rice, we evaluated the food safety and nutritional content of transgenic rice in cooperation with the Chinese Center for Disease Control and Prevention starting in 2001. The test items included nutritional composition analysis, nutritional bioavailability evaluation, immunotoxical assessment, teratogenicity test, a 90-day-feeding subchronic toxicity study in rats, and 60-day-feeding studies in pigs. Up to now, all the measured variables of the transgenic rice were similar to those of the nontransgenic parental rice, indicating substantial equivalences of nutritional bioavailability and food safety between transgenic rice and nontransgenic rice. Our results suggested that the use of insect-resistant transgenic hybrid rice represented a sustainable alternative control method that may facilitate integrated pest management in rice production systems long dominated by the use of broad-spectrum insecticides.

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Notes

Authors' addresses: Song-Biao Chen, Zhao-Yang Deng, Hong-lin Xu, Hong-Li Zhai, Xiao-Li Wei, Wan-Kui Gong, Xiang Liu, Guifang Xiao, and Zhen Zhu, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101; Jun Su, Chang-Quan Hu, Jing-Wan Yan, Hua-An Xie, Feng Wang, and Zai-Jie Chen, Fujian Provincial Key Laboratory of Agricultural Genetic Engineering, Fujian Academy of Agriculture Sciences, Fuzhou 350003; Qiang Fu, China National Rice Research Institute, Hangzhou 310006; Yue-Ren Li, Plant Protection Institute, Fujian Academy of Agriculture Sciences, Fuzhou 350003, China. Feng Wang: tel.: (86-10-87884694), fax: (86-10-87884694), email: wf@fjage.org. Zhen Zhu: tel.: (86-10-64852890), fax: (86-10-64852890), email: zzhu@genetics.ac.cn.

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Understanding the molecular mechanism of rice pollen development

Zhang Dasheng, Tan Hexin, and Zhang Dabing

Male sterile lines are critical for hybrid rice breeding, and evidences showed that the rice male sterile lines used have various defects during pollen development, for instance, abnormal tapetum degradation. However, the molecular mechanism of rice pollen development remains poorly understood. To understand this, we identified six key regulators for rice pollen development using the forward genetics approach. Among these, *TDR* (*Tapetum Degeneration Retardation*) and *OsMS6* have been shown to regulate tapetum degeneration by triggering a programmed cell death (PCD) process during pollen development. Four other genes have abilities to regulate lipid metabolism, sugar transport, and signal transduction during pollen maturation. In addition, expression profiling using microarray experiments revealed down-regulated and up-regulated genes in the *tdr* mutant. Some target genes have been shown to be directly regulated by TDR through a chromatin immunoprecipitation (ChIP) assay. The revealed network controlling rice pollen development is discussed, along with its possible use for developing new male sterile varieties for hybrid rice breeding.

Keywords: male sterile, hybrid rice, genes, pollen development, tapetum

The life cycle of flowering plants alternates between the generation of diploid sporophytes and haploid gametophytes, and male pollen grains/gametophytes are produced in the anther locule of the stamen, which requires cooperative interactions between sporophytic structure and gametophytic tissue (Scott et al 1991, Goldberg et al 1993, McCormick 1993, Ma 2005). The anther has four lobes with similar structure attached to a central core with connective and vascular tissue. When anther morphogenesis is completed, the microsporocytes of each anther lobe are surrounded by four somatic layers, that is, the epidermis, the endothecium, the middle layer, and the tapetum (Goldberg et al 1993). As the innermost of the sporophytic layers of the anther wall, the tapetum directly contacts the developing microspores, and plays an essential role in development from microspores to pollen grains (Pacini 1990, Shivanna et al 1997).

Rice (*Oryza sativa*) is one of the most important crops feeding the world, and it has become an excellent monocot model crop for genetic, molecular, and genomic studies (IRGSP 2005). Understanding the molecular mechanism of rice anther and pollen development is crucial for future hybrid rice breeding. Molecular studies have identified several genes participating in controlling anther and pollen development in rice, including the genes regulating cell differentiation, meiosis, pollen development, and anther dehiscence (Fig. 1) (Tan et al 2007).

During early anther development, a putative leucine-rich-repeat receptor kinase encoding the gene *MSP1* (*MULTIPLE SPOROCYTE1*) plays a critical role in normal anther cell differentiation, especially in controlling early sporogenic development. *MSP1* is an ortholog of *EXS/EMS1* in *Arabidopsis*. In addition to the *msp1-1*, *msp1-2*, and *msp1-3* mutants, we identified another *msp1* mutant, named *msp1-4*, with 10 base pairs (bp) of deletion between 758 bp and 767 bp in the *MSP1* open reading frame (ORF) (Wang et al 2006). Analysis of the *msp1* mutants revealed that *MSP1* is required for restricting the number of cells for further male and female sporogenesis, and a reduction/loss of *MSP1* activity resulted in an extra number of both male and female sporocytes (Nonomura et al 2003, Wang et al 2006). Moreover, the *msp1* mutants formed abnormal anther wall layers without the tapetal layer, and microsporocyte development was retarded during meiotic prophase I, causing complete male sterility. Recently, *OsTDL1A*, a rice *TPD1*-like gene, has been shown to bind the leucine-rich-repeat domain of MSP1 to restrict sporocyte numbers, but this function is limited for the ovule (Zhao et al 2008).

Like other flowering plants, rice sporocytic cells undergo meiosis to generate haploid microspores required for sexual reproduction. Several rice genes have been identified as involved in meiosis such as *PAIR2* (*HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS2*), *PAIR1*, and *OsRad21-4*. *PAIR2* is the homolog of *HOP1* in *Saccharomyces cerevisiae* and *ASY1* in *Arabidopsis* encoding a HORMA-domain protein, which regulates chromosome synapsis at meiosis I during the male development (Nonomura et al 2004a, 2006). PAIR1 is a novel protein predictably containing two coiled-coil motifs, two basic regions, and a nuclear localization signal sequence, and it is required for chromosome pairing and cytokinesis during both male and female gamete development (Nonomura et al 2004b). The rice *OsRad21-4* encodes an ortholog of yeast Rec8 protein, which is essential for efficient meiosis, and *OsRad21-4* knock-down lines displayed abnormal homologous chromosome pairing and uneven distribution of chromosomes during meiosis (Zhang et al 2006). ARGONAUTE (AGO) members have been shown to be crucial for RNA-mediated silencing in plants, and, within the rice genome, 18 copies of predicted AGO family members were predicted. Intriguingly, one rice *AGO* gene, *MEIOSIS ARRESTED AT LEPTOTENE1* (*MEL1*), has been shown to control chromosome condensation during early meiotic stages, and the *mel1* mutant develops abnormal multinucleated and vacuolated pollen mother cells. Also, abnormal development of female germ cells was observed (Nonomura et al 2007), suggesting that RNA-mediated gene silencing plays an essential role during germ cell development in rice.

Cell differentiation and subsequent degradation in the tapetum coincide very well with anther postmeiotic development, and premature or delayed degradation of the tapetum may cause male sterility. Programmed cell death (PCD) is considered to

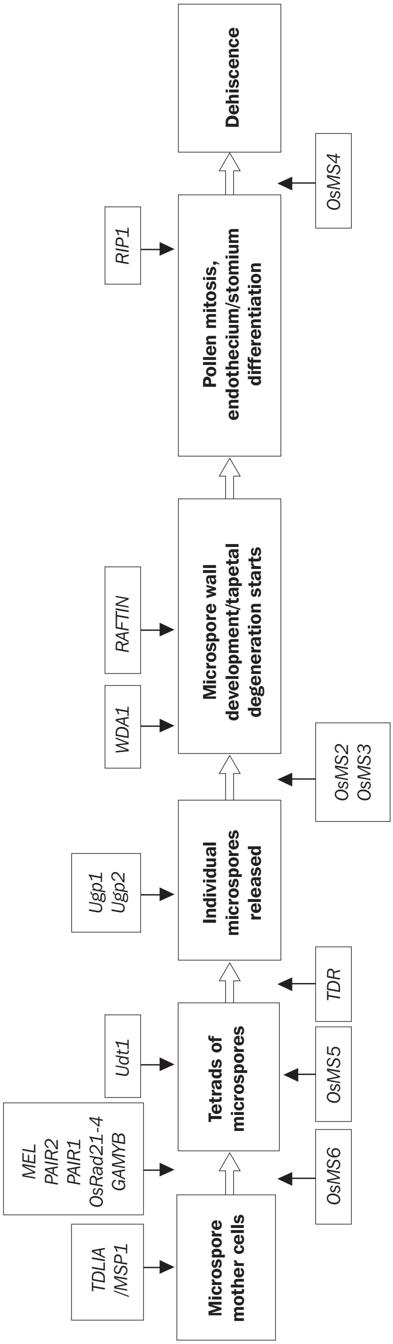

Fig. 1. A diagram for the genes involved in rice anther and pollen development. The open arrows indicate development transition. The genes listed above were reported by other laboratories, and the genes below were the ones found in our laboratory.

provide cellular contents supporting pollen wall development and to allow subsequent pollen formation and release. However, the molecular mechanism regulating tapetum PCD in plants still remains poorly understood. Investigations on key transcription factors during rice anther development and pollen formation are helpful to reveal the regulation and function of transcriptional control. A key gene regulating the early differentiation and development of tapetal cells of rice is *Undeveloped Tapetum1* (*Udt1*), which encodes a putative basic loop-helix-loop (bHLH) transcription factor. The *Udt1* mutant displayed undifferentiated tapetum and delayed degeneration of anther layers with aborted microspores (Jung et al 2005). TDR (Tapetum Degeneration Retardation), a newly found putative basic helix-loop-helix (bHLH) protein in rice, has been shown to be a positive regulator of tapetal PCD (Li et al 2006). Additionally, some other genes have been shown to control postmeiotic tapetum development and microspore development. For instance, the *ABORTED MICROSPORE (AMS*) gene, the ortholog of *TDR* in *Arabidopsis*, plays a key role in programmed tapetum and microspore development (Sorensen et al 2003). In addition, the *Arabidopsis MALE STERILITY1* gene encoding a protein with a PHD finger motif is required for proper tapetum function and normal microspore formation (Wilson et al 2001, Ito and Shinozaki 2002, Ito et al 2007, Yang et al 2007). Moreover, *AtMYB103* is essential for the development of tapetum, pollen, and trichome (Higginson et al 2003). OsGAMYB is another transcription factor regulating rice pollen development, loss-of-function of *OsGAMYB* results in loss of α-amylase expression in the endosperm with GA treatment, the mutant microspores could not stick to the tapetal cells at the tetrad stage, and the microspore mother cells displayed abnormal development. Meanwhile, the mutant tapetum cells expanded aberrantly (Kaneko et al 2004, Tsuji et al 2006).

One important role of the tapetum is providing nutrients for pollen development, for instance, supplying enzymes for callose dissolution and materials to form a well-organized pollen wall. Rice tapetum belongs to the secretory type which provides lipidic molecules for pollen wall development via secreting oil bodies named Orbicules (Ubisch bodies), which are spheroid structures of approx. 1 μm. *RAFTIN* is preferentially expressed in tapetal cells, and accumulated in Ubisch bodies, and reduction of *OsRAFTIN1* produced abnormal pollen wall development, resulting in male sterility (Wang et al 2003).

As a sink organ, the anther requires a supply of nutrients, particularly carbohydrates, from source tissues for pollen development and maturation. Disturbances in carbohydrate partitioning or metabolism within the anther frequently cause abnormal pollen development, and eventually male sterility. UDP-glucose pyrophosphorylase (UGPase) is involved in sucrose degradation by catalyzing UDP-glucose formed by sucrose synthase to glucose-1-phosphate to maintain anther development (Winter and Huber 2000). One rice UGPase gene, *Ugp1*, has been shown to be critical for callose deposition from the tetrad, creating novel thermosensitive male sterile lines (Chen et al 2007).

Myosins are actin-based molecular motor proteins that are related to eukaryotic motility such as cytokinesis, muscular contraction, cell shape maintenance, and vesicle transport (Mermall et al 1998, Berg et al 2001). O*smyoXIB* (*Oryza sativa* myosin XI B), a rice myosin gene, was first demonstrated in controlling pollen development by sensing environmental factors (Jiang et al 2007). One *Ds* insertion mutant of *OsmyoXIB* displayed abnormal pollen development under short daylength (SD) conditions because the OSMYOXIB-GUS fusion protein was localized only in the anther epidermal layer, although this protein is normally distributed in the anther wall layers, connective tissues, and miscrospores during pollen development under long daylength (LD) conditions. *Rice Immature Pollen 1* (*RIP1*) is homologous to the proteins with five WD40 repeats, and the *rip1* mutant displayed delayed development of microspores from the vacuolated stage, causing male sterility (Han et al 2006).

The biosynthesis of very long chain fatty acids (VLCFAs) is essential for wax and ether lipid synthesis for anther development in flowering plants. The rice *WDA1* (*Wax-deficient anther1*) gene encoding one putative enzyme with higher similarity to *Arabidopsis* CER1 is mainly expressed in the anther epidermal cell and putatively involved in the decarboxylation pathway for the production of VLCFAs. *wda1* displayed a smaller anther with reduced wax crystals (Jung et al 2006), suggesting the essential role of VLCFAs during anther development in rice.

Identification and genetic analysis of male sterile mutants in rice

To understand the molecular mechanism in rice pollen development, we treated 3,000 grams of seeds of japonica cultivar 9522 background with ^{60}Co γ-ray. Then, 5,963 lines of M_2 progeny were transplanted in a paddy field, and 666 mutants of M_3 progeny showed abnormal development in several organs such as the flower, leaf, and seed. Isolation and genetic analysis for these mutants would pave the way for further cloning of important genes in rice development (Chu et al 2005, 2006, Liu et al 2005, Chen et al 2006).

Among these mutants, we isolated about 100 male sterile mutants. Among these lines, we selected 20 mutants for further genetic analysis and gene isolation. We have now cloned 6 key regulators for rice pollen development (Fig. 1). As mentioned above, we employed a map-based cloning approach and revealed TDR as a master regulator during rice tapetum development and degeneration. The *tdr* mutant displayed degeneration retardation of the tapetum and middle layer, as well as a collapse of microspores. The *TDR* gene is mainly expressed in the tapetum and it encodes a putative basic/helix-loop-helix protein that is likely localized in the nucleus. More interestingly, two genes, *OsCP1* and *OsC6*, encoding a cysteine protease and a protease inhibitor, respectively, have been shown to be likely direct targets of TDR by chromatin immunoprecipitation analyses and the electrophoretic mobility shift assay (Li et al 2006).

As a complex wall system in flowering plants, the pollen outer wall mainly consists of aliphatic sporopollenin. Recently, we revealed the function of TDR in regulating aliphatic metabolism for rice pollen development. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) observations indicated that the *tdr* mutant developed a defective pollen wall. Also, the *tdr* anther has altered levels of aliphatic compositions, in particular, less accumulation of fatty acids, primary alcohols, alkanes, and alkenes, and an abnormal increase in secondary alcohols with carbon length from C29 to C35 in *tdr* (Zhang et al 2008). This evidence suggests that TDR plays a key role in lipid transport and metabolism for pollen wall formation in rice.

OsMS6 is another key gene regulating tapetum degeneration by triggering a PCD process during pollen development, and the *osms6* tapetum showed abnormally early degeneration (data not shown). In addition to *TDR* and *OsMS6*, we identified four other genes, *OsMS2*, *OsMS3*, *OsMS4*, and *OsMS5*, regulating lipid metabolism, sugar transport, and signal transduction during rice pollen development and maturation.

TDR is one master regulator during rice anther development

To reveal the role of transcriptional control of TDR during pollen development, we did expression profiling between the *tdr* mutant and wild-type counterpart by microarray assay. Some 236 genes showed statistically significant differences between the wild type and *tdr*, including 154 up-regulated genes and 82 down-regulated genes (Zhang et al 2008). Also, a high proportion of the genes altered in *tdr* were related to metabolism, transcription, cell wall/membrane/envelope biogenesis, posttranslational modification, and signal transduction. The changed metabolism-related genes in *tdr* mainly included carbohydrate transport and metabolism, energy production and conversion, amino acid transport and metabolism, lipid metabolism, as well as secondary metabolite biosynthesis. This finding was consistent with the fact that the tapetum is known to be metabolically active and to play a major role during pollen development. Therefore, TDR seems to be a crucial component at a key step in the regulatory network responsible for normal tapetum development (Fig. 2).

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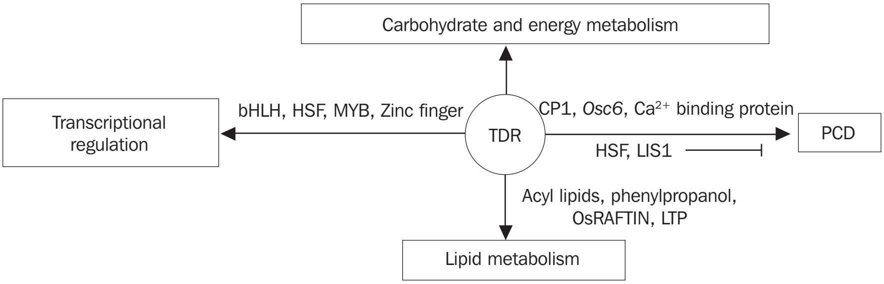

Fig. 2. Proposed model for the role of TDR during rice pollen development. The arrow indicates the implementation function of *TDR* regulating the genes during pollen development.

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Notes

Authors' address: School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China. Email: zhangdb@sjtu.edu.cn.

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Progress in molecular breeding of super hybrid rice by transferring genomic DNA of distant relatives

Zhao Bingran and Yuan Longping

The gene pool used in rice breeding programs needs to be expanded by combining regular breeding methods and molecular techniques to make new breakthroughs. Elite maintainer lines, restorer lines, and combinations of super hybrid rice have been developed by genomic DNA transformation with the Spike-stalk Injection Method from distant relatives, including *Oryza minuta* and *Echinochloa crus-galli*, into parental lines of hybrid rice. Analysis of molecular markers, DNA sequences, and Southern blotting has revealed that high DNA polymorphism exists between variant lines and their receptors, indicating that a special DNA fragment from distant relatives may be integrated into the genome of rice. Therefore, transformation of genomic DNA from distant relatives to the plant of a target receptor may open an avenue for creating new rice germplasm, and developing super hybrid rice.

Keywords: super hybrid rice, molecular breeding, genomic DNA transformation, germplasm

Based on progress made in China's Super High-Yielding Hybrid Rice Program by reaching the yield target of phase I (10.5 t ha^{-1}) in 2000 and phase II (12 t ha^{-1}) in 2004, a phase III super hybrid rice breeding program was proposed, in which yield was targeted at 13.5 t ha^{-1} on a large scale by 2010, and approaches of biotechnology combined with morphological improvement and the use of intersubspecific heterosis are emphasized (Yuan and Zhao 2004).

Genes from distant relatives conditioned resistance to diseases and pests (Brar and Khush 1997, Lee et al 1999, Yan et al 2001, Zhai et al 2002), yield (Ku et al 1999, Xiao et al 1996), grain quality (Ye et al 2000), cytoplasmic male sterility (Bijoya et al 1999), and other phenotypes, and these have been transferred into cultivated rice through gene engineering (Ku et al 1999, Lee et al 1999, Ye et al 2000), protoplast fusion (Brar and Khush 1997, Liu et al 1999), or wide hybridization (Xiao et al 1996) combined with MAS (marker-assisted selection) or the embryo rescue technique (Lee et al 1999, Yan et al 2001). But these genes are only a very small part of the genes of interest in distant relatives, and it is generally difficult to produce hybrids between rice and its distant relatives with these methods.

To develop super hybrid rice, it is necessary to expand the gene reservoir and to create new genetic germplasm with biotechnology. Genomic DNA of distant relatives has been transferred into parental lines of hybrid rice, and several restorer and maintainer lines have been developed at the China National Hybrid Rice Research and Development Center.

Genomic DNA transformation

We have established a new and simple procedure called the Spike-stalk Injection Method (SIM) (Zhao et al 1998), which is a modified technique based on the procedure invented by Pena et al (1987). When a recipient of rice undergoes meiosis, 50 µL of exogenous genomic DNA is injected into the uppermost internode of a stem, just under the panicle base. The DNA has a density of 450 µg mL^{-1} of SSC solution (in 1 L of SSC solution, also with distilled water and with 8.77 g NaCl, 0.186 g Na_2 EDTA, 5.88 g Tribasic; the pH is 7), and is mostly 25–50 kb in length. After the SIM treatment, variants usually occur at a rate from 10^{-3} to 10^{-2} in the first generation (D_1). Diverse variant lines have been developed from the transformation of genomic DNA of *Oryza minuta*, *Echinochloa crus-galli*, *Panicum maximun*, *Sorghum sudanense*, maize, algae, and so on.

Elite maintainer and restorer lines developed

Genomic DNA of *O. minuta* was introduced into the stem of V20B (an elite maintainer line of three-line hybrid rice). Variants were found from the D_1 generation. A new sterile line (Yewei A) and its maintainer line (Yewei B) (Fig. 1) have been derived from a D_1 variant plant. Another sterile line, Zi-100A, and its maintainer line, Zi-100B, have been developed using Yewei B as one of its parents. Zi-100A (and Zi-100B) not only has good grain quality, but it also has strong resistance to rice blast and tolerance of high temperature. A series of new elite maintainer lines has yet to be developed from Zi-100B.

When genomic DNA of *E. crus-galli* (a C_4 plant) was introduced into R207 (a restorer line of three-line hybrid rice), variants occurred in the first generation (D_1). A new elite stable strain, RB207-1, was developed from one D_1 variant plant through the pedigree method. RB207-1 had 286 grains per panicle and 32 g for 1,000-grain weight, whereas R207 had only 205 grains and 23 g, respectively (Fig. 2), but the tillering ability of RB207-1 was the same as that of R207.

Super hybrid rice combinations developed

A new medium indica hybrid rice combination, Ziyou1007 (Zi-100A/RB207-1), with high yield potential, good quality, rice blast resistance, and early maturity, has been developed. Ziyou1007 yielded 16.2% higher than the check Jinyou 207 in regional

Fig. 1. Plants of V20B (left), Yewei B (middle), and *O. minuta* (right).

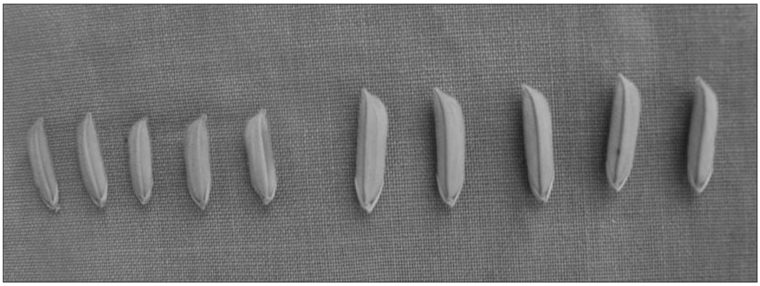

Fig. 2. Rice grain of R207 (the five grains to the left) and RB207-1 (the five grains to the right).

yield trials of Hunan Province in 2005-06, and yielded 13.5 t ha^{-1} on demonstration plots in Xupu County of Hunan in 2007. It was certified by the crop variety appraisal committee in 2007, and approved as a super hybrid rice of Hunan Province in 2008 (Fig. 3).

Genetic polymorphism between new developed lines and their parents

SSR, AFLP, RAPD, and DNA sequence analysis performed among Yewei B, V20B, and *O. minuta*, and among RB207-1, R207, and *E. crus-galli*, found about 5% polymorphism of molecular markers between variant lines and their original receptor lines,

Fig. 3. Plants of Jinyou207 (left) and Ziyou1007 (right).

and found specific DNA fragments from distant relatives that might be integrated into the rice genome (Zhao et al 2000, 2004, Xing et al 2004).

For instance, 2,117, 2,111, and 2,827 products (DNA bands) were amplified, respectively, in V20B, Yewei B, and *O. minuta* with 32 AFLP primer pairs. Among these, most were the same, whereas 184 products were different: 95 bands disappeared and 89 bands increased when comparing the products in Yewei B with those in V20B. So, the polymorphism rate between Yewei B and V20B was 4.4% [(89 + 95)/(2,117 + 2,111)]. As for the increased bands, 22 perhaps came from *O. minuta* and 67 bands existed either in the receptor or in the donor. The polymorphism rate between RB207-1 and R207 was also 4.4% [(105 + 82)/(2,132 + 2,155)], coincidently (Xing et al 2004).

The total number of products amplified with 83 RAPD primers was 397, 399, and 420 in V20B, Yewei B, and *O. minuta*, respectively. Polymorphic products between V20B and Yewei B were found in the amplification products of 15 primers (such as OPC-13, OPH-17, OPE-18, and OPG-11, among others): Yewei B has 14 extra bands compared with V20B: of these, perhaps 6 came from *O. minuta* (Fig. 4), 8 were present in either the donor or its receptor, and 12 bands disappeared. The polymorphism rate between Yewei B and V20B was 6.6% [(12 + 14)/(397 + 399)].

The special products of Yewei B and *O. minuta* (not in V20B) amplified with the same primer of OPG-11 were cloned and sequenced (Zhao et al 2001). We found that the two special DNA fragments not only were both 975 bp in length, but were also homologous in sequence. Their similarity was 97% except for 29 bp. In contrast,

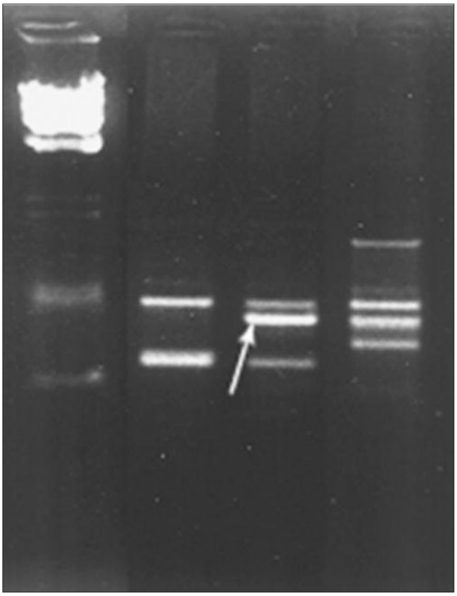

Fig. 4. RAPD patterns amplified with primer OPG-11. A pair of special bands are shown in a variant strain (the middle band) and in a donor; samples in different lanes from left to right are M1: DNA molecular weight marker II; 1: V20B (recipient); 2: Yewei B (variant); 3: *O. minuta* (donor).

no significant homologous DNA fragment with the two fragments was found in all data libraries of the rice genome existing so far.

Southern blotting analysis of the target RAPD fragment has been performed. Primers are designed according to the above sequences of the target RAPD fragment obtained from Yewei B (or *O. minuta*). The results of PCR showed that special DNA fragments were amplified from Yewei B and *O. minuta*, but not from V20B. Hybridization patterns between the target DNA fragment and the genomes of the three materials presented in Figure 5 showed that a special DNA fragment did exist in both Yewei B and *O. minuta*, but not in V20B (Zhao et al 2005).

Discussion

Besides this work of ours, new germplasm of rice and other crops was created through transformation of genomic DNA of their distant relatives with the pollen-tube-pathway technique (Zhou et al 1993). Southern blotting analysis proved primarily that those special segments of *Zizania latifolia* and *Leymus racemouses* were transferred into rice and wheat (Liu et al 2000, Miao et al 2000), respectively.

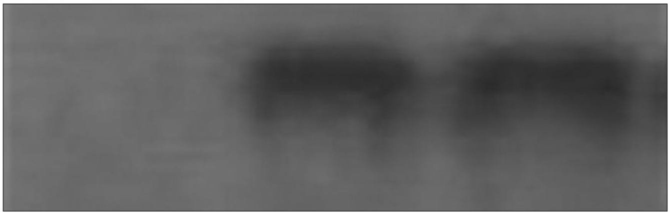

Fig. 5. Southern blotting analysis of Yewei B, V20B, and *O. minuta*. This shows that a special DNA fragment existed in both Yewei B and *O. minuta*, but not in V20B. Samples (genome DNA) in different lanes are 1: V20B (recipient); 2: Yewei B (variant); 3: *O. minuta* (donor).

In most cases, the relationship between cultivated species and distant relatives is too far apart to make hybrids using regular approaches. Although much more work needs to be done about the mechanism, as proved in our study, genomic DNA transformation is an efficient approach for breeders to use with distant relatives to create new rice germplasm, and even to develop super hybrid rice combined with regular breeding methods.

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Authors' address: China National Hybrid Rice Research and Development Center, Changsha 410125, China. E-mail: brzhao652@hhrrc.ac.cn; lpyuan@hhrrc.ac.cn.

Cloning and analysis of several phytohormone-related genes in super hybrid rice parental lines

Zhigang Huang, Ruozhong Wang, Hesong Li, Xianguo Qing, and Langtao Xiao

Cloning functional genes in super hybrid rice parental lines is of great importance for the super hybrid rice industry. Based on a database search and bioinformatics analysis, several phytohormone-related genes were suucessfully cloned and sequenced. Auxin receptor TIR1 was cloned from Zhu 1S by the RT-PCR method and designated as *OsTIR1* (GenBank accession number EU40058). *OsTIR1* is 2,219 bp in length and its complete open reading frame (ORF) is 1,764 bp encoding a protein of 587 amino acid residues. *OsTIR1* contains both an F-box motif and a leucine-rich repeat domain. An ethylene-responsive element-binding protein gene (accession number EF507537) and partial sequence ABA receptor (Mg-chelatase H subunit, CHLH) gene (accession number EU569725) were also cloned and submitted to GenBank. Further functional analysis will be conducted to acknowledge their importance in the growth and development of hybrid rice.

Super hybrid rice parental lines are special and important breeding materials. Cloning functional genes is of great importance for the super hybrid rice industry. Phytohormones are key developmental regulators. Alterations in phytohormone responses have been responsible for several important agricultural advances, such as the breeding of semidwarf varieties and increased grain production (Ashikari et al 2005, Silverstone and Sun 2000).

Genetic studies in *Arabidopsis* have led to the identification of a number of genes that function in auxin signaling. Two recent papers demonstrate that TIR1 is the auxin receptor, which plays a key role in the Aux/IAA degradation pathway of the 26S proteasome and combines with auxin directly in the SCFTIR1 complex (Dharmasiri et al 2005, Kepinski and Leyser 2005). Abscisic acid (ABA) is a vital phytohormone that mainly regulates stomatal aperture and seed development. *Arabidopsis* ABAR/CHLH is an ABA receptor that specifically binds ABA and mediates ABA signaling as a positive regulator in seed germination, postgermination growth, and stomatal movement (Shen et al 2006).

Although the discovery of these receptors is exciting in the complex story of phytohormone regulation, we are just beginning to comprehend the whole picture of developmental control following phytohormone perception. Also, many aspects of phytohormones are yet to be uncovered, such as signal transduction and universality in single or double cotyledon plants. Here, we would like to explain the latest progress in the cloning and analysis of phytohormone-related genes from super hybrid rice parental lines.

Results

Cloning and analysis of *OsTIR1*

Based on a Blast search and bioinformatics analysis, *OsTIR1* was cloned from Zhu 1 S by the RT-PCR method (GenBank accession number EU40058). *OsTIR1* is 2,219 bp in length and its complete open reading frame (ORF) is 1,764 bp encoding a protein of 587 amino acid residues (Fig. 1). Homology searches of the Protein Sequence Data Bank revealed extensive homology to other known plant *TIR1* genes (Fig. 2). The identity and homology between *OsTIR1* and *AtTIR1* are 61% and 77%, respectively. Protein secondary and tertiary structure predictions also show that *OsTIR1* and *AtTIR1* have significant homology (Fig. 3). Structural analysis revealed that *OsTIR1* had conserved motifs of the *TIR1* gene such as an F-box motif and a leucine-rich repeat domain (Fig. 4).

Cloning and analysis of *OsABP1*

In order to study super rice auxin-binding protein 1 (*OsABP1*), its putative cDNA sequence (J013102J06) was obtained from a nucleotide sequence database through bioinformatics analysis, and the ORF encodes a presumptive protein containing 206 amino acid residues. Blast against the current GenBank DNA and protein sequence database revealed significant homology with the ABP1 of other plants, and the functions of the presumptive protein resemble those of the ABP1 of other plants. Based on this sequence, ABP1 cDNA of super rice was amplified by RT-PCR. A 624-bp sequence was obtained after sequencing, and it is nearly in accord with an electronic searching outcome except for 22 nucleotides. The sequence was submitted to GenBank, and its accession number is AY968674.

Cloning and analysis of *OsCHLH*

The putative *OsCHLH* sequence was also obtained by bioinformatics analysis. It has extensive homology with other known plant *CHLH* genes. The identity and homology between *OsCHLH* and *AtCHLH* are 82% and 89%, respectively. The predictions of its secondary structure, transmembrane helices, and localization in cells all show that it is the homolog of *AtCHLH* in rice. Both of them have a conserved magnesium-chelatase domain. The specific primers for *OsCHLH* were designed subsequently and used to amplify in RT-PCR. After a T-A clone and sequencing, a 1,350-bp sequence was obtained and submitted to the GenBank database. Its accession number is EU569725.

```
1     CGATCCGTTGCAGCATGGGGCGCGGCGGCTCGCGCGCGGCGTGCGCCGCCGCGGCGCCGCCGTGGCACTCGCTCCCGGACGAGGTCTGG
                       M  G  R  G  G  S  R  A  A  C  A  A  A  A  P  P  W  H  S  L  P  D  E  V  W
90    GAGCACGCCTTCTCCTTCCTCCCCGCCGCCGCGGACAGGGGCGCCGCGGCGGGGGCGTGCAGCTCGTGGCTCCGCGCCGAGCGCCGGTCG
         E  H  A  F  S  F  L  P  A  A  A  D  R  G  A  A  A  G  A  C  S  S  W  L  R  A  E  R  R  S
180   CGCCGCCGCCTCGCCGTCGCCAACTGCTACGCCGCCGCGCCGCGGGACGCCGTCGAGCGGTTCCCGTCCGTGCGCGCCGCCGAGGTCAAG
         R  R  R  L  A  V  A  N  C  Y  A  A  A  P  R  D  A  V  E  R  F  P  S  V  R  A  A  E  V  K
270   GGCAAGCCCCACTTCGCCGACTTCGGCCTCGTCCCCCCCGCCTGGGGCGCCGCCGCGGCGCCGTGGATCGCCGCCGCCGCCGACGGGTGG
      G  K  P  H  F  A  D  F  G  L  V  P  P  A  W  G  A  A  A  A  P  W  I  A  A  A  A  D  G  W
360   CCGCTGCTCGAGGAGCTCAGCTTCAAGCGCATGGTCGTCACCGACGAGTGCCTCGAGATGATCGCCGCGTCCTTCAGGAACTTCCAGGTG
      P  L  L  E  E  L  S  F  K  R  M  V  V  T  D  E  C  L  E  M  I  A  A  S  F  R  N  F  Q  V
450   CTCCGCCTCGTCTCCTGCGACGGCTTCAGCACCGCGGGCCTCGCCGCCATTGCTGCCGGTTGCAGACACCTAAGAGAACTTGACCTGCAA
      L  R  L  V  S  C  D  G  F  S  T  A  G  L  A  A  I  A  A  G  C  R  H  L  R  E  L  D  L  Q
540   GAGAACGAGATTGAGGATTGTTCTATTCATTGGCTCAGCCTCTTCCCGGAATCGTTCACTTCTCTAGTAACTCTAAACTTTTCATGCTTA
      E  N  E  I  E  D  C  S  I  H  W  L  S  L  F  P  E  S  F  T  S  L  V  T  L  N  F  S  C  L
630   GAGGGGGAGGTCAATATCACTGTACTTGAACGGTTAGTGACCAGATGTCACAACCTGAAGACTCTTAAGCTCAACAATGCTATCCCCCTT
      E  G  E  V  N  I  T  V  L  E  R  L  V  T  R  C  H  N  L  K  T  L  K  L  N  N  A  I  P  L
720   GACAAGCTTGCTAGCCTCCTTCATAAGGCTCCTCAGCTAGTTGAACTCGGAACTGGCAAATTCTCTGCTGATTACCATTCCGATCTGTTT
      D  K  L  A  S  L  L  H  K  A  P  Q  L  V  E  L  G  T  G  K  F  S  A  D  Y  H  S  D  L  F
810   GCAAAGCTGGAGGCGGCGTTTGGAGGTTGTAAAAGCTTGAGAAGGCTTTCTGGGGCTTGGGATGCTGTTCCAGATTATCTGCCAGCATTC
      A  K  L  E  A  A  F  G  G  C  K  S  L  R  R  L  S  G  A  W  D  A  V  P  D  Y  L  P  A  F
900   TATTGTGTATGTGAAGGCCTCACATCACTTAATCTGAGTTATGCTACTGTGCGAGGTCCTGAGCTCATCAAATTCATTAGCAGATGCAGA
      Y  C  V  C  E  G  L  T  S  L  N  L  S  Y  A  T  V  R  G  P  E  L  I  K  F  I  S  R  C  R
990   AATTTGCAACAATTATGGGTGATGGACCTCATTGAGGATCATGGTTTAGCTGTTGTGGCATCATCTTGCAATAAACTTCAAGAGTTGCGG
      N  L  Q  Q  L  W  V  M  D  L  I  E  D  H  G  L  A  V  V  A  S  S  C  N  K  L  Q  E  L  R
1080  GTCTTCCCTTCTGACCCTTTTGGTGCAGGATTCTTGACTGAAAGAGGTCTTGTTGATGTCTCTGCAAGTTGTCCAATGTTGGAGTCAGTG
      V  F  P  S  D  P  F  G  A  G  F  L  T  E  R  G  L  V  D  V  S  A  S  C  P  M  L  E  S  V
1170  CTCTACTTCTGCAGACGGATGACAAATGAGGCACTTATTACCATTGCAAAGAACCGTCCCAACTTCACTTGCTTCCGCCTATGCATCCTT
      L  Y  F  C  R  R  M  T  N  E  A  L  I  T  I  A  K  N  R  P  N  F  T  C  F  R  L  C  I  L
1260  GAGCCACACACTCCAGACTACATCACACGGGAGCCTCTTGATGCAGGTTTCAGCGCCATTGTGGAGTCATGCAGGGGCCTTAGGCGTCTC
      E  P  H  T  P  D  Y  I  T  R  E  P  L  D  A  G  F  S  A  I  V  E  S  C  R  G  L  R  R  L
1350  TCTATCTCAGGCCTTCTCACAGATCTTGTGTTTAAATCCATTGGGGCACATGCTGATCGTCTTGAGATGCTTTCAATCGCCTTCGCTGGG
      S  I  S  G  L  L  T  D  L  V  F  K  S  I  G  A  H  A  D  R  L  E  M  L  S  I  A  F  A  G
1440  AACAGCGACTTGGGCCTGCATTACATCCTCTCAGGCTGCAAGAGCCTGAAGAAACTGGAGATCAGGGACTGCCCATTTGGTGATAAGCCA
      N  S  D  L  G  L  H  Y  I  L  S  G  C  K  S  L  K  K  L  E  I  R  D  C  P  F  G  D  K  P
1530  TTGCTGGCGAACGCAGCAAAGCTGGAGACAATGCGATCCCTTTGGATGTCGTCGTGCTTGTTGACCCTGGGCGCATGCCGACAGCTTGCA
      L  L  A  N  A  A  K  L  E  T  M  R  S  L  W  M  S  S  C  L  L  T  L  G  A  C  R  Q  L  A
1620  CGCAAGATGCCCCGCCTTAGTGTGGAGATCATGAACGATCCTGGAAGGTCATGCCCCTTGGATTCGCTTCCGGATGAAACACCTGTTGAG
      R  K  M  P  R  L  S  V  E  I  M  N  D  P  G  R  S  C  P  L  D  S  L  P  D  E  T  P  V  E
1710  AAACTGTACGTCTACCGGACGATCGCAGGTCCAAGGTCCGACACACCAGCCTGCGTCCAGATTGTGTAGGGGGCAGTATGTGCGGTATCG
      K L Y V Y R T I A G P R S D T P A C V Q I V *
1800  GTATGCGGGTGTTATGGAGGTATTTATTTTCAATCTCGATTAGTACCTTCAAACACCTTCCTCGCATAACATAGAATTGTTTCCTTCCAA
1890  GTAGAAAGGCTGATGGTTGGATAGACTTGTCATGATCTCTACTCGGCTACAAAACTGTTGCAGGTGTAGAATGAAAATTCACAGCCTTTT
1980  TCAGGTGTTAATAACTGTAGGAGGAGACTGGATGAATTACAGAAAACTACTAGATAGCATCATGAGAAAACTTCGGTTTGTTTTCTTAGC
2070  GTGTTTAGCACTTTGTATGTATAAAAATATGGCATCCATTTGTACTGTACTAGTGGAAAATTTGTATCTGGAGCTGAGCTTTGTCACCTC
2160  TACTTTTTTGACCTAAATTATGAATTGTTGTTGCTTCAGCAAAAAAAAAAAAAAAAAAAA
```

Fig. 1. TIR1 cDNA sequence and predicted amino acid sequence.

Conclusions

In this research, we have analyzed and cloned three phytohormone-related genes in super hybrid rice parental lines. The prokaryotic expression and purification of protein are under way. Functional analysis will be conducted further to acknowledge their importance in the growth and development of rice.

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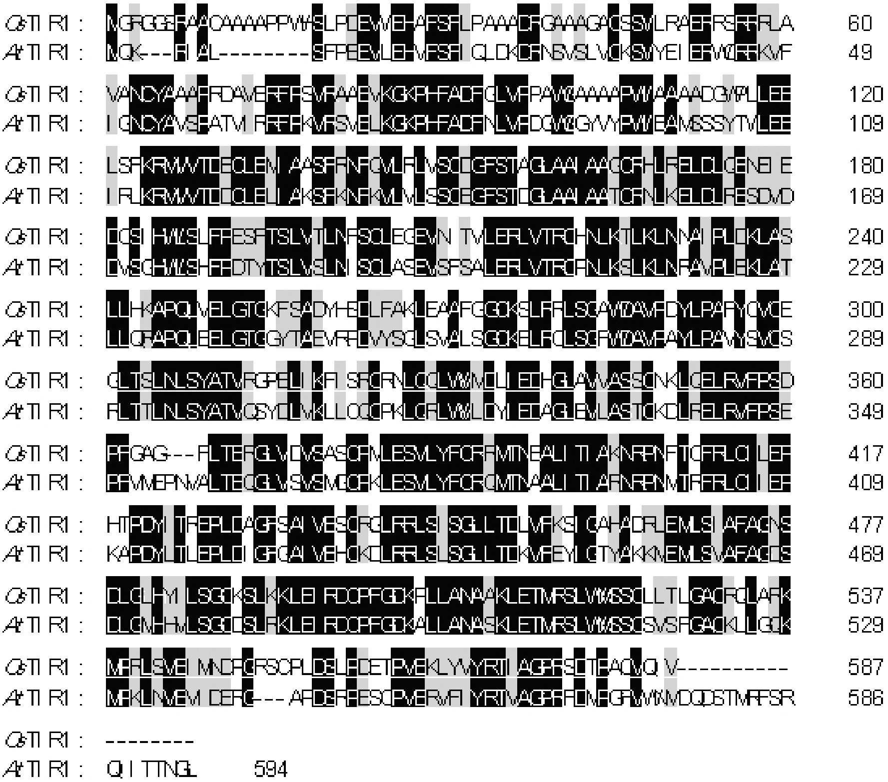

Fig. 2. Alignment of *OsTIR1* and *AtTIR1*.

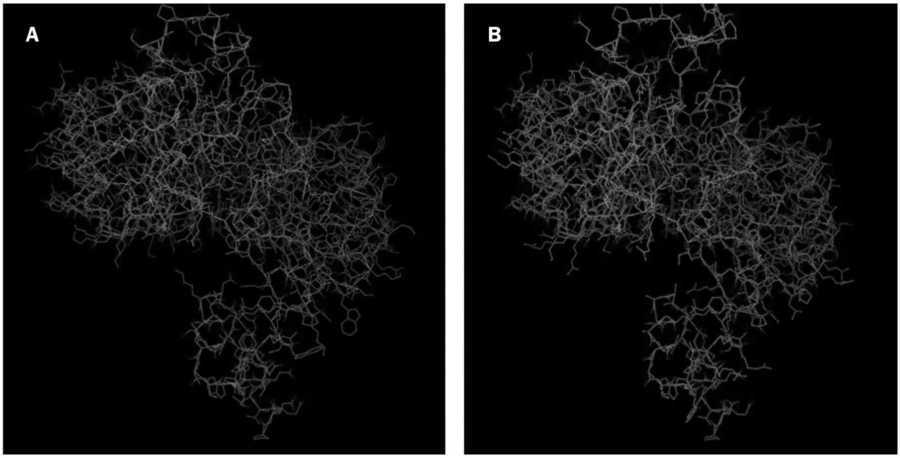

Fig. 3. Protein tertiary structure prediction of *AtTIR1* and *OsTIR1*.

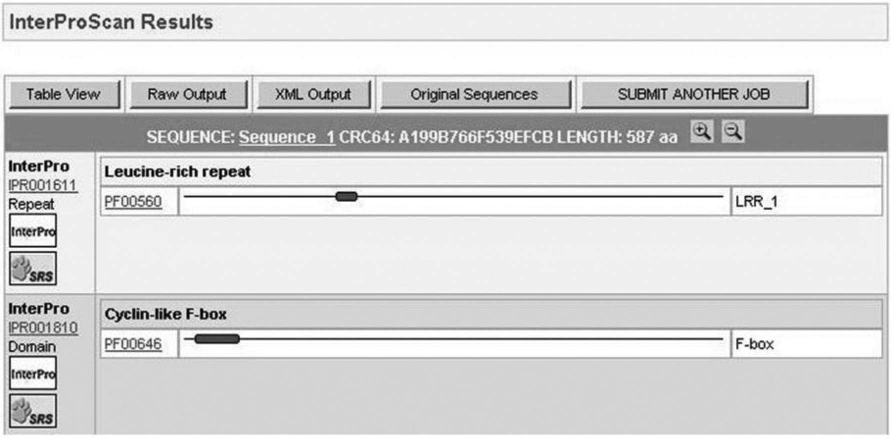

Fig. 4. Functional domain prediction of *OsTIR1*.

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Notes

Authors' addresses: Zhigang Huang, Ruozhong Wang, Hesong Li, and Langtao Xiao, Hunan Provincial Key Laboratory of Phytohormones and Development, Hunan Agricultural University, Changsha 410128, China; Xianguo Qing, National Center of Hybrid Rice Research and Development, Changsha 410125, China; email: langtaoxiao@163.com.

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Marker-assisted selection for brown planthopper resistance genes in rice

Huaxiong Qi, Jinbo Li, Mingyuan Xia, Guangcun He, Bingliang Wan, and Zhongping Zha

The brown planthopper (BPH) is an important pest of rice in Asia. One crucial measure to control BPH is to breed cultivars with durable resistance by identifying new BPH resistance genes and pyramiding major resistance genes. B5 is a rice line derived from wild rice *Oryza officinalis*. It is highly resistant to BPH biotypes 1 and 2 collected in China. The BPH resistance genes *Bph14* and *Bph15* have been identified in B5 and mapped onto rice chromosome 3 and chromosome 4, respectively. Marker-assisted selection (MAS) involves the use of molecular markers that are tightly linked to or cosegregating with the target genes, providing an effective means for the selection of BPH resistance genes in rice breeding programs. In this study, we used B5 as the donor of the BPH resistance gene and performed MAS for the genetic improvement of BPH resistance.

Methods

In our rice breeding program, elite restorer lines 9311, 1826, and M087 were used as recipients of the BPH resistance genes in B5. The bacterial blight (BB)–resistant line CBB23 was the disease resistance donor used for pyramiding BPH and BB resistance genes. Screened simple sequence repeat (SSR) markers were used to select BPH resistance genes in the segregating populations of crosses between resistance gene donors and recipients. To evaluate BPH resistance, 18 hybrids were obtained from crosses between six rice introgression lines carrying homozygous BPH resistance genes and three sterile lines (Guangzhan 63S, Peiai 64S, and Yuetai A). Both mass seedling screening tests and field tests were done to evaluate BPH resistance. Susceptible cultivar Taichung Native 1 (TN1) and resistant line B5 were used as negative and positive controls, respectively.

Polymorphic PCR-based markers for the parents were screened according to the mapped regions of *Bph14* and *Bph15* and molecular markers tightly linked to *Bph14* and *Bph15*. The *Bph14*-linked SSR marker MRG2329 and the *Bph15*-linked SSR marker MS5 were used to select for *Bph14* or *Bph15* in segregating populations, respectively.

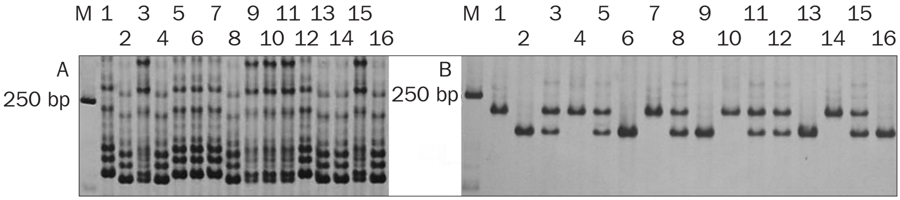

Fig. 1. PCR analysis of *Bph14*-linked marker MRG2329 (A) and *Bph15*-linked marker MS5 (B). M = marker; 1 = B5; 2 = 9311; 3 = F_1; 4–16 = plants of segregating populations.

Table 1. Reaction of six parents and six introgression lines to BPH.[a]

Line	Cross	Gene	RG	
			MSST	FT
C506	9311/B5//M087	*Bph14*	0	1
C422	9311//9311/CBB23///1826/B5	*Bph14*	0	0
C511	9311/B5//M087	*Bph15*	0	0
C508	9311/B5//M087	*Bph14, Bph15*	0	1
C518	9311//9311/CBB23///1826/B5	*Bph14, Bph15*	0	1
P043	B5-10	*Bph14, Bph15*	0	0
TN1			9	
1826			9	
9311			9	
CBB23			9	
M087			9	

[a]RG = resistance grade; MSST = mass seedling screening test; FT = field test.

Results

PCR analysis showed that MRG2329 was tightly linked to *Bph14* and MS5 was tightly linked to *Bph15* (Fig. 1). We performed MAS of the *Bph14* and *Bph15* gene using MRG2329 and MS5 in our study, respectively. Through MAS at the seedling stage and selection of agronomic traits at the adult plant stage, we obtained six introgression lines that carried the BPH resistance gene(s) and showed stable agronomic traits.

BPH resistance in the six introgression lines and 18 hybrids was determined in mass seedling screening tests and field tests (Tables 1, 2, Fig. 2). The BPH-resistant control (B5) showed a score of 0, while the BPH susceptible control (TN1) and the other parents had a score of 9. All six introgression lines were highly resistant to BPH. Of the 18 hybrids, those crossed with *Bph14*/*Bph15* pyramided genes had higher resistance than those with a *Bph14*- or *Bph15*-single gene. Hybrids from Guangzhan 63S

Table 2. Reaction of hybrids from different sterile and restorer lines to BPH.

Hybrid	RG (MSST)		RG (FT)	
	Mean	Range	Mean	Range
H-C506	5.7	3–9	7.0	5–9
H-C422	5.0	3–7	7.0	5–9
H-C511	4.3	3–7	4.3	3–7
H-C508	2.0	0–3	4.3	1–7
H-C518	2.0	0–3	3.0	0–9
H-P043	1.0	0–3	2.3	0–7
H-Yuetai A	4.0	3–7	3.0	0–7
H-Peiai 64S	5.0	3–9	8.0	7–9
H-Guangzhan 63S	3.0	1–3	3.0	0–5

Fig. 2. Field test showing hybrid resistance and susceptibility to BPH.

were more resistant than those coming from Peiai 64S and Yuetai A. Results of field tests showed that hybrids from Peiai 64S were moderately susceptible to susceptible to BPH. However, the resistance of all hybrids was inferior to that of the introgression lines. The lines with homozygous genes had higher resistance than the lines with heterozygous genes.

Conclusions

The results indicate that introgression lines containing the genes *Bph14*, *Bph15,* or both were highly resistant to BPH, and that hybrid plants derived from these introgression lines displayed different levels of resistance with sterile lines in different genetic background. Additionally, these results suggest that BPH resistance in current rice cultivars can be further increased through MAS. The two markers linked with *Bph14* and *Bph15* can be effectively used in breeding programs for BPH resistance.

Notes

Authors' addresses: Huaxiong Qi, Jinbo Li, Mingyuan Xia, Bingliang Wan, and Zhongping Zha, Food Crops Research Institute, Hubei Academy of Agricultural Sciences, Wuhan 430064, China; Guangcun He, College of Life Sciences, Wuhan University, Wuhan 430072, China.

ISSR analysis of genetic diversity of rice germplasm with long panicles and big grains

Liang Kangjing, Yao Xiaolan, Lin Jing, Wei Xinyu, Wang Naiyuan, and Huang Yuxian

The genetic diversity of 56 specific rice germplasm accessions with long panicles (30.73–46.3 cm) and big grains (30.7–70.63 g) was analyzed using inter simple sequence repeats (ISSR). This rice was derived from the IRRI new plant type strains and then developed by crossing and radiation. A total of 80 pairs of ISSR primers were applied to screen for polymorphism, with nine of them being tested to produce polymorphic bands. The nine pairs of primers generated a total of 70 bands, of which 47 were polymorphic. Percentage polymorphic bands (PPB) was 66.18%; genetic similarity coefficient (GS) varied from 0.368 to 0.962, with an average of 0.665; genetic diversity index (H) was 0.233; the number of alleles (Na) was 1.662; and the effective number of alleles (Ne) amounted to 1.414. Clustering analysis showed 56 accessions that could be classified into four groups with *GS* of 0.64: the first cluster consisted of nine accessions; the second and third clusters contained six accessions, respectively; and the fourth group had 35 accessions. The results provided the scientific basis for further studies on their molecular mechanism and use in breeding.

Keywords: rice, ISSR markers, genetic diversity, rice germplasm with long panicles and big grains, cluster analysis

Rice breeding practices have proved that breeding success depends on finding and using effective special materials. Rice germplasm with long panicles and big grains attracted great attention among domestic and overseas scholars (Rabiei et al 2004, Fan et al 2006, Li et al 2004, Song et al 2007, Takai et al 2005, Wan et al 2006, Xie et al 2006). Thus, we have selected and bred 56 cultivars having these characters from IRRI new plant type strains by crossing and radiation. The genetic diversity of these 56 specific rice germplasm accessions was analyzed by using inter simple sequence repeats (ISSR) and the genetic types of this rice germplasm were revealed on the molecular level. The purpose of this study was to provide a basis for selecting germplasm for high-yield breeding.

Table 1. Means of yield-related traits of 56 accessions with long panicles and big grains.

Character	Range	Average value	Standard deviation	Coefficient of variation
Weight per plant (g)	5.72–27.93	15.88	5.78	37.38
Weight of 1,000 grains (g)	30.70–70.63	40.75	7.66	18.80
Panicle length (cm)	30.07–46.30	34.88	3.46	9.91
Grains per panicle (no.)	79.33–215.33	140.20	24.90	17.76
Filled grains (no.)	26.67–175.0	78.43	26.90	34.30
Seed-setting rate (%)	18.0 –90.4	55.98	15.44	27.59
Length of grain (mm)	10.10–15.87	12.97	1.33	10.29
Width of grain (mm)	2.53–4.30	3.09	0.38	12.21
Thickness of grain (mm)	2.10–3.10	2.41	0.17	7.17

Materials and methods

Test materials

Fifty-six rice strains (Table 1) were grown in the field of the College of Crop Sciences of the Fujian Agriculture and Forestry University. About 0.1 g of fresh leaves from each plant was collected, frozen with liquid nitrogen, and immediately stored in sealed plastic bags.

DNA extraction and PCR amplification

Genomic DNA of the samples was extracted according to protocol. About 0.1 g of leaf was ground into powder form in a mortar with liquid nitrogen. This powder was mixed with 1.1 mL of 2% CTAB in an eppendorf tube, then shaken at 1,300 rpm at 65 °C for 1 h, followed by centrifugation at 10,000 rpm for 5 min. The supernatant was transferred into another tube. An equal volume of chloroform-isoamyl alcohol (CI, 24:1) was added into the tube after it cooled to room temperature. After being mixed by gentle inversion, the mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was mixed with 400 μL of isopropanol. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. The DNA pellet was then recovered. The DNA was washed twice with 75% ethanol and centrifuged at 10,000 rpm for 5 min at 4 °C. The DNA that remained was air-dried, re-suspended in 100 μL of sterile pure water, and then stored at 4 °C for later use.

A total of 80 ISSR primers obtained from Sangong Biotechnology Co. Ltd. (Table 2) was screened and nine primers were selected for the ISSR analysis. The mixture (10 μL) for PCR contained 0.8 μL $MgCl^2$ (1.5 mmol L^{-1}), 0.2 μL dNTPs (10 mmol L^{-1}) (Promega Co.), 0.3 μL primers (40 μmol L^{-1}), 0.5 μL template genomic DNA, and 0.5 UTaq DNA polymerase. PCR amplification was performed on a peltier thermal cycler T-gradient thermoblock (Biometra, Inc., USA) with pre-denaturation

Table 2. Sequences of nine primers used in ISSR analysis and their amplification products.

Primer	Sequence (5′-3′)	Bands (no.)	Polymorphic bands (no.)	Percentage of polymorphic bands (%)	Size range (bp)
7	AGAGAGAGAGAGAGAGT	9	7	77.8	260–2,000
27	ACACACACACACACACG	12	10	83.3	330–1,600
36	AGAGAGAGAGAGAGAGCTA	8	7	87.5	280–1,650
40	GAGAGAGAGAGAGAGACTT	8	4	50	340–1,600
41	GAGAGAGAGAGAGAGACTC	5	2	40	250–1,750
44	CTCTCTCTCTCTCTCTAGC	7	5	71.4	240–2,000
45	CTCTCTCTCTCTCTCTAGG	9	5	55.6	480–1,800
55	ACACACACACACACACCTT	6	4	66.7	300–1,600
73	GACAGACAGACAGACA	6	3	50	350–1,400

at 94 °C for 10 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, extension at 72 °C for 2 min, and a final extension step at 72 °C for 10 min. PCR amplification products were separated by electrophoresis on 8% polyacrylamide gels (20 V/cm, 120 min) and visualized by silver staining. The gels were finally scanned.

Data analysis

Amplified fragments were scored either as 1 (for presence of a band) or 0 (for the absence of a band). The fragments that were similar in the samples were the common bands, whereas those that differed were the polymorphic ones. The matrix of scores was then subjected to statistical analysis using the program POPGENE (version 1.32). The number of alleles (Na), the number of effective alleles (Ne), Nei's gene diversity (H), and percentage of polymorphic bands (PPB) were calculated. A distance program, NTSYS-pc (version 2.0), was used to calculate Jaccard similarity coefficients. These coefficients were used as operational taxonomic units to construct a dendrogram using the unweighted pair group method.

Results

Amplified bands

A total of 70 bands were amplified, of which 47 were polymorphic. The PPB of the population was 67.1%. The amplified bands of one ISSR marker varied from 6 to 12. Of the nine ISSR primers, ISSR primer 27 amplified the most bands, while primer 41 amplified the least. Figure 1 shows the amplified bands using primer 27. No amplified

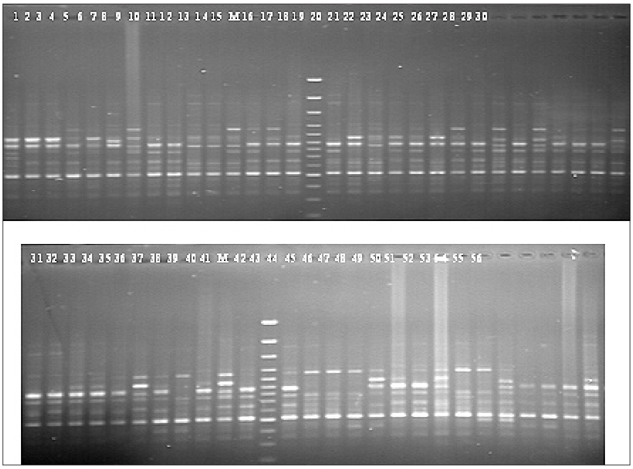

Fig. 1. Amplification products generated by ISSR primer 27.

Table 3. Analysis of genetic variation generated by ISSR markers.

Number of alleles (Na)	Effective number of alleles (Ne)	Percentage of polymorphic loci (PPB) (%)	Gene diversity index (H)
1.662	1.414	66.18	0.233

band was observed using the blank check, indicating the validity of the amplification results.

Genetic diversity of *Oryza sativa* L.

The amplified polymorphic bands varied from one population to another. Band size ranged from 240 to 2,000 bp, with an average of 19 bands per primer. The values of Na, Ne, PPB, and H were 1.662, 1.414, 66.18%, and 0.233, respectively (Table 3). From these, we can conclude that these rice accessions are genetically diverse.

Clustering analysis

An UPGMA dendrogram produced using the Jaccard coefficients of the tested accessions is shown in Figure 2. The 56 accessions were classified into four clusters with a genetic similarity coefficient (GS) of 0.64. The dendrogram indicated that the GS varied from 0.368 to 0.962 (average of 0.665 and range from 0.07 to 1.29). The fourth cluster included 35 accessions; the other clusters included their respective 6 accessions. The differences in the average values of yield-related traits in the four clusters were large— cluster I had a greater number of grains than the others, cluster II had higher 1,000-grain weight and width, and cluster IV had longer grains (Table 4).

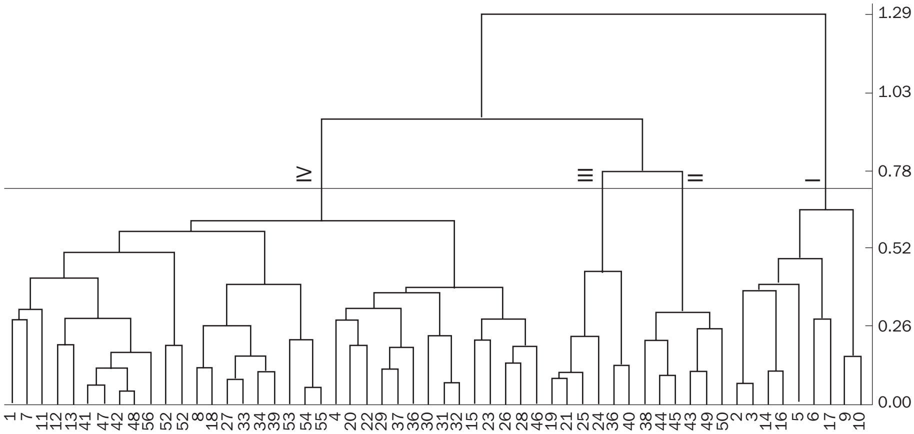

Fig. 2. Results of cluster analysis of 56 rice varieties as determined by UPGMA.

Table 4. The average values of yield-related traits in four groups.

Character	Cluster			
	I	II	III	IV
Weight per plant (g)	20.35	11.87	15.61	15.46
Weight of 1,000 grains (g)	35.01	45.11	38.40	41.87
Panicle length (cm)	32.54	33.34	33.56	35.97
Grains per panicle (no.)	159.59	140.67	138.28	135.47
Filled grains (no.)	90.37	63.89	74	78.62
Seed-setting rate (%)	56	45.75	55.17	57.87
Length of grain (mm)	11.54	12.7	12.64	13.43
Width of grain (mm)	2.90	3.64	3.06	3.05
Thickness of grain (mm)	2.31	2.49	2.35	2.43
Strains (no.)	9	6	6	35

Discussion

As identification is affected by environmental factors, the affinity of germplasm was difficult to assess through plant morphology. Thus, the analysis of genetic diversity and affinity by modern molecular biology is extremely important. Using a primer length of 15–18 bp and annealing temperature of 50–55 °C, the characteristics of the ISSR marker guarantee the repeatability and specificity of the amplified bands, increasing the accuracy of classification. This confirms the results from other studies, suggesting the reliability and stability of clustering analysis of germplasm based on ISSR markers. This method could provide reliable information for selecting parents for breeding high-yielding rice.

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Notes

Authors' address: College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China.

Molecular tagging of the thermo-sensitive genic male sterility gene in fine-grain indica rice

D. Kavithamani, S. Robin, S. Manonmani, D. Malarvizhi, R. Pushpam,
K. Mohana Sundaram, and T.S. Raveendran

Molecular tagging of thermo-sensitive genic male sterility (TGMS) was attempted for the development of TGMS parental lines through marker-assisted rapid generation advancement. A stable TGMS donor, TS 29, was crossed with one fine-grain popular variety, CO(R)49. The F_2 population was raised during the winter season and the selfed seeds were harvested from 400 individual plants. DNA was extracted from all the plants. The stubbles were left and the sterile plants were identified. The F_3 population was assayed for pollen viability and it ranged from 0 to 100%. DNA samples collected from the parents and extremely fertile and sterile individual progenies were identified. The corresponding F_2 plants' DNA was used for carrying out bulk segregant analysis. One SSR marker, RM214, on chromosome 7 was putatively identified as being linked to the trait.

Rice continues to be the single most important source of calories for a majority of the human population in the world. India annually plants rice on a total area of 43 million ha, which produces an average of 125 million tons of rice. Under current rice consumption, production needs to reach 158 million tons, with a growth rate of 2.4%, to satisfy the population, which could grow at 1.9% per annum. Notwithstanding past achievements in rice production that made the country self-sufficient, the future years warrant refined approaches to overcome the already attained yield plateau and stagnant genetic potential. Rice hybrids have a yield advantage of 15–20% over the best conventionally bred varieties. China's success in commercial hybrid production clearly demonstrates that hybrid rice is now the most significant practical tool for increasing global rice production. The exploitation of hybrid rice through three-line breeding involving a cytoplasmic male sterile parent is cumbersome, which restricts the use of any varieties or elite lines as parental lines due to the selective occurrence of restorer genes. However, in a TGMS two-line hybrid system, any fertile line can be used as a pollen parent (PP); therefore, the frequency and dimension of heterotic hybrids are higher.

TGMS lines are usually sterile at high temperature and fertile at low temperature. TGMS-based hybrids involve simpler seed production methods involving only two lines—a TGMS and a genetically complementary pollen parent (restorer line). The monogenic recessiveness of the TGMS gene to be integrated into classical breeding involves a long gestation cycle, which requires the substitution of environments for sterility observation and seed production since the gene is under the influence of the environment. DNA markers that are absolutely heritable and environmentally insensitive can be used efficiently to tag the TGMS gene for using linked markers as indirect selection tools.

Materials and methods

A fresh cross combination was developed by mating the most stable TGMS line, TS 29, with the agronomically improved adapted high-yielding medium slender grain variety CO(R)49. The F_2 generation was raised under a sterility-restrictive environment favoring fertility reversion and around 400 individual plants were randomly selected for characterization of TGMS genes. DNA was extracted from all 400 F_2 plants and was preserved. The F_3 seeds (progenies) collected from the individual plants were raised under sterility-causing conditions in the plains (Coimbatore) in ear-to-rows. The plants in an individual progeny were assessed for pollen fertility. Based on F_3 family performance, DNA bulks of fertile, sterile, and heterozygote for each of 10 plants were made from the corresponding F_2 plants. A set of 50 SSR markers putatively located in the vicinity of any one of the six *tms* genes reported in rice was targeted initially. Parental polymorphism was followed by bulk segregant analysis.

Results and discussion

The F_2 seed material harvested from the fixed true hybrids was raised at Coimbatore in the late rabi season. This environment was used to favor seed set in all the F_2 plants whether they were homozygous or heterozygous for the TGMS gene. Populations of 400 plants in the cross were ensured with self-pollination after assessing pollen fertility. The F_2 population segregated for duration and plant height besides panicle and grain characteristics. Although the climate was not the most conducive for sterility induction, the population also segregated for pollen fertility ranging from 0 to 100% (Fig. 1). The plants with absolute sterility were identified and stubble planted. Spikelet fertility was also recorded, which ranged from 0 to 100% (Fig. 2).

A polymorphism survey between CO(R)49 and TS 29 indicated that the parents were polymorphic only for nine primers, accounting for only 18% SSR polymorphism. The nine primers were screened individually to corroborate the allelic pattern of the sterile F_2 plants and the TGMS parent TS 29. Among the polymorphic primers, initial analysis revealed that one microsatellite primer, RM214 on chromosome 7, was found to exhibit a common SSR allele similar to TS 29 in all eight sterile F_2 plants (Fig. 3) but was nonallelic in CO(R)49. This genomic region was already reported to be associated with the TGMS trait, especially *tms2* (Maruyama et al 1991).

Several TGMS genes in rice have been reported from China, Japan, and the International Rice Research Institute (IRRI), in the Philippines. The TGMS trait was identified to be controlled by a recessive gene (Borkakati and Virmani 1996). Recently,

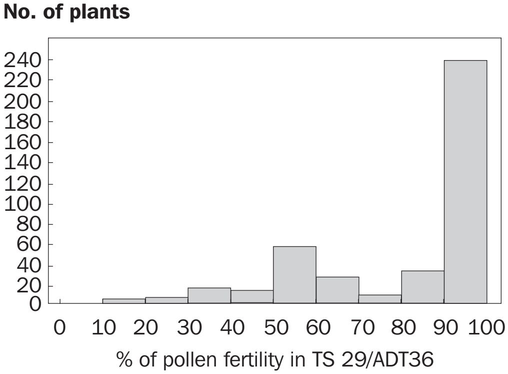


Fig. 1. Frequency distribution of pollen fertility.

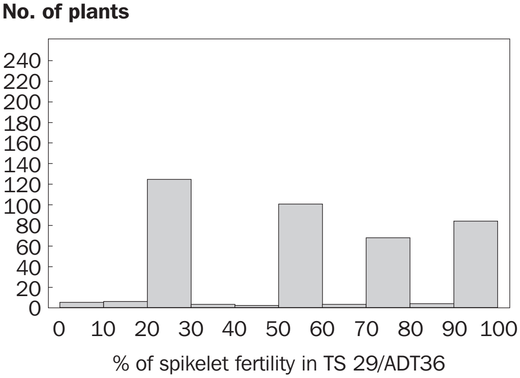
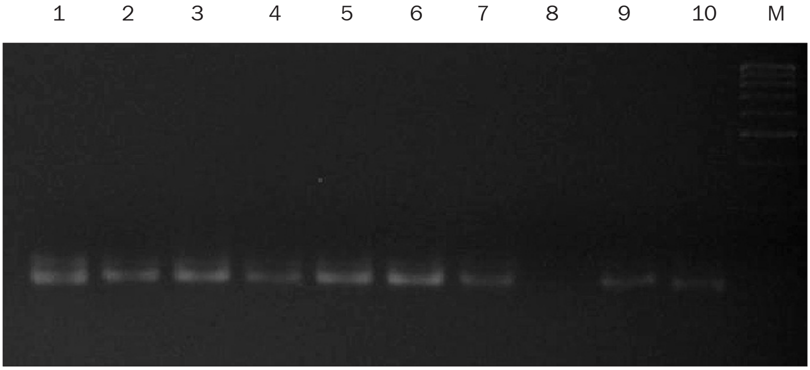


Fig. 2. Frequency distribution of spikelet fertility.



M = 100-bp ladder, 1 = CO(R)49, 2–9 = sterile plants, 10 = TS 29.

Fig. 3. Polymorphic survey for TGMS gene tagging.

Ku et al (2003) showed that programmed cell death of premature tapetum is associated with male sterility of TGMS in rice. Later, several other TGMS genes were added. The work of Rongbai et al (2005) resulted in the identification of two pairs of major *tms* genes in UPRI 95-140 TGMS, nonallelic to any of the known *tms* genes, and these genes were reported to be located on chromosomes 3 and 7, tentatively assigned as *tms6* and *tms7,* respectively. Analysis by various workers indicated that there was scope for creating new TGMS genes in a genetic background that can be exploited in our interest.

Among the several alleles identified for TGMS expression, *tms2* was the most studied gene as it was already mapped on chromosome 7 by using RFLP markers (Yamaguchi et al 1997). Many microsatellite markers associated with *tms2* genes are readily available. One marker, RM 11 (115–120 bp in size), was found to be associated with *tms2* and it is approximately 5 cM away from *tms2* physically. Three thermosensitive male sterility (TGMS) genes, *tms2*, *tgms*, and *tms 5*, were pyramided using linked microsatellite markers. In our study, the search for more *tms* genes is continuing and, on molecular tagging, the different genes will be pyramided to develop a sterile fine-grain-type parent for use in the ongoing hybrid rice breeding program targeted toward the development of new hybrids with highly commercial grain type.

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Notes

Authors' address: Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore- 641 003, Tamil Nadu, India; email: robin.tnau@gmail.com.

Improving hybrid rice grain quality

Grain quality of hybrid rice: genetic variation and molecular improvement

Ming-Hong Gu, Qiao-Quan Liu, Chang-Jie Yan, and Shu-Zhu Tang

Hybrid rice was first released in 1974 and now accounts for more than half of the annual rice-planting area in China and is widely grown worldwide. Hybrid technology has greatly increased yield, but grain quality needs to be improved much more. In rice endosperm, the starch comprises about 90% of the dry matter, and grain quality is greatly affected by starch composition and structure. Therefore, starch biosynthesis might play a crucial role in the formation of rice quality, especially cooking and eating quality. In our studies, the allelic variation of 18 genes involved in starch synthesis, as well as the diversity of grain cooking and eating quality, was carefully investigated among representative germplasm accessions, including some important parents for hybrid rice production. The effects of the variation on grain quality were first studied through association analysis, and then confirmed by using near-isogenic lines and/or transformants. In this study, the genetic variation of these starch synthesis–related genes (SSRGs) and their effects on grain quality are presented. The strategies and application of molecular techniques for quality improvement of hybrid rice are also discussed.

Keywords: grain quality, hybrid rice, starch synthesis–related genes (SSRGs), allelic variation, molecular improvement

Rice is a staple food crop for about half of the global population. About 70% of the population eats rice as the main food. With rapid economic development in the past three decades, much more attention has been paid to rice grain quality in Asia.

China is one of the biggest rice producers in the world. Both indica and japonica rice (*Oryza sativa* L.) are cultivated on a large scale and the total amount of rice grain produced annually accounts for more than 190 million tons. Since 1974, the planting area of hybrid rice has been growing rapidly because of its higher yield and good adaptation, especially for hybrid indica rice (Yuan 1987). It now accounts for about 55% of the total rice-planting area in China. Besides yield, grain quality is also highly appreciated. Though much work has been done on improving quality, most hybrid rice still leaves much to be desired. Grain quality is a complex character, not only because of the various components in the endosperm, including starch, protein, fat, and so on, but also because of ploidy difference. The endosperm is a triploid tissue although the embryo is diploid tissue. In addition, the grain quality of hybrid rice to some extent is quite different from that of common varieties released from pure-line selection in conventional breeding. First, the grains of hybrid rice are the offspring of hybrid plants of two parental varieties (male sterile and restorer lines). Both parents make a genetic contribution to the grains. Second, the grains harvested from hybrid rice plants are mixed individuals in the F_2 generation and segregation in quality characters is unavoidable, which depends on the genetic divergence between the parents of the hybrid. Moreover, environmental factors, such as temperature, water supply, and soil fertility, also play an important role in quality determination. As mentioned above, to improve the grain quality of hybrid rice, it is necessary to consider all aspects, of which the genetic improvement of parental varieties is the most essential.

The most consumed rice grains in Asia are endosperm that is usually processed as cooked rice. It is common that people from different areas have different tastes, which depend on nationality, climate, and traditional preferences. Generally speaking, people in northeast Asia, such as northern China, Japan, and Korea, prefer japonica rice with relatively short and round grains and stickier and softer texture after cooking. On the other hand, people in South and Southeast Asia, such as in southern China, India, Vietnam, Thailand, Indonesia, and Malaysia, prefer indica rice that is usually longer, has more slender grain, and is relatively fluffy after cooking. It is clear that, for developing varieties to meet the various demands for rice grain quality, the key point is to explore the genetic mechanisms of grain quality diversity in rice.

Evaluating rice grain quality

To evaluate rice grain quality, various methods have been extensively used. In general, three characters are regularly used to evaluate the cooking quality of rice: amylose content (AC), gelatinization temperature (GT), and gel consistency (GC).

Generally speaking, the AC of commercialized varieties varies from 14% to 17% in japonica rice and from 14% to 25% in indica rice. Many researchers have recognized that AC is one of the most important determinants for cooking and eating quality. Cooked rice with low AC tends to be moist, tender, and cohesive, whereas cooked rice with high AC is dry and fluffy in texture (Juliano 1985). GC refers to the running distance of hot rice gel in controlled temperature in a test tube. It can be categorized as hard, medium, and soft according to the distance from short to long (about 40 mm and 60 mm, respectively). GT is evaluated indirectly by alkali spreading value (ASV). Based on the values of AC, GC, and GT, the cooking and eating quality of different rice varieties can be divided into several categories (Li et al 1990). Varieties with fine cooking and eating quality are usually characterized by medium AC (15–17%), soft GC (>60 mm), and lower GT (ASV approx. 6.0). But, a significant difference can be quite common in cooked rice within varieties in the same category evaluated by AC, GC, and GT (Li et al 1990). To solve this problem, an RVA (rapid viscosity analyzer) profile was introduced for rice quality evaluation (Bason 1994). Research on RVA indicated that any single parameter was insufficient to characterize varieties for cooking and eating quality. But three parameters, breakdown viscosity (BDV), setback (SBV), and consistency of viscosity (CSV), combined together, could give great help to evaluating the cooking and eating quality of rice (Gu et al, unpublished data).

Although the cooking and eating quality of rice grain can be characterized by AC, GC, and GT together with the parameters of RVA profile, the characteristics described here are the physical properties. Apart from texture, the flavor of cooked rice is sometimes even important for evaluation of cooking and eating quality. And, all the chemical components in the endosperm, including major and minor components of starch, protein, fat, fatty acid of lipid, and aromatic substance, may contribute to the quality formation of cooked rice. To evaluate quality correctly, human testing is still necessary as the final step in addition to physico-chemical evaluation methods.

Starch synthesis–related genes (SSRGs) and their allelic variation

As mentioned, rice quality is a complex character, and controlled by many components. However, in terms of the composition of rice endosperm, starch, including amylose and amylopectin, accounts for about 90% of the milled rice. Much evidence strongly suggested that both the composition and fine structure of starch play a key role in determining rice quality (Juliano 1985).

Major genes are involved in the starch synthesis pathway

Starch is an insoluble polymer of glucose residues, and is a major storage product of rice grain. The starch granule is a complex structure with a hierarchical order composed of two distinct types of glucose polymers. The first is amylose, comprising largely unbranched α-(1→4)-linked glucan chains; the second is amylopectin, a larger and highly branched glucan polymer produced by the formation of α-(1→6)-linkages between adjoining straight glucan chains (see the review by James et al 2003). The AC and the length of the chain and its distribution in the starch granule have been proven to affect physico-chemical properties as well as cooking and eating quality.

In plants, the substrate for starch synthesis is ADP-glucose (ADPGlc), which is largely produced in cytosol with ADP-glucose pyrophosphorylase (AGP) catalyzed, and then transported into the plastids for starch synthesis. AGP is heterotrimeric, consisting of two large subunits (AGPL) and two small subunits (AGPS) encoded by at least six different genes. AGP activity is shown to be regulated by allosteric effectors, such as Pi and Mg^{2+}.

In the process of starch biosynthesis, glucan chain elongation is accomplished by starch synthases (SS), which catalyze the transfer of the glucosyl moiety of the soluble precursor ADPGlc to the reducing end of pre-existing α-(1→4)-linked glucan primers to produce the insoluble glucan polymers amylose and amylopectin. Rice has multiple isoforms of SS, containing up to five isoforms according to their conserved sequences. The major classes of SS can be broadly distributed into two groups: the first group is primarily involved in amylose synthesis, including granule-bound starch synthase I (GBSSI) and GBSSII; the second group is principally confined to amylopectin biosynthesis. GBSSI is encoded by *Waxy* genes, specifically functioning in the plastid in the elongation of amylose in storage tissue, such as endosperm; however, GBSSII, encoded by another gene, is found to elongate amylose in nonstorage organs. In addition to the role of amylose elongation, it is found that GBSSI is also responsible for the extension of long glucans within the amylopectin fraction.

For amylopectin synthesis, glucan elongation is accomplished by the second SS group, soluble starch synthases (SSS). Rice has eight SSS isoforms: SSS I, SSS IIa (SSS II-3), SSS IIb (SSS II-2), SSS IIc (SSS II-1), SSSI IIa (SSS III-2), SSS IIIb (SSS III-1), SSS IVa (SSS IV-1), and SSS IVb (SSS IV-2). Of these, SSS I (Fujita et al 2006), SSS IIa (Umemoto et al 2002, Nakamura et al 2005), and SSS IIIa (Fujita et al 2007) have been characterized from biochemical studies using their mutants or transformants. The accumulated knowledge showed that SSS I preferentially synthesized DP (degree of polymerization) 7 to 11 chains by elongating DP 4 to 7 short chains of glycogen or amylopectin (Fujita et al 2006), and the further extension to produce longer chains that extend between clusters is catalyzed by SSS II and/or SSS III. Meanwhile, some studies strongly suggested that SSS I, SSS IIa, and SSS IIIa are coordinated in the process of amylopectin synthesis.

Starch branching enzyme (SBE) is the only enzyme that can introduce α-1,6 glucosidic linkages into α-polyglucans in plants. Plants have two types of SBE: SBE I and SBE II. In rice, SBE1 (SBE I) and SBE3 (SBE IIb) have been found to be responsible for the formation of branching. Starch debranching enzyme (DBE) that directly hydrolyzes α-1,6 glucosidic linkages of α-polyglucans is divided into two types, isoamylase (ISA) and pullulanase (PULL) in rice. They differ in substrate specificity. To date, three genes, *ISA1*, *ISA2*, and *ISA3*, were found to encode isoamylase.

It is well known that many isoforms of SSS and SBE are involved in starch synthesis. Each enzyme has a unique role in determining starch fine structure. Although the functions of each enzyme have been roughly understood, the information that can be directly applied in a rice breeding program is absent, except for *Wx* and *SSSII-3,* which are responsible for AC and GT traits, respectively. Therefore, it is very important to exploit the major genes that are mainly responsible for grain quality determination. To solve these problems, the allelic variation on SSRGs should be fully understood; this would facilitate the selection of useful alleles for quality improvement in a breeding program.

Allelic variation of SSRGs

To date, only two genes, *Wx* and *SSS II-3*, had been studied and understood in terms of allelic variation. On the *Wx* locus, Sano (1984) had reported that the two functional alleles, Wx^a and Wx^b, which are associated with high (22–29%) and low (12–19%) AC, respectively, are predominantly distributed in indica and japonica rice, respectively. In addition, the *Wx* gene was successfully cloned by Wang et al (1990). The very low expression of the Wx^b allele has been shown to result from the inefficient splicing of intron 1 due to a G-to-T mutation at the 5′ splice site (Bligh et al 1998, Cai et al 1998). In 2003, the *wx* allele in glutinous rice was sequenced, and a 23-bp duplication occurred in exon 2 and resulted in a loss of function (Wanchana et al 2003). Ayres et al (1997) also reported that there was a CT repeat in the 5′-untranslated region of the *Wx* gene, and hence the *Wx* gene was divided into several haplotypes on the basis of CT repeats, suggesting that unknown alleles might be present at the *Wx* locus in rice. Recently, Mikami et al (2008) identified five putative alleles (Wx^a Wx^{in}, Wx^b, Wx^{op}, and *wx*) on the basis of an investigation of near-isogenic lines, and conducted a comparison of nucleotide sequences of five alleles among 18 rice accessions. The results showed that the sequence variation could be reflected in phenotype alterations.

The *SSSII-3* locus (also named *ALK*) was proven to be responsible for GT variation in rice. Umemoto et al (2002) mapped the *ALK* gene on chromosome 6, and revealed that *SSSII-3* played a role in the elongation of short chains of DP<10, which leads to the production of intermediate chains (DP 13–22). SSSII-3 activity is hindered in japonica rice, resulting in an amylopectin structure difference between the starches of indica and japonica rice. This gene was cloned through the map-based cloning approach (Gao et al 2003), and a mutation of G_{264} to C_{264} was shown to be associated with GT alteration in rice.

Except for *Wx* and *SSSII-3* loci, global understanding of allelic diversification on other SSRGs is absent so far. To survey the allelic variation on the SSRGs, we and our co-workers had successfully sequenced 18 SSRGs (*AGPL1, AGPL2, AGPS, Wx, GBSSII, SSSI, SSSII-1, SSSII-2, SSSII-3, SSSIII-1, SSSIII-2, SSSIV-1, SSSIV-2, SBE1, SBE3, SBE4, ISA1, PULL*) in 13 representative cultivars (Tian et al, unpublished). Haplotype analysis was conducted on the basis of sequence variation. The results indicated that there are several putative alleles at most of these SSRGs. For example, there are two alleles at the locus of *SBE1, SBE3,* and *ISA1*, respectively. Subsequently, the molecular markers for each locus had been developed to detect the allelic variation among much more germplasm. In total, 49 molecular markers had been developed in our research (Yan et al, unpublished). It should be noted that the allele estimation is based solely on sequence variation; whether this can be reflected in phenotypic alterations remains to be studied.

The effects of allelic diversification of SSRGs on rice grain quality

Association analysis

Association analysis, also known as association mapping or linkage disequilibrium mapping, is a method that relies on linkage disequilibrium to study the relationship between phenotypic variation and genetic polymorphisms (Breseghello and Sorrells 2006, Bao et al 2006). In our study, based on the sequence variation on 18 SSRGs of 13 cultivars, association analysis was conducted in our research group to search for the major genes responsible for grain quality alterations. The results from single-gene analysis indicated that the *Wx* gene was the major one associated with AC. Several others were also found to be associated with AC, but their F values were much lower than that of *Wx*. So, we preferred to think that *Wx* was the major gene controlling AC. The results from interaction analysis indicated that two other genes, *PULL* and *SSSII-3*, interacted with *Wx* to control AC. The results of single-gene analysis also indicated that six SSRGs were significantly associated with GC, among which *Wx* was thought to be the major one. Moreover, further analysis showed that four genes play regular roles interacting with *Wx* to control GC in rice. With an analysis of GT, the associated value of *SSSII-3* was much higher than others, so *SSSII-3* was thought to be the major gene controlling GT. Seven other genes were also found to associate with GT. Taken together, we can conclude that *Wx* and *SSSII-3* played a key role in determining the cooking and eating quality of rice grain, whereas other SSRGs might act as minor genes or regulators.

Near-isogenic line (NIL) analysis

It has been widely accepted that a NIL is an ideal material for precisely studying the function of target genes and their genetic effect as it can erase the interference caused by genetic noise. Rice grain quality is a typical quantitative trait with a complex genetic system, easily affected by environmental factors. So, it is very important to study the genes' effect, especially the functional difference of multiple alleles by using NILs. Recently, Mikami et al (2008) have successfully constructed five NILs for five alleles on the *Wx* locus, and precisely estimated the functional difference of five putative alleles. To investigate the status of allelic diversification on SSRGs, two representative cultivars, Guichao 2 (inferior quality, indica) and Suyunuo (good quality of waxy variety, japonica), were selected as a recurrent parent and donor parent, respectively, to develop NILs after selection assisted by the molecular marker developed above. Therefore, many NILs were obtained, including *SBE, SBE3, ISA1, PULL,* and *SSSI*. Consequently, the genetic effects of the variation of eight SSRGs were preliminarily analyzed after investigating the variation in starch physico-chemical properties, including AC, GT, and RVA profiles, among the NILs and their recurrent parent. The preliminary results showed that several NILs behaved differently in AC and/or RVA profiles when compared with their recurrent parent Guichao 2. This implied that the alleles on SSRGs between Guichao 2 and Suyunuo functionally differentiated, which resulted in different genetic effects.

Molecular improvement of hybrid rice quality

Molecular marker-assisted selection

In China, the poor quality of indica hybrid rice is often due to a high AC in its parent lines. For example, AC in Zhenshan 97 (ZS) and in Long-te-fu (LTF), the key Chinese female lines to produce indica hybrid rice, becomes dramatically high (25–30%). As a result, the derived hybrid rice quality is not satisfactory. Therefore, AC down-regulation to a reasonable level in endosperm is a major objective for the improvement of rice grain quality, especially of indica hybrid rice.

As showed and discussed above, the amylose in rice endosperm is synthesized by GBSSI, which is encoded by the *Wx* gene. Research from Cai et al (1998) showed that AC was correlated with the ability to excise intron 1 from the leader sequence of the *Wx* transcript, and a single nucleotide polymorphism (G or T) located at the first nucleotide of the splice donor site of the *Wx* intron 1 might be the key factor respond-

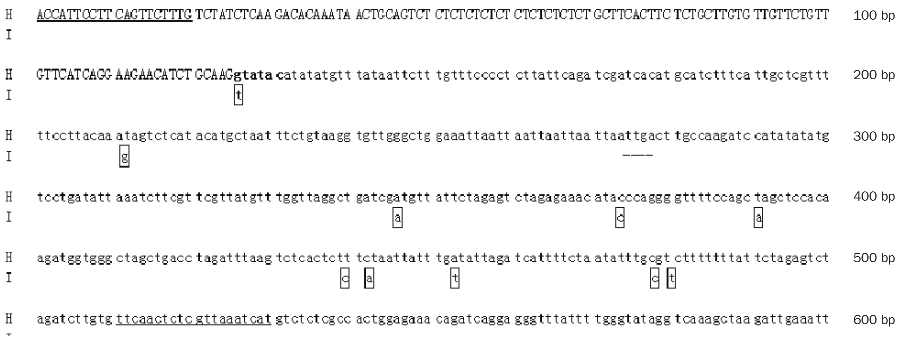

Fig. 1. Comparison of the *Wx* exon 1-intron 1 junction sequences from rice cultivars with high (H) and intermediate (I) amylose content (AC). Exon and intron sequences are shown in uppercase and lowercase letters, respectively. The complete sequence is given only for cultivars with high AC, and only those that differ from the corresponding nucleotides are shown in boxes for cultivars with an intermediate level of AC. The dashes (–) indicate nucleotide deletions in the intermediate AC cultivar, compared to the corresponding sequence of the high-AC cultivar. The sequences in bold represent the recognition site by *Acc*I in the high-AC cultivar, where it is not due to the G-to-T mutation in the case of the intermediate-AC cultivar. The primers used for CAPS marker PCR-*Acc*I determination are underlined.

ing to alternative splicing. Thus, a CAPS marker, named PCR-AccI, was subsequently developed to identify whether the nucleotide at this site is G or T, which could easily infer the AC in a genetic or breeding material (Cai et al 2002, Fig. 1). This is especially important for breeding programs that attempt to improve rice quality by MAS. By using the developed molecular marker and its assistance in selection, we successfully introgressed the *Wx*-TT locus of rice cultivars with good quality and intermediate AC into LTF-B and ZS-B (Liu et al 2006a). These were subsequently introduced into their relevant male sterile lines (LTF-A and ZS-A) to generate improved indica hybrids. In the selected lines LTF(tt) and ZS(tt), AC declined to a relatively low amount (15%) (Table 1). Consequently, the hybrids crossed from the selected lines had dramatically reduced amylose content. In field trials, the agronomic performance in the improved lines and their hybrids was examined and compared with that of the originals (Table 1). The other key factors involved in rice cooking and eating quality were also improved in the selected lines and their hybrids.

With the same marker of the *Wx* gene and MAS procedure, the AC of several other rice varieties was also improved (Table 1), such as indica restorer line Teqing (Liu et al 2006b). Teqing, either as an elite conventional cultivar with high yield or a male parent for two-line indica hybrid rice production, made a great contribution to rice production at the end of the 20th century in China. However, the AC in the endosperm of Teqing seeds is high. In the selected lines, the AC in the endosperm declined to a relatively low 15% from the 28.5% of the originals (Table 1). Consequently, the two-line hybrids derived from the selected lines and Pei'ai 64S also showed reduced amylose. In field trials, the agronomic performance in the improved lines and their hybrids was seen to be comparable with that of the originals except for a negative

Table 1. Comparison of cooking and eating quality between target lines and quality-improved lines after marker-assisted selection with the *Wx* genic PCR-*Acc*I marker.[a]

Target line	Line (hybrid) name	AC (%)	GC (mm)	GT (ASV)
Long-te-fu	LTF-B (wild-type)	27.8	60.0	7.0
	LTF(tt)-B (MAS)	17.9**	110.5**	3.7**
	LTF-A/MH63 (hybrid, wild-type)	22.5	34.8	5.4
	LTF(tt)-A /MH63 (hybrid, MAS)	16.1**	82.5**	3.7*
Zhenshan 97	ZS-B (wild-type)	26.8	40.0	6.4
	ZS(tt)-B (MAS)	15.5**	98.2**	2.6**
	ZS-A/MH63 (hybrid, wild-type)	21.4	61.0	4.5
	ZS(tt)-A/MH63 (hybrid, MAS)	16.0**	82.0*	2.4*
Teqing	Teqing (wild-type)	28.5	32.0	4.5
	Teqing-TT (MAS)	15.0**	75.0**	2.5*
	Pei'ai 64S/Teqing (hybrid, wild-type)	24.7	33.5	4.3
	Pei'ai 64S/Teqing-TT (hybrid, MAS)	19.2*	50.5**	3.8

[a]* and ** mean least significant differences at 0.05 and 0.01 probability levels, respectively, when compared with the original parent; no label means no significant difference.

impact on grain weight. Simultaneously, the other key factor involved in rice cooking and eating quality, GC, was also improved by the effect of reduced AC in the selected lines and their hybrids.

Transgenic approach

Up to now, SSRGs have been identified in rice. Thus, we could regulate the expression of specific SSRGs by using a transgenic approach to manipulate starch composition and/or structure as well as grain quality in target genotypes. So, it is possible to alter the synthesis of amylose in endosperm by manipulating *Wx* gene expression in order to improve the cooking and eating quality of rice. The antisense *Wx* gene was used to decrease AC in japonica rice (Shimada et al 1993, Terada et al 2000) and in Chinese japonica rice varieties (Chen et al 2002, Liu et al 2003). In our results, various degrees of reduction in amylose content, up to 96%, were found in the seeds of transgenic rice with the antisense *Wx* gene driven by the very promoter of the *Wx* gene. Consistently, opaque white seeds, similar to glutinous rice, were observed in several transgenic lines of japonica rice (Liu et al 2003). In transgenic lines derived from indica rice, which usually have high amylose, a significant reduction in amylose content was also found in the endosperm, but the reduction was lower than that observed in transgenic japonica rice. Genetic analysis demonstrated that the transgenes and improved amylose content

Table 2. The AC, GC, and GT performance of transgenic hybrid rice with the antisense *Wx* gene.[a]

Maintainer line/hybrid	AC (%)	GC (cm)	GT (ASV)
Maintainer line			
LTF-B (wild-type)	27.17	3.00	6.80
L25-B	8.09**	10.10**	5.20*
L18-B	23.16*	3.20	6.65
Hybrid			
LTF-A/YH559 (Teyou 559)	24.28	4.67	3.71
L25-A/YH559	10.06**	10.80**	2.77*
L18-A/YH559	21.16**	5.05*	3.54

[a]* and ** mean least significant differences at 0.05 and 0.01 probability levels, respectively, when compared with the original parent; no label means no significant difference.

are stably inherited (up to the fifteenth generation) in these transgenic lines.

It is imperative and more useful to apply this technique to indica hybrid rice. Toward this end, an antisense *Wx* gene was constructed and introduced into indica rice strains, including Long-te-fu B (LTF-B) and Zhenshan 97B, the widely used maintainer lines for producing hybrid rice in China (Chen et al 2002, Liu et al 2003). We have successfully lowered the AC in mature seeds of the target lines to as low as 7% (Table 2, Chen et al 2002). Two transgenic maintainer lines (L25B and L18B), derived from one of the key maintainer parents of an indica hybrid rice in China, LTF-B, were selected and the antisense *Wx* gene was subsequently introduced into the male sterile counterpart LTF-A to generate improved indica hybrids (Liu et al 2005). The transgenic indica hybrids crossed from the selected transgenic male sterile lines and tested restorer lines were tested for quality and agronomic performance under normal field conditions. Our results demonstrated that the reduction in AC in the homozygous transgenic maintainer lines was stably passed down in five successive generations and improved quality was also found in their relevant transgenic hybrids produced. The other two key characters of rice cooking and eating quality, gel consistency (GC) and gelatinization temperature (GT), were also improved in the grain of both the transgenic maintainer lines and their relevant hybrid rice (Table 2, Liu et al 2005). In addition, no change was observed for most of the agronomic characters of the transgenic maintainer lines and the relevant transgenic hybrids. Although the grain weight of the transgenic versions decreased, the grain yield of either the homozygous transgenic parent lines or the transgenic hybrids was similar when compared with that of the wild-type controls. These results suggest that the transgenic approach is an effective way to obtain rice lines with both improved quality and high yield, especially for indica hybrid rice.

Through the manipulation of the genes encoding starch branching enzymes (SBE), scientists have bred several novel varieties with high amylose content in maize and potato. But, up to now, this wasn't achieved in rice. In our study, by down-regulation of SBE expression via antisense RNA or RNA interference, we generated several novel transgenic rice lines with greatly increased apparent AC (AAC), up to more than 50%. This dramatic increase in AAC resulted in a novel starch rich in resistant starch (Liu et al, unpublished).

Characteristics and relationship of quality between hybrid rice and its parents

Rice quality is defined in terms of endosperm traits and differs in several distinct ways when compared with other plant traits such as plant height, heading date, etc. Endosperm is a triploid tissue as it developed from the fusion of two polar nuclei from the female and one sperm nucleus from the male. Therefore, the expression of rice quality in endosperm is controlled by a triploid genetic model. This feature leads to four genotypic combinations (*AAA, AAa, Aaa,* and *aaa*) in endosperm of hybrid rice with two different alleles, *A* and *a* in parents. Moreover, endosperm quality in the F_1 hybrid will differ between a cross from two different genotypes and their reciprocal cross (Mo 1990). In addition, the occurrence of a fertilized egg is the beginning of a new generation, so the endosperm on a rice plant represents the next generation of its maternal plant. For hybrid rice, the endosperm is on the F_2 generation as it forms on the F_1 hybrid plant. So, endosperm quality will be segregated among the seeds from the cross between two different genotypes, especially for hybrid rice, which is always made from two parents with distinct diversification.

As rice grain quality is controlled by quantitative trait loci expressed in the genome of triploid endosperm and the maternal plant, many genetic analyses had indicated that the inheritance of rice grain quality is complex. Genetic control for rice grain quality involves the genetic effects from genes of both endosperm and its maternal plant, which could be further partitioned into dominance and additive components, and the genetic parameters are also subject to modification by the interactions between genotype and environment (Shi et al 1997). Thus, quality expression in rice endosperm is quite complex, particularly for the quality of hybrid rice.

Perspectives for improving hybrid rice quality

With regard to specific gene expression in either triploid endosperm or the diploid maternal plant, as well as controlled by QTLs, it was truly difficult to improve rice grain quality. In addition, there might be an interaction between starch quality and other seed components, such as protein and fat content and composition. Thus, it is very difficult to improve rice grain quality, especially hybrid grain quality, because of the distinct genetic difference between the female and male parents. But, with the development of biotechnology and high-density marker linkage maps in rice, a series of studies for QTL mapping and functional analysis of grain quality formation have been conducted, and the roles of each SSRG in grain quality determination are elucidated.

Therefore, it is possible to improve grain quality, especially starch-related quality, via modern biotechnology such as MAS and the transgenic approach described above and, more recently, molecular designing. In particular, it is important to combine the power of both molecular and conventional breeding approaches.

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Notes

Authors' address: Key Laboratory of Plant Functional Genomics of Ministry of Education, Agricultural College, Yangzhou University, Yangzhou 225009, Jiangsu Province, China. Ming-Hong Gu, e-mail: gumh@yzu.edu.cn.

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Hybrid rice quality in China

Zhu Zhi-We, Min Jie, Zhang Bo-Ping, Chen Neng, and Wang Dan-Ying

Hybrid rice is a major crop produced in China. Its planting area covers 62% of the total area planted to rice. Thus, control of hybrid rice quality has become a major goal. Statistical analyses of rice quality data for major hybrid rice lines from 1985 to 2004 showed that the rice quality of indica hybrid rice improved quickly because of improvements in male sterile lines. When japonica hybrid rice quality was compared in different locations, lines planted in the north had much lower quality than those in the south. Among the different test parameters evaluated, head rice, chalky grain rate, and degree of chalkiness were found to be key factors in increasing hybrid rice quality. Results also suggest that improving male sterile lines will be a major way to increase hybrid rice quality. However, high tolerance of unfavorable environments was also important.

Hybrid rice has been developed in China for about 40 years, turning the country into a major player in the rice-growing world. According to China's National Agro-Tech Extension and Service Center (NATESC), about 62% of the rice grown in the country is hybrid rice and about 85% of indica rice cultivated in 2006 was indica hybrid rice. The grain quality and yield of hybrid rice in northern China, however, are not as good as conventional rice, so more hybrid rice is planted in the middle and southern parts of China. The goal for hybrid rice breeding in China is twofold. The first breeding goal was to develop a hybrid rice line with higher yield and higher resistance, meeting the needs of 1.3 billion people. After 1990, breeders turned to obtaining rice hybrids with higher yield, higher grain quality, and higher resistance in order to meet the needs of rice consumers who demanded better cooking quality, rice cakes, rice noodles, and so on. About 30% of the hybrid rice lines are of high grain quality, with japonica hybrid rice having better grain quality than its indica counterpart.

The national rice quality standard in China

The national rice quality standard, described in "NY/T 20-1986 *High Grain Quality Rice*," was established in 1986. It is used to evaluate the quality of rice varieties. The evaluation results are accepted by the National Crop Variety Approval Committee

Table 1. Grain quality standards (NY/T 593) for indica rice.[a]

Grade	HR (%)			CD (%)	T grade	AC (%)	QV (%)
	LG	MG	SG				
1	≥50	≥55	≥60	≤2	1	17–22	≥75
2	≥45	≥50	≥55	≤5	≤2	17–22	≥70
3	≥40	≥45	≥50	≤8	≤2	15–24	≥65
4	≥35	≥40	≥45	≤15	≤3	13–26	≥60
5	≥30	≥35	≥40	≤25	≤4	13–26	≥55

[a] HR = head rice, CD = chalkiness degree, T = translucency, AC = amylose content, QV = quality value, LG = long grain, MG = medium grain, SG = short grain.

and this constitutes an important step for grain quality improvement of hybrid rice. In 2002, this standard was modified to "NY/T 593-2002 *Cooking Rice Variety Quality*" (Table 1). The major changes made were amending the evaluation rule and setting up a synthesis evaluation method so that grain quality could be evaluated by using several indices for specific rice samples. Also, indica rice was divided into three classes on the basis of grain length, and low head rice rate was defined for long-grain rice as this class cracks easily. The grade was changed from 2 to 5 with grades 1 to 3 reflecting high grain quality and grades 4 and 5 describing medium grain quality.

The traits of rice grain quality evaluated in China are milling quality, appearance, cooking and eating quality, nutrition, and taste.

- Milling quality has three indices: brown rice rate (BR), milled rice rate (MR), and head rice rate (HR).
- Appearance has three indices: chalky grain rate (CG), chalkiness degree (CD), and translucency (T). The lower the CD and the higher the T, the better.
- Cooking and eating quality has three indices also: gelatinization temperature (expressed in terms of alkali spreading value [AV]), gel consistency (GC), and amylose content (AC).
- Nutrition quality uses protein content (PC) as the only index.

The taste evaluation (GB/T 15682-1995 *Rice-Determination of Cooking Test Quality*) is done involving at least 10 people. Using a control, people score rice samples for odor, color, shape, palatability, and cooled rice flavor. The total score is calculated with a maximum score of 100.

According to the contribution to grain quality, some indices were considered key indices in NY/T 593. They are head rice rate, chalkiness degree, translucency, amylose content, and quality value (QV) (Tables 1 and 2). An integrated evaluation of rice samples is done using these five indices; a certain grain quality grade equals a certain scoring range of the five indices.

Table 2. Grain quality standards (NY/T 593) for japonica rice.

Grade	HR (%)	CD (%)	T grade	AC (%)	QV (%)
1	≥72	≤1	1	15–18	≥85
2	≥69	≤3	≤2	15–18	≥80
3	≥66	≤5	≤2	15–20	≥75
4	≥63	≤10	≤3	13–22	≥70
5	≥60	≤15	≤3	13–22	≥65

Head rice rate was divided into three classes according to grain length. Long grain (LG) has a length more than 6.5 mm, medium grain (MG) is between 5.6 and 6.5 mm long, and short grain (SG) is less than 5.6 mm long.

Quality value includes the testing score and taste score. The tasting score is the sum of the index scores, the maximum being 100. First, the testing data are found in Tables 3 and 4. Next, the testing score is obtained from Table 5. The sum of all testing scores is then determined. The formula for quality value is

$$i = \frac{t_1 + t_2}{k} \times 100$$

where i = quality value, t_1 = total testing score, t_2 = total tasting score, and k = constant, the sum of the maximum scores of testing and tasting, 200.

The development of hybrid rice grain quality in China

Three-line indica hybrid rice was used in China for the first time in 1973. There were some predominant lines—such as Nanyou No. 2, Nanyou No. 6, Shanyou No. 2, Shanyou No. 6, Weiyou No. 2, and Weiyou No. 6, which were accepted by farmers. All these indica hybrid rice lines dominate because of their high yield and resistance. But they have poor grain quality—high amylose content, short gel consistency, and high chalkiness contribute to poor taste. From 1984 to 1995, some male sterile lines with good grain quality were developed, such as Yuetai A, Jin 23A, Yue 4A, Tianfeng A, Bo A, and Zhong 9A. They made good progress in improving the grain quality of indica hybrid rice. The hybrid combinations Honglianyou No. 6, Jinyou 207, Yueyou 9113, Tianyou 998, Boyou 253, and Zhongyou 253 reached first-grade grain quality under NY/T 593. These were planted on more than 333,000 ha in China in 2007.

Compared with three-line indica hybrid rice, the adoption of japonica hybrid rice was very slow; less than 3% of the japonica rice area was covered by japonica hybrid rice. This was because japonica hybrid rice has no remarkable advantage over japonica conventional rice in yield or grain quality. NATESC data showed that plant-

Table 3. Testing index for indica rice.

Index		Grade				
		1	2	3	4	5
BR (%)	LG	≥81	79.0–80.9	77.0–78.9	75.0–76.9	<75
	MG	≥82		78.0–79.9	76.0–77.9	<76
	SG	≥83	81.0–82.9	79.0–80.9	77.0–78.9	<77
MR (%)	LG	≥73	71.0–72.9	69.0–70.9	67.0–68.9	<67
	MG	≥74	72.0–73.9	70.0–71.9	68.0–69.9	<68
	SG	≥75	73.0–74.9	71.0–72.9	69.0–70.9	<69
HR (%)	LG	≥50	45.0–49.9	40.0–44.9	35.0–39.9	<35
	MG	≥55	50.0–54.9	45.0–49.9	40.0–44.9	<40
	SG	≥60	55.0–59.9	50.0–54.9	45.0–49.9	<45
CG (%)		≤10	11–20	21–30	31–60	>60
CD (%)		≤2	2.1–5.0	5.1–8.0	8.1–15.0	<15
T grade		1	2	3	4	5
AV grade		≥6	5.0–5.9	4.0–4.9	3.0–3.9	<3
GC (mm)		≥70	60–69	50–59	40–49	<40
AC (%)		17–22	15.0–16.9 or 22.1–24.0	13.0–14.9 or 24.1–26.0	11.0–12.9 or 26.1–28.0	<11 or >28
PC (%)		≥10	9.0–9.9	8.0–8.9	7.0–7.9	<7

Table 4. Testing index for japonica rice.

Index	Grade				
	1	2	3	4	5
BR (%)	≥84	82.0–83.9	80.0–81.9	78.0–79.9	<78
MR (%)	≥77	75.0–76.9	73.0–74.9	71.0–72.9	<71
HR (%)	≥72	69.0–71.9	66.0–68.9	63.0–65.9	<63
CG (%)	≤10	11–20	21–30	31–60	>60
CD (%)	≤1	1.1–3.0	3.1–5.0	5.1–10.0	>10
T grade	1	2	3	4	5
AV grade	7	6.0–6.9	5.0–5.9	4.0–4.9	<4
GC (mm)	≥80	70–79	60–69	50–59	<50
AC (%)	15–18	13.0–14.9 or 18.1–20.0	11.0–12.9 or 20.1–22.0	9.0–10.9 or 22.1–24.0	<9 or >24
PC (%)	≥9	8.0–8.9	7.0–7.9	6.0–6.9	<6

Table 5. Scores of testing index.

Grade	BR	MR	HR	CG	CD	T	AV	GC	AC	PC
1	10	5	15	5	15	10	5	10	15	10
2	8	4	12	4	12	8	4	8	12	8
3	6	3	9	3	9	6	3	6	9	6
4	4	2	6	2	6	4	2	4	6	4
5	2	1	3	1	3	2	1	2	3	2

ing area of some japonica hybrid rice, such as Liuyou No. 1 and Liuyou C bao, has been more then 6,000 ha since 1993.

Two-line hybrid rice developed faster than three-line hybrid rice recently. In 1987, rice breeders in China began two-line hybrid rice research. In 1996, the planting area of Peizashanqing, a two-line hybrid rice, reached 64,000 ha. In 2002, the area planted to another two-line hybrid, Liangyoupeijiu, amounted to 825,000 ha, becoming the most widely planted rice variety in China.

Generally, the grain quality of two-line hybrid rice is better as it has lower amylose content and longer gel consistency and good taste.

Table 6 shows the planted area and quality of the 10 most popular rice varieties. From the list, we can infer that

1. With the development of indica hybrid rice, conventional indica rice varieties were replaced by indica hybrid rice.
2. The japonica rice area increased because of the cultivation of conventional japonica rice. Japonica hybrid rice is still being underdeveloped.
3. All of the hybrid rice is indica. Few varieties of hybrid rice were planted to large areas in the 1990s.
4. From 1999 to 2006, the grain quality of indica hybrid rice improved rapidly, with two-line hybrids playing an important role. Liangyoupeijiu and Yangliangyou No. 6 are two good examples.

Grain quality of main hybrid combination series

In the last 35 years, Chinese rice breeders have bred about 10 types of male sterile lines with hundreds of individual CMS lines. We selected hybrid combinations from 15 rice male sterile lines that have been used widely, and analyzed their grain quality. We tested 1,036 samples in our laboratory from 2000 to 2003.

Generally, the male sterile lines have a key role conferring rice grain quality traits, but it is also important to have feasible restorer lines. The coefficient of variation (CV) of brown rice rate, milled rice rate, grain length, and grain length/grain width in all the series of hybrid rice combinations is smaller; these indices are inherited basically from male sterile lines. The main difference in grain quality in hybrid rice lines

Table 6. Grain quality and area planted to top 10 rice varieties.[a]

	1983			1993		
	Variety	Area (000 ha)	Quality	Variety	Area (000 ha)	Quality
Hybrid rice	Shanyou No. 2	2,041	I,LQ	Shanyou 63	4,868	I,MQ
	Weiyou No. 6	1,051	I	Shanyou 64	749	I,MQ
	Shanyou No. 6	905	I,MQ	D you 63	713	I,MQ
	Shanyou No. 3	222	I	Weiyou 64	625	I
	Siyou No. 6	74	I,MQ	Shanyougui 99	398	I,HQ
	Aiyou No. 1	72	I,HQ	Shanyougui 33	297	I,LQ
	Shanyou No. 8	70	I	Boyou 64	285	I,MQ
	Nanyou No. 3	66	I	Weiyou 46	285	I,LQ
	Siyou No. 30	57	I,MQ	Shanyou No. 10	281	I,LQ
	Nanyou No. 2	54	I	S64	247	I,LQ
Conventional rice	Guichao No. 2	1,431	I,LQ	Zhefu 802	446	I,LQ
	Guangluai No. 4	1,110	I,LQ	Wuyujing No. 3	431	J,HQ
	Yuanfengzao	717	I,LQ	Jingxian 89	329	I,MQ
	Guichao No. 13	681	I,MQ	Xiangzaoxian No. 7	320	I,LQ
	Shuanggui No. 1	571	I,LQ	Wuyujing No. 2	253	J,HQ
	Hong 410	557	I,MQ	Zhe 733	206	I,LQ
	Xianfeng No. 1	413	I,MQ	Qishanzhan	199	I,LQ
	Nanjing No. 11	408	I,MQ	Qiguizao 25	173	I,LQ
	Xiangzaoai No. 9	391	I,LQ	Ewan No. 5	173	I,HQ
	Guanger 104	324	I,MQ	Hejiang 19	160	J,HQ

[a] I = indica rice, J = japonica rice, LQ = lower grain quality, MQ = medium grain quality, HQ = high grain quality.

in the same series as the hybrid rice combinations lies in head rice rate, chalky grain rate, and degree of chalkiness (Table 7) (for the high CV value).

Of those 15 hybrid rice combination series, Chuanyou, Yixiangyou, II you, Neiyou, and Liangxi were found to have higher grain quality as they had low chalkiness, long gel consistency, and acceptable amylose content. As to short-shaped grains, the II you series had good milling quality. The series of Mianyou, Zhongyou, T you, and Jinyou had better appearance.

	1999			2006		
	Variety	Area (000 ha)	Quality	Variety	Area (000 ha)	Quality
Hybrid rice	Shanyou 63	1,439	I,MQ	Liangyoupeijiu	771	I,HQ
	Gangyou 22	1,151	I,MQ	Jinyou 402	535	I,LQ
	II you 501	617	I,MQ	Jinyou 207	461	I,HQ
	II you 838	515	I,LQ	Jinyou 463	417	I,MQ
	Xieyou 46	424	I,LQ	Fengliangyou No. 1	400	I,MQ
	Shanyou 46	311	I,LQ	Gangyou 725	284	I,MQ
	Weiyou 46	303	I,LQ	Jinyou 974	240	I,MQ
	Teyou 63	274	I,MQ	II you 838	235	I,LQ
	Shanyou 77	263	I,MQ	Yangliangyou No. 6	205	I,HQ
	Shanyoudouxi No. 1	247	I,MQ	II youming 86	189	I,MQ
Conventional rice	Wuyunjing No. 7	614	J,HQ	Kongyu 131	700	J,HQ
	Wuyujing No. 3	343	J,HQ	Xudao No. 3	320	J,MQ
	Wuyunjing No. 8	327	J,MQ	Longjing 14	241	J,HQ
	Suijing 3	293	J,MQ	Jijing 88	204	J,HQ
	Jiayu 948	281	I,MQ	Wujing 15	185	J,MQ
	Zaofeng No. 9	225	I,HQ	Wuyunjing No. 7	171	J,HQ
	Kendao No. 8	219	J,HQ	Wuyujing No. 3	170	J,HQ
	Hejiang 19	211	J,HQ	Yanfeng 47	142	J,MQ
	Zhongyouzao 81	191	I,HQ	Ningjing No. 1	141	J,MQ
	Liaojing 454	155	J,HQ	Yujing No. 6	136	J,HQ

Main grain quality features of hybrid rice

Compared with conventional rice, indica hybrid rice has better milling quality and japonica hybrid rice is not so special.

The data in Table 8 came from 8,390 rice variety samples tested in our laboratory from 1985 to 2002. Indica hybrid rice had higher milling quality than indica conventional rice, but appearance and cooking quality were lower. The frequency of chalky grain of indica hybrid rice in the second grade was 15% only, so chalkiness is a key element of grain quality for indica hybrid rice. The low frequency in gel consistency implies indica hybrid rice's low-grade taste.

Table 7. Average values and coefficients of variation of grain quality in 15 hybrid rice combination series.[a]

Series		BR (%)	MR (%)	HR (%)	GL (mm)	L/W	CG (%)	CD (%)	T grade	Av grade	GC (mm)	AC (%)	PC (%)
Shanyou	Av	80.7	72.6	44.8	6.1	2.4	77	18.3	2.6	5.2	50	21.2	9.6
n=128	CV	1.7	2.8	34.1	4.0	7.9	27.3	52.5	24.4	16.6	23.4	9.3	11.6
Mianyou	Av	81.1	73.5	49.6	7.1	3.1	34	5.2	1.3	5.4	62	21.7	8.9
n=35	CV	1.9	1.9	19.3	3.5	5.2	58.7	65.5	40.3	15.2	24.9	19.7	13.4
Zhongyou	Av	80.8	72.9	51.2	6.8	3.1	34	6.5	2.0	5.5	57	21.5	9.8
n=75	CV	1.5	2.1	24.4	5.6	8.4	58.5	79.8	33.7	16.0	24.8	13.2	11.8
Chuanyou	Av	81.1	73.8	51.2	6.5	2.7	49	8.2	1.8	5.0	68	18.4	9.9
n=34	CV	1.3	1.8	22.0	8.5	11.8	53.2	71.4	28.1	20.3	21.0	18.8	10.2
Yixiangyou	Av	80.2	73.0	48.9	6.8	3.0	28	4.6	1.7	5.7	72	16.9	9.4
n=44	CV	0.9	1.7	34.5	6.5	7.4	62.5	77.2	43.1	18.3	21.4	21.6	14.0
K you	Av	81.1	73.3	46.6	6.8	2.9	56	10.4	2.3	5.9	58	22.6	9.6
n=44	CV	1.4	2.1	28.2	4.4	5.5	43.9	80.8	32.0	11.2	25.7	5.8	14.0
T you	Av	80.9	73.3	56.7	6.5	2.8	47	7.8	1.6	5.8	57	21.0	9.9
n=44	CV	1.3	2.2	17.5	6.1	11.4	53.3	77.9	38.4	16.4	24.3	11.9	12.6
Jinyou	Av	81.4	73.3	48.9	6.8	3.1	50	8.4	1.8	5.4	56	22.3	9.6
n=85	CV	1.4	2.1	29.8	4.2	6.6	44.1	78.0	40.5	18.1	25.2	8.6	15.1
D you	Av	81.3	73.6	49.7	6.7	2.9	53	10.3	1.9	5.6	54	22.1	9.1
n=99	CV	1.2	1.9	22.9	5.4	7.9	42.8	71.3	32.2	13.3	25.1	11.2	13.3
Gangyou	Av	80.9	73.2	49.0	6.1	2.4	70	14.6	2.8	5.1	49	21.2	9.2
n=28	CV	1.2	1.7	26.4	4.0	3.9	24.7	42.2	26.5	18.5	28.5	8.6	16.2
Teyou	Av	81.1	73.7	55.6	6.1	2.5	81	19.2	2.7	6.3	49	21.7	9.7
n=56	CV	1.5	2.7	21.5	4.4	8.2	26.1	50.1	27.9	11.6	29.2	9.4	9.9
Xieyou	Av	81.3	73.7	51.5	6.7	2.8	66	14.4	2.2	5.6	61	21.8	9.3
n=46	CV	1.2	2.1	23.8	6.1	9.8	29.6	57.3	25.5	18.3	29.3	11.5	13.5
II you	Av	80.7	73.6	57.3	6.1	2.5	51	10.2	2.2	5.6	51	21.7	9.9
n=99	CV	1.4	2.5	21.6	4.1	7.2	38.9	59.7	28.9	15.9	29.0	9.8	13.2
Neiyou	Av	81.2	73.6	51.0	7.1	3.1	23	3.9	1.4	5.3	70	15.8	10.7
n=25	CV	0.8	1.6	26.4	6.3	8.6	53.5	76. 9	35. 2	13.5	19.9	20.8	12.7
Liangxi	Av	81.0	73.4	51.2	6.6	3.0	40	7.0	2.1	5.1	71	18.9	9.8
n=194	CV	1.7	2.4	30.8	7.2	8.3	58.1	97.1	33.4	22.0	22.2	24.3	12.0

[a] GL = grain length, L/W = grain length/grain width, Av = average, CV = coefficient of variation.

Table 8. Frequency of rice quality indices meeting the NY/T 593 criteria.

Index	Indica conventional rice n=3,280		Indica hybrid rice n=2,607		Japonica conventional rice n=2,219		Japonica hybrid rice n=284	
	First grade	≥Second grade	First grade	≥Second grade	First grade	≥Second grade	First grade	≥Second grade
BR	17.4	65.9	36.7	87.2	36.0	87.9	46.1	92.3
MR	24.7	65.9	48.4	84.9	17.8	91.9	22.2	74.3
HR	44.4	56.9	52.1	65.1	33.7	55.0	29.9	55.6
CG	16.0	31.8	4.6	15.0	17.3	36.3	17.6	34.5
CD	22.5	42.8	10.9	31.7	16.1	45.2	17.3	39.8
T	19.5	57.7	20.9	72.2	43.0	83.5	40.5	80.1
AV	61.6	82.7	36.6	68.7	92.4	97.5	89.4	94.7
GC	43.0	54.9	32.1	46.9	41.3	72.7	47.5	78.5
AC	15.5	57.8	40.6	75.4	51.2	83.7	65.5	94.0
PC	49.2	77.1	38.6	66.3	39.6	73.6	41.5	72.2

The grain quality of japonica hybrid rice is the same as that of conventional japonica rice and better than that of indica hybrid rice, except in terms of milled rice rate and head rice rate. This explains the small area planted to japonica hybrid rice.

Head rice rate, chalkiness, translucency, and gel consistency in hybrid rice are unstable.

The data on grain quality of Shanyou 63, D you 527, and Liangyoupeijiu are shown in Table 8. Tested in our laboratory from 2000 to 2003, these samples came from different provinces in middle and southern China. Shanyou 63 and D you 527 are varieties with medium grain quality; Liangyoupeijiu has high grain quality and is a two-line variety. All of them had high CVs in head rice rate, chalkiness, translucency, and gel consistency. These grain quality indices can be readily affected by weather, environment, and cultivation techniques. As cultivation technique can be controlled by the farmer, it is very important to breed hybrid rice that can tolerate adverse conditions.

The key index for the grain quality of indica hybrid rice is chalkiness, which becomes a deciding factor for indica hybrid rice grading. Compared with conventional rice, the chalkiness of indica hybrid rice is very large (Table 8). Some varieties of indica hybrid rice have very low chalkiness, but the highest CV would reduce the grades of some samples (Tables 7 and 9).

In terms of frequency of rice quality indices meeting the NY/T 593 (Table 8), gel consistency is the second key index for grain quality of indica hybrid rice. Short gel consistency would not give indica hybrid rice such a palatable taste.

Table 9. Average values and coefficients of variation of grain quality of hybrid rice.

Variety		BR (%)	MR (%)	HR (%)	GL (mm)	L/W	CG (%)	CD (%)	T grade	AV grade	GC (mm)	AC (%)	PC (%)
Shanyou 63	Av	81.2	73.5	51.6	6.1	2.4	72.4	16.8	2.4	5.2	62.5	20.8	10.1
n=61	CV	1.0	1.7	16.6	2.5	4.3	21.6	40.7	25.5	10.0	22.7	5.7	11.1
D you 527	Av	80.4	72.0	46.1	7.0	3.0	66.9	17.7	2.3	5.4	64.8	21.9	8.4
n=28	CV	1.1	1.9	18.9	3.1	3.5	25.3	55.8	23.6	11.6	20.3	5.7	11.2
Liangyoupeijiu	Av	80.5	72.5	53.0	6.6	2.9	44.2	8.2	1.9	5.9	80.1	21.3	9.3
n=36	CV	1.2	2.1	14.3	3.5	4.0	34.1	51.9	31.9	9.5	13.4	5.9	9.7

Conclusions

Guided by national standards and breeding goals, grain quality improvement efforts for hybrid rice have made great progress. Male sterile lines play an important role in enhancing grain quality. It is crucial that male sterile lines have corresponding quality as in restorer lines. The key grain quality indices—head rice rate, degree of chalkiness, gel consistency, and amylose content—emphasize the need to improve the grain quality of all kinds of rice. Improvement of chalkiness in hybrid rice is especially important. The grain quality of japonica hybrid rice is the same as that of japonica conventional rice, so it is not a drag on developing japonica hybrid rice.

Notes

Authors' address: China National Rice Research Institute, Hangzhou, Zhejiang, China.

Improving grain quality in hybrid rice

Feng Wang, Wuge Liu, Yisheng Han, and Zhenrong Liu

Grain quality is a major objective for many breeding programs. Because the ultimate economic product of hybrid rice is a bulk of segregating endosperms of the F_2 generation, the quality traits of hybrid rice are co-determined by both female and male parental lines. This makes hybrid rice breeding for fine grain quality more complicated and challenging. With the rapid development of biotechnology, inheritance of the main quality traits has been elucidated and most of them have been precisely mapped on linkage groups with molecular markers. Based on the understanding of the nature of the inheritance of the quality attributes and the mapping of the genes, strategies for breeding high-quality hybrid rice have been proposed, and a highly efficient breeding platform needs to be established by pyramiding all the desirable grain quality traits into the elite parental lines of hybrid rice with both traditional backcross and molecular marker–assisted selection to accelerate the development of fine-quality hybrid rice. Some MS lines, such as Taifeng A, Tianxiang A, and Yuefeng A, and restorer lines Guanghui 998 and Guanghui 122, as well as a series of heterotic combinations with better grain quality traits have been developed.

Rice is one of the most important crops in the world as it provides staple food for almost half of the world's population. Hybrid rice has a yield advantage of more than 30% over conventional pure-line varieties (Yuan 1994). Its potential for increasing rice production and productivity has motivated many countries to attempt to exploit this technology. However, seeds borne on F_1 rice hybrids are F_2 seeds. Most of the quality traits, such as endosperm translucency, chalkiness, amylose content (AC), gelatinization temperature (GT), and gel consistency (GC), are bound to show genetic segregation due to the endosperm being formed by fertilization and union of the 2n gamete of the female parent and the n gamete from the male parent (Virmani 1994). Thus, both female and male parental lines have effects on the grain quality traits of hybrid rice. This makes the improvement of grain quality of hybrid rice more complicated and challenging.

Since the widely used male sterile (MS) lines Zhenshan 97A, V20A, II-32A, D62A, Longtepu A, You I A, and Bo A have short or bold grains, high amylose content, hard gel consistency, and chalky endosperm, the hybrid rice developed by using these lines also has the same drawbacks in quality traits as the parental lines (Zhou et al 2003, Hu 2007). As people become better off, poor grain quality will be one of the main factors hindering a wider acceptance of hybrid rice by some rice-growing farmers.

Therefore, it is of importance for facilitating the development of hybrid rice and enhancing the economic benefit for rice-growing farmers to study the genetic basis of quality traits and to develop high-quality parental lines with both traditional breeding methods and modern biotechnology.

The genetics of quality traits and strategies for improvement

Grain size, shape, and weight

Since rice is consumed and processed mainly in whole-kernel form, grain length, breadth, shape, and weight are always of foremost importance for its market price (Virmani 1994). Long and slender grain is generally preferred for indica rice by a majority of consumers in China, the United States, and most Asian countries (Unnevehr et al 1992, Juliano and Villareal 1993). Grain length, breadth, and shape are determined by the maternal genotype and long grain length is dominant over short grain length (Xu et al 1995, Li et al 2000). Fu et al (1994) reported that the grain shape of hybrid rice is mainly determined by the MS line and grain length, breadth, and weight are co-determined by both the male and female parent.

Many QTLs associated with grain size, shape, and weight, such as *qGL-1*, *qGL-3*, *qGL-3a, grl1-1, GS3*, *gl3, gs3, gb3, grb7-1, grb7-1,* and *gw8.1*, have been mapped on chromosomes 1, 3, and 8, respectively (Wan et al 2005, 2006, Amarawathi et al 2008, Rabiei et al 2004, Fan et al 2006, Xie et al 2006). Among these, *qGL-3* and *qGL-3a* were mapped to the same interval on chromosome 3 and the restorer line IR24 allele at *qGL-3a* increased grain length by an average of 0.28 mm across four environments (Wan et al 2005, 2006). *GS3* is a QTL with a major effect on grain size and weight; it was detected in a widely used restorer line of hybrid rice (Minghui 63) and mapped into a 1-cM region delimited by an indel marker (GS09) and SSR marker (MRG5881) on chromosome 3 (Fan et al 2006). It has been demonstrated that *GS3* is a recessive gene that can increase grain length and grain weight. Incorporating this long-grain allele into the medium-grain parental line by using molecular marker–assisted selection, to make both parents carry the long-grain allele, could improve both the yield and grain quality of rice hybrids.

Endosperm appearance

Endosperm appearance is primarily decided by amylose content. Endosperm varies from waxy or dull to translucent as amylose content increases (Kumar et al 1994). Khush et al (1988) crossed a parent having a different endosperm appearance ranging from opaque to translucent. The appearance of the endosperm of seeds borne on hybrid plants varied in crosses involving waxy or dull endosperm, on the one hand,

and translucent endosperm, on the other. Therefore, to obtain hybrids with a uniform grain appearance, both parents should have the same endosperm type.

Endosperm appearance also varies in degree of chalkiness, which is referred to as white center, white belly, or white back depending upon its location on or within the endosperm (Virmani 1994). White centers and white bellies are reported to be governed by a single recessive gene and white bellies are also reported to be controlled by a dominant gene (Nagai 1958) and by polygenes (Chang and Somrith 1979). He et al (1999) analyzed QTLs for chalkiness by using a doubled-haploid (DH) population derived from an anther culture of the hybrid ZYQ (indica)/JX17 (japonica) and they detected three QTLs (*qPGWC*-8, *qPGWC*-12, and *qSWC*-3) for ratio of chalky grain and square of the white core on chromosomes 3, 8, and 12, respectively.

Xu et al (1989) reported that a significant difference existed in chalkiness score between the F_1 and its reciprocal cross F_1 when both parental lines had different chalkiness. If using a variety with less chalkiness as a female parent and a variety with more chalkiness as a male parent, the F_1 will have less chalkiness, and low chalkiness is partially dominant over high chalkiness. Zhu et al (2002) also reported that the male sterile line (female parent) had greater effects on chalkiness than the restorer line (male parent) in hybrid rice. Therefore, it is crucial to develop and use a male sterile line with less or no chalkiness in a breeding program for hybrid rice.

Amylose content, gel consistency, and gelatinization temperature

Amylose content is one of the major factors that influence cooking and eating quality. Grains harvested from hybrid rice plants are bulk F_2 seeds and they show segregation for amylose content. Thus, the amylose content of hybrid rice is actually the mean value of the bulk F_2 seeds and is reported to generally be between that of the parents. Hybrids with desirable AC can be bred by carefully selecting proper parents (Kumar et al 1994).

Amylose content is genetically controlled by a major gene (*Wx*) on chromosome 6 and some minor QTLs (*qAC-2* and *qAC-5*) on chromosomes 2 and 5, respectively (He et al 1999, Tian et al 2005, Sun et al 2006). The major gene *Wx* has already been sequenced (Wang et al 1990). Bligh et al (1995) found that there was a simple repeat sequence $(CT)_n$ in the gene, and then developed a simple sequence repeat (SSR) marker 484/485 (RM190) based on it. Bligh et al (1995), Ayres et al (1997), and Shu et al (1999) analyzed various rice varieties/lines with this SSR marker, and detected more than eight alleles at the locus of RM190 in the *Wx* gene. Varieties carrying various alleles have different AC. The number of repeat sequences is highly correlated with the amylose content of rice. Therefore, SSR marker RM190 is useful in molecular marker–assisted selection for improving the AC of parental lines in hybrid rice.

Gel consistency in rice determines the softness or hardness of cooked rice after cooling. Hard GC is dominant over intermediate or soft GC. Similarly, intermediate GC was found to be dominant over soft GC in analysis of F_1 seeds (Kumar et al 1994). GC in rice is found to be genetically controlled by a major-effect QTL (*qGC-6*) on chromosome 6 and some minor-effect QTLs (*qGC-2* and *qGC-7*) on chromosomes 2 and 7, respectively (Tian et al 2005, Sun et al 2006, He et al 1999). The major-effect

QTL *qGC-6* is located in the vicinity of the waxy locus on chromosome 6. Lanceras et al (2000) used a RIL population derived from KDML105 × CT9993 to analyze the QTLs for grain quality traits and also found that the QTLs for AC and GC were near each other. This provides a perfect explanation for why a variety with low AC generally has soft GC. The coincidence of QTLs in the vicinity of the *Wx* gene for AC and GC may be due to pleiotropy or linkage (Lanceras et al 2000).

Gelatinization temperature is known to affect the cooking quality of rice. It is also reported to be genetically controlled by major- and minor-effect QTLs. The major genes (QTLs) *alk*, *qGT-6*, and/or *qASS-6* have been mapped to the same interval on chromosome 6 (Gao et al 2003, Tian et al 2005, He et al 1999). Just like AC and GC, one major gene and some modifier genes governed GT in rice (Lanceras et al 2000, Sun et al 2006).

According to what was mentioned above, AC, GC, and GT are controlled by the waxy locus and/or the tightly linked genomic region. The segment harboring *Wx* and *alk* genes on chromosome 6 plays a very important role in determining the cooking and eating quality of rice. Thus, AC, GC, and GT for the parental lines of hybrid rice could be improved by substituting the targeted segment (allele) on chromosome 6 with a desirable one from a high-quality parent through backcross breeding and molecular marker–assisted selection. Using this method, Zhou et al (2003) successfully improved a high-AC maintainer (Zhenshan 97B) through substituting the segment harboring the high-AC *Wx* allele with a segment harboring the low-AC *Wx* allele from restorer Minghui 63 that has medium AC, soft GC, and high GT. The improved line has not only low AC but also soft GC and high GT as the donor.

Aroma

Aroma is an important quality characteristic of high-quality rice. It is reported to be controlled by a single recessive gene (*fgr*) on chromosome 8 (Sood and Siddiq 1978, Ahn et al 1992, Lorieux et al 1996, Li et al 2006). Many kinds of markers tightly linked with *fgr*, such as RG28 (Ahn et al1992), RSP04 (Jin et al 2003), SCU015RM (Cordeiro et al 1996), and GR01 (Li et al 2006), have been developed. In order to improve the precision of marker-assisted selection (MAS) for the fragrance gene in rice, a functional marker (GRFM04) has been developed based on the deletion of 8 bp in the DNA sequence of the *BAD2* gene, namely, the fragrance gene, in fragrant rice varieties (Wang et al 2008). This provides a powerful measure for efficiently incorporating the recessive fragrance gene into parental lines of hybrid rice and finally developing aromatic hybrid rice through MAS breeding.

Advances in improving grain quality in hybrid rice

Improving quality traits by using MAS

Tianfeng A/B is a newly developed elite MS/maintainer line. It has long grains, high resistance to blast, and good combining ability. By using it, more than 10 heterotic hybrids have been released for commercial production in China. However, although it has high amylose content, it has no aroma. In order to lower its AC and add aroma

Table 1. Some quality traits of the improved hybrid rice lines.[a]

Improved lines	GL (mm)	GB (mm)	L/B	Brown rice (%)	Milled rice (%)	AC (%)	GC (mm)	Alkali value (grade)	Aroma
D211	9.6	2.5	3.8	71.4	67.4	15.7	72	2.0	Moderate
D221	9.5	2.5	3.8	81.3	73.5	15.5	73	2.0	Strong
D223	9.6	2.5	3.8	80.8	73.0	15.6	72	2.1	Strong
D225	10.3	2.5	4.0	80.7	72.8	15.2	76	2.0	Strong
D238	10.0	2.4	4.0	79.8	71.5	13.0	81	2.0	Strong
Yuefeng B (donor)	9.9	2.4	4.1	82.0	73.7	17.4	75	5.8	Strong
Tianfeng B (recipient)	9.2	2.4	3.8	81.2	73	27.0	32	3.8	None

[a]GL = grain length; GB = grain breadth; L/B = length/breadth ratio; AC = amylose content; GT = gelatinization temperature.

into it, a maintainer (Yuefeng B) with fragrance and low amylose content was used as donor of the low-AC allele and *fgr* gene. Through four backcrossings and two selfings and using MAS based on the tightly linked SSR marker RM190 of the *Wx* gene and the functional marker GRFM04 of the fragrance gene *fgr*, the low-AC allele and *fgr* were successfully introgressed into the maintainer Tianfeng B and five improved lines obtained. The results of chemical analysis showed (Table 1) that the AC of the improved lines D211, D221, and D223 ranged from 13.0% to 15.7%, which was much lower than that of Tianfeng B, which was 27.0%, and their GC ranged from 72 to 81 mm, which was much higher than that of Tianfeng B. This indicates that the low-AC allele from Yuefeng B also influences GC. All the improved lines had moderate or strong aroma after the *fgr* gene was introgressed. However, the alkali value of the improved lines was about grade 2.0, lower than that of both the donor and recipient. The other agronomic traits of improved lines showed no significant difference from those of the original Tianfeng B. By backcrossing one of the improved lines (D221) with the MS lines, a new MS line, Tianxiang A, with low AC, soft GC, and strong aroma, was developed. This indicated success using MAS with the SSR marker RM190 and the functional marker GRFM04 to improve the quality traits of AC, GC, and aroma for parental lines in hybrid rice.

MS lines with fine grain quality

Most of the MS lines used for commercial production in China have short or bold grains, chalky endosperm, high amylose content, and hard gel consistency. This leads to the hybrid combinations derived from these lines usually having poor grain quality. Therefore, breeding fine-quality MS lines is of prime importance. Yuefeng A and Taifeng A are two newly developed MS lines in Guangdong, China. Compared with the previously developed Xieqingzao A, both MS lines have slender grain, less or no

Table 2. Quality traits of elite male sterile lines and their hybrids.

Quality trait	Taifeng A	Yuefeng A	Xieqingzao A (check)	Taifengyou 3922	Taifengyou 368	Fengyou 998	Fengyou Xiangzhan	Shanyou 63 (check)
Brown rice (%)	81.6	80.3	80.7	83.8	83.8	82.6	79.2	82.6
Milled rice (%)	–	71.2	71.4	75.4	–	76.4	72.6	76.2
Head rice (%)	74.1	28.2	24.6	52.4	70.4	66.5	63.5	67.9
Kernel length (mm)	8.1	6.7	6.5	8.7	7.3	7.0	7.1	6.3
Kernel breadth (mm)	1.8	1.8	2.3	1.8	1.9	1.94	2.0	2.5
Kernel shape (L/B)	4.3	3.7	2.8	4.8	3.8	3.6	3.5	2.5
Chalky kernels (%)	1	0	100	10	17	30	7	74
Chalkiness (%)	0.1	0	25.2	3.9	6.0	5.4	0.7	16.1
Translucency (grade)	1	1	3	1	–	2	1	2
Gel consistency (mm)	82	92	58	53	80	83	84	44
Amylose content (%)	13.9	14.3	23.4	21.3	16.5	17.0	13.5	20.1
Aromatic	No	Yes	No	No	No	Yes	Yes	No

chalkiness, translucent endosperm, low amylose content, and soft gel consistency (Table 2). Taifeng A has long kernel length of 8.1 mm and its head rice recovery can reach 74.1%. Yuefeng A has aroma in both leaf and kernel. Hybrids derived from these two MS lines, such as Taifengyou 3922, Taifengyou 368, Fengyou 998, and Fengyouxiangzhan, also have better appearance and eating and cooking quality than Shanyou 63, a widely grown hybrid in China.

Table 3. Grain quality of elite restorer lines and their hybrids.

Quality trait	Guanghui 998	Guanghui 122	Qiuyou 998	Huayou 998	Tianyou 998	Tianyou 122
Brown rice (%)	80.6	81.9	80.9	80.0	78.9	81.3
Milled rice (%)	71.8	73.7	73.7	–	–	–
Head rice (%)	52.1	58.8	56.5	64.2	61.5	34.6
Kernel length (mm)	6.9	6.8	6.2	–	–	–
Kernel breadth (mm)	2.2	2.2	–	–	–	–
Kernel shape (L/B)	3.4	3.1	3.0	2.9	3.1	3.0
Chalky kernels (%)	22	16	16	6.0	10.0	15
Chalkiness (%)	3.6	3.3	2.5	0.6	2.5	3.8
Translucency (grade)	2	2	2	–	–	–
Gel consistency (mm)	76	76	63	80	65	54
Amylose content (%)	15.5	15.3	22.9	17.6	22.1	19.1

Restorer lines with fine grain quality

Restorer lines are another parent determining the grain quality of hybrid rice. Breeding new restorers possessing fine grain quality is of importance also. Much progress has been made in Guangdong Province. Some restorer lines with better grain quality, such as Guanghui 998, Guanghui 122, Guanghui 290, and Guanghui 368, have been developed and widely used in recent years (Table 3). A series of hybrids, such as Tianyou 998, Boyou 998, Qiuyou 998, Fengyou 998 (Fengyousimiao), Tianyou 122, Boyou 122, Yueza 122, Shanyou 122, Tianyou 368, and Tianyou 290, have been developed and widely planted on more than 2 million hectares in southern China.

Conclusions

Both parents co-determine the grain quality of hybrid rice; thus, the improvement of parental lines in desirable quality traits is of importance in breeding high-quality hybrid rice. To date, the inheritance of the main quality traits has been elucidated and most of them have been precisely mapped on linkage groups with molecular markers. This makes it possible to deploy a “breeding by design” strategy and to pyramid the favorable genes or QTLs conferring the desirable quality traits into elite parental lines, especially MS lines, by marker-assisted breeding to establish a highly efficient platform for hybrid rice breeding and to accelerate the rapid development of fine-quality hybrid rice.

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Notes

Authors' address: Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, Guangdong, China; email: fwang1631@163.com.

Technology of hybrid seed production

Cultivation techniques for high-yielding hybrid rice seed production

Zhou Chengshu, Liu Aimin, and Xiao Cenling

This paper explains the four stages of hybrid rice seed production in China. It describes the main cultivation technology for Chinese hybrid rice seed production from three aspects: cultivation and composition of male and female populations, synchronization of flowering, and raising the rate of outcrossing seed set. A future way for large-scale standardization and mechanization for Chinese hybrid rice seed production is proposed.

Seed production technology is a key to the success of hybrid rice. According to statistics, the area planted for hybrid rice seed production was 50,000 ha in Hunan Province in 1976, with a yield of 324 kg ha^{-1}. The area ratio between seed production and hybrid rice cultivation was 1:15. In 1990, the area of hybrid rice seed production was 17,000 ha in Hunan Province, with a yield of 2,745 kg ha^{-1}, and the area ratio between seed production and hybrid rice cultivation was 1:120. From 1990 to 2007, the area of hybrid rice seed production in China was 80,000 to 100,000 ha and the yield was 2,550 kg ha^{-1} to 3,750 kg ha^{-1}, which ensured a supply for the seed demand for hybrid rice commercial cultivation.

The four stages of research and application of hybrid rice seed production technology in China

The technical exploration stage (from 1973 to 1978)

The yield of seed production increased from the initial 90 kg ha^{-1} to 450 kg ha^{-1}. The key techniques at this stage were the arrangement of a secure period for flowering and sowing time, adjusting flowering date, proper composition of the parental population, raising the rate of outcrossing seed set, roguing, and purification. The way to use leaf number interval, time interval, and effective accumulated temperature interval to determine seed sowing time was advanced. A series of integrated technologies was also advanced, including the three factors for secure heading and flowering (temperature, sunlight, and relative humidity), applying fertilizer and chemicals to control and adjust florescence, cutting the canopy leaves, removing the flag-leaf sheath, and spraying a low dosage of GA_3 to improve out-crossing posture and strict isolation.

The technical development stage (from 1979 to 1983)

Seed production yield was raised from 450 to 1,500 kg ha^{-1}. Research was based on biological characteristics of the parental lines of three-line hybrid rice, studying GA_3 technology as the core to developing high-yielding parental populations, and using a different method for cultivating the male and female parents separately, expanding the row ratio between male and female lines, nurturing a parental population with a harmonious number of spikelets and flowering period, and spraying GA_3 to reduce the degree of panicle included in the flag-leaf sheath of sterile lines. High-yielding technology was integrated by cultivating high-yield populations, spraying GA_3 at the proper time with a proper dosage to eliminate leaf cutting and leaf sheath stripping.

The technical maturity stage (from 1984 to 1990)

The yield of seed production was raised from 1,500 to 2,700 kg ha^{-1}, and the average yield in 1995 surpassed 3,000 kg ha^{-1} at many seed production bases in Hunan Province under spring or summer seasons. In some high-yielding seed production areas, the yield reached 4,500 kg ha^{-1}, whereas the highest yield reached 7,386 kg ha^{-1}. The primary research objective at this stage was to raise the percentage of outcrossing seed set of the female line and a high-yielding model was formed as "yielding 3 t ha^{-1} as the goal, increasing female capacity as a basis, and enhancing pollen density as a core, with integrated techniques as support." The outcrossing seed set surpassed 50% and the highest reached 85.2% in some high-yielding plots. At the same time, theoretical research on the cultivation of hybrid rice seed production was maturing, and a theory of "outcrossing cultivation of rice" was established.

Two-line hybrid rice seed production technology research stage (from 1989 to 1995)

The fertility expression of photo-thermo-sensitive genic male sterile (PTGMS) rice was a response to environmental conditions and, with the genetic drift of critical temperature for fertility reversing, a primary study on the techniques of secure two-line hybrid seed production was done based on high yield with high-quality seed production technology for three-line hybrid rice. PTGMS lines can be used with a lower critical temperature, with operational procedures for producing core seed and foundation seed of PTGMS lines. A series with secure high yield and high-quality seed production techniques was formed, including using a safety index to select a location as a seed plot in which environmental conditions could satisfy the requirements for a secure period for fertility reversing, by arranging the date for a secure period in the sensitive stage of the seed production season, adopting cultivation methods to ensure male sterility expression, and monitoring fertility expression in the field. As a result, the yield of two-line hybrid rice seed production was as high as that of three-line hybrids and seed quality reached national standards.

The characteristics of cultivation technology of hybrid rice seed production in China

Outcrossing of rice

Two parental lines (a sterile line and a restorer line) were cultivated alternating with each other symbiotically and they must flower at the same time. The sterile line can form seed by accepting pollen from the restorer line through supplementary pollination. Then, hybrid rice seed production is called outcrossing cultivation. Techniques for establishing a high-yielding parental population included using different cultivation methods according to different characteristics between two parents, such as growth duration, tillering ability, and response to fertilizer, along with synchronization of flowering, the proportion of the parental population, outcrossing posture, and the weather during flowering and pollination. This needs a consideration of the requirement for ensuring female sterility expression stably for two-line hybrid rice production.

A large number of hybrid combinations in seed production

The current rice-farming system adopted in China includes double-season rice and single-season rice. Hundreds of hybrid rice varieties are used in commercial production, and different varieties are planted in corresponding areas and are classified by ecological types, mainly as japonica and indica types. Indica types are divided into photosensitive and nonphotosensitive types, and nonphotosensitive types include early-season rice, single-season rice, and late-season rice. Early-season hybrid rice is divided into middle-maturing and late-maturing types. Late-season hybrid rice is divided into early-maturing and late-maturing types. Different types of hybrid rice are adopted in corresponding areas for seed production.

The sowing time interval between parents varied a lot with different types of hybrid rice combinations for seed production. For instance, the sowing time interval for Xianglianyou68 was 11 days, whereas for Xinxiangyou63 it was about 60 days.

Seed production covers four seasons

After 30 years of development, five seed production districts had been established from 17°N (Sanya City in Hainan Province) to 33°N (Salt City in Jiangsu Province), with a coverage of five seasons.

1. Spring, summer, and autumn season are adopted in central-southern China and the northern part of southern China, including Hunan Province, Jiangxi Province, a part of Guangxi Province, and Fujian Province, with bases located in middle- or low-elevation hilly area.
2. Summer-season seed production is used in the southwest part of China, including Chongqing and Sichuan provinces.
3. Late-summer season seed production is followed in Salt City of Jiangsu Province, eastern China.
4. Spring- and autumn-season seed production is popular in southern China, including Guangdong, Guangxi, and Fujian provinces.

5. Winter-season seed production is used only in the southern part of Hainan Province, where rice grows under tropical conditions.

Current status of cultivation techniques for hybrid rice seed production

The cultivation techniques of hybrid seed production adopted in China are based on a labor-intensive model characterized by many labor inputs, labor-intensiveness, precise field management, and corresponding cultivation methods according to the growth and development differences between male and female lines.

The composition of parental populations for seed production

The composition ratio of parental populations was determined by the row ratio and planting density. The land ratio between males and females was 1:3–4, the spikelet ratio was 1:2.5–3.0, and the outcrossing seed-setting rate of female parents was above 50%. The specific technical indicators of parental populations were as follows:

Item	Hills ha^{-1} (10,000)	Basic seedlings ha^{-1} (10,000)	Maximal seedlings ha^{-1} (10,000)	Effective panicles ha^{-1} (10,000)	Total spikelets ha^{-1} (10,000)
Male	2.7–4.2	45–75	180–225	90–140	12,000
Female	33–40	180–225	375–450	300–375	30,000–37,500

The morphological characters of male parents were vigorous plants, strong and longer leaves with deep green color, more tillers, more panicles and spikelets, and a longer heading period with longer bloom duration (10–15 days).

The morphological characters of female parents were vigorous plants with erect leaves and short flag leaf, fewer panicles developed from late tillers, and an ample number of seedlings, panicles, and grains. Heading was uniform with shorter bloom duration (8–10 days).

Cultivation techniques for male parents

1. Techniques for raising seedlings

   Four main techniques were used for raising seedlings: the water-bed method, water-bed with two-phase method, floppy-pad in paddy field, and floppy-pad on dry land. Different techniques were adopted according to different combinations, different locations, and different seasons.
2. Techniques for fertilizer application

   Two to three fertilizer applications were especially used for the male parent based on the same quantity of fertilizer being applied to the female parent. The best way was deep dressing of fertilizer into the soil in the form of a ball mixed with soil and placed in between the hills of the male parent. The time to apply fertilizer to the male parent varies by type of parental lines, which was determined by growth duration, sowing time interval between parents, and the time of A-line transplanting.

3. Planting model of the male parent

   There were four main planting models: single row, double narrow rows, double small rows, and double wide rows. Different planting models were applicable for different types of hybrid combinations. Generally, single row, double small rows, and double wide rows were better adapted for the combinations with male parents with longer growth duration and large pollen load, whereas double narrow rows were better for the combinations with male parents with shorter growth duration, weaker in tillering, and shorter sowing interval between parents or the combination that the female's growth duration is longer than the male parent's. Different planting models were adopted in different seed production areas.

The row ratio and pollination methods were adopted according to the planting model of the male parent. The row ratio for the single-row planting model was usually 1:10–12, and 2:10–12 for double narrow rows, 2:10–14 for double small rows, and 2:16–18 for double wide rows.

Rope pulling or a single pole was often used for pollinating a single row, double narrow rows, and double small rows, whereas the double-pole method was adopted for double wide rows.

Ridge planting was adopted for the male parent when in combination with inverted sowing time interval (the sowing time of the male parent was later than that of the female).

Ditch planting was adopted for the male parent when the female parent was directly seeded.

Wider planting was adopted for the male parent with long growth duration, stronger tillering ability, and high panicle-bearing rate, and transplanting density can be 20 × 27–30 cm and 1–2 seedlings per hill. Narrow planting was adopted for the male parent with short growth period, weaker tillering ability, and lower panicle-bearing rate, and transplanting density can be 15 × 20 cm and 3–4 seedlings per hill.

Cultivation techniques for the female parent

1. Planting models

   Transplanting and direct seeding were the two methods used for female parents. Transplanting seedlings includes water-bed seedlings, floppy-pad in paddy field seedlings, and dry (paddy) younger seedlings. The younger seedlings were usually transplanted at a seedling age of 10–13 days, with leaf number of 2.5–3.5, whereas the water-bed seedlings had a seedling age of 16–20 days, with leaf number 4.5–5.5.

   To establish a female population with enough panicles and spikelets and with uniform heading and flowering, transplanting enough basic seedlings with a planting density of 14 × 14–17 cm and 2–3 seedlings per hill was an essential method for obtaining a population with more than 70% of panicles coming from the main culm.

2. Fertilizer technology

   The total fertilizer application was divided as follows:

   A heavier dosage used as basic fertilizer: 80% of N, 100% of P, and 40% of K were applied before transplanting.

   Light topdressing with 20% of N and 20% of K applied about 5 days after transplanting.

   A supplementary application of 30% of K and a small amount of P and other microelement fertilizer at the 5th stage of panicle differentiation and the initial heading stage (foliar spraying).

   The quantity of fertilizer required for seed production varied a lot with different sterile lines, for example, the N requirement for Xinxiang A and Jing23 A was 120–150 kg ha^{-1} whereas 150–180 kg ha^{-1} was required for Fengyuan A and Zhenxian97 A, but 200–225 kg ha^{-1} was required for II-32 A.
3. Irrigation

   Irrigation management was the way to control the population development in hybrid seed production and could also help to increase outcrossing seed set. From the late tillering stage to the 3rd stage of panicle differentiation of the female parent, the field should be keep sun-dried to control later tillers and then increase the uniformity of panicle heading, controlling the length of canopy leaves, reducing obstacles to pollination, and improving resistance to lodging. The field should be irrigated with standing deepwater during the booting stage and flowering period to maintain the field with higher relative humidity, which is favorable for maintaining the stigma vitality of the female parent and the pollen vitality of the male parent. This is also good for raising the simultaneity rate of florescence between the male and female. The field should be irrigated intermittently with shallow water at all other times.

Techniques for raising the rate of outcrossing seed-setting of the female parent

Choose appropriate season and location. Seed bases should have favorable ecological conditions for growing rice. In China, the seed bases were basically concentrated in the basin and valley area of hills and mountains after more than 20 years of optimization. The selected seed bases had concentrated land and relatively flat topography, sufficient sunshine, fertile soil, and a perfect irrigation and drainage system. The environmental conditions had appropriate temperature, humidity, and sunshine during the flowering and pollination period.

Techniques used for obtaining synchronization. To obtain synchronization, three methods were used in seed production to determine the sowing interval between the male and female lines. The leaf number difference method was mainly adopted when the sowing interval was more than 15 days, the time (days) difference method was usually applied when the sowing interval was less than 15 days, and the accumulated effective temperature (EAT) difference method was more accurate but not so convenient because it needed special equipment for recording daily temperature. All these methods could ensure synchronization of heading and flowering. To obtain full synchronization with a sufficient pollen supply from the male parent, three different planting models were applied in practice, one-phase, two-phase, and three-phase planting of male parents according to the heading rate and the length of the flowering period of the pollen parent.

Many factors can influence days from sowing to initial heading, such as parents' seed quality (days from sowing to heading of newly produced seeds were about 2–3 days longer than those of stored seed), different ways of raising seedlings (water-bed seedlings take about 2–3 days longer than small seedlings by floppy-bed on dry land), transplanting density (lower density with longer duration), fertilizer application (higher dosage of N with longer duration), and water management (often-dried fields have longer duration than ones kept with deep standing water). To reduce the heading difference from cultivation practices between plots within a seed base, adopting technical rules of cultivation is proposed when seed production activity involves many contract farmers.

Synchronization can be forecast through investigation on parents' growing status, leaf number in the main culm, leaf emergence rate, and the morphological characters of panicle differentiation. Several methods are used to check synchronization, such as stripping young panicles, checking the remaining leaf number of the main culm, checking the corresponding leaf number using panicle differentiation, calculating and comparing EAT data between male and female parents, and checking the days from sowing up to the present day.

Regulating heading and flowering date was a unique technique especially used in hybrid rice seed production. According to the results of forecasting and checking panicle development, measures with deep standing water or potassium to promote early heading, and nitrogen and drought to delay heading, were taken to adjust the heading and flowering date by extending or shortening flowering duration.

Techniques used to improve outcrossing posture. The panicle neck of sterile lines could not be fully elongated, usually with one-fourth to one-third of the panicle concealed in the flag-leaf sheath. In that case, the number of pollinated spikelets of the female would be reduced. Cutting off the upper part of canopy leaves and removing the flag-leaf sheath were often adopted to expose the panicles in the 1970s. Because of the success of a study on GA_3 application, the panicles of the female parent could be fully exserted with all of the spikelets exposed, and the outcrossing ability of the female parent improved markedly.

The main technical indicators of the ideal outcrossing posture for seed production are as follows: the panicle layer of male parents is 10–20 cm higher than that of the female, the height of the male parent is about 90–100 cm, the exserted length of panicle necks is about −2–10 cm, grain exposed rate was more than 95%, full panicle exposed rate was more than 90%, and the panicle was above the flag leaf (the distance between the top of panicles and flag leaf was more than 5 cm, or the extended angle of the flag leaf was greater than 45°).

The main techniques to improve outcrossing posture for hybrid rice seed production is spraying GA_3 and cutting the top of canopy leaves when they are longer.

Techniques used for supplementary pollination. The four main methods used for supplementary pollination are as follows: pulling a rope to drive pollen, sweeping a single pole to drive pollen, pushing a single pole to drive pollen, and pushing double poles to drive pollen. Different methods are adopted by different seed bases, and also applied according to different planting models for the male parent. Rope pulling is the most efficient method and it requires less labor, whereas bamboo pole methods are commonly adopted at places where labor is sufficient.

Development of cultivation technology for hybrid rice seed production in China

A mature system of cultivation techniques for hybrid rice seed production with stabilized seed bases has been established in China since research and practices began more than 30 years ago. But this was based on labor-intensive farming practices, and seed production was scattered on a small scale, had more labor input, was labor-intensive, and had a low level of mechanization and higher production costs. The ability to withstand a bad harvest induced by abnormal climate was weak, with unstable seed yield and quality. As China's industrialization and urbanization have developed, the population engaged in agriculture has decreased markedly. The labor-intensive model for seed production was not conducive to a stable and sustainable development of seed bases, which has become an obstacle to the development of hybrid rice in China. Therefore, to continue to develop hybrid rice, seed production must be large-scale and standardized. Popularizing mechanization with the corresponding techniques of chemical control should be a tendency of seed production, such as broadcast seeding or mechanical transplanting, spraying, harvesting and drying, using slow-release fertilizer, chemical weeding, regulating heading and flowering, controlling plant height to prevent lodging, reducing split seeds and sprouting on panicles, etc. The goal is to establish a new system of cultivation techniques with low labor input and economies of scale.

Notes

Authors' addresses: Zhou Chengshu, Hunan Hybrid Rice Research Center; Liu Aimin, Hunan Longping Seed Co. Ltd.; Xiao Cenling, Hunan Agricultural University.

Using herbicide lethality in hybrid rice

Q.S. Zhu, Q.J Yang, D.W. Zhang, S.M. Wang, J. Dong, C. Feng, and J.B. Zhu

This paper discusses the theory and significance of new seed production. Results indicate that by adopting a mixed seeding production method, the male parent could be killed and female parent survive by spraying 9,000 mL of 25% bentazone water every hectare after pollination, and the production of hybrid seeds could reach 6,690 kg ha^{-1}. The development of this technique has prospects.

With improved seed production technology and parental outcrossing traits, the yield of hybrid rice seed production has increased from hundreds of kilograms per hectare to a national average of 2.5 t ha^{-1} during the past three decades in China. However, the current protocol of seed production is based on technology established 30 years ago, that is, female and male parents are transplanted separately in different rows at a certain ratio, and they are harvested separately. The complexity of seed production has become a limiting factor for wide-scale adoption of hybrid rice because of much labor required, difficulties in controlling purity, and mechanical operations.

In 1984, Norin 8 m, a mutant from japonica rice variety Norin 8, was found to be lethally susceptible to bentazone (3-isopropyl-1H-benzo -2,1,3-thiadiazine-4(3H)-ketone-2,2-dioxide), which is the ingredient of herbicides such as Basagran, Bentazone, etc., that are widely used in rice fields for weed control. The discovery of bentazone lethality, which was genetically controlled by a pair of recessive genes, opened a path for an innovative system of hybrid rice seed production. The key of the technology is to integrate the lethality into restorer lines that have the same or similar number of days to flowering as cytoplasmic male sterile (CMS) lines so that seeds of female and male parents can be blended, planted, or transplanted together. After pollination, bentazone herbicide is sprayed in the field to eliminate male plants and all plants left in the field are CMS plants bearing hybrid seeds, which can be bulk harvested. The advantages of this technology are the easier operation of seed production, use of less labor, and adaptation to mechanical seed production on a large scale, as well as increased hybrid seed yield and purity. After 21 seasons in 10 years of breeding, we successfully developed many CMS restorer lines with bentazone lethality and different maturities to synchronize various CMS lines. Heterotic hybrids were developed by using these restorer lines, and applied to production commercially.

The donor parent for lethality was a mutant line, Norin 8 m, which is lethally susceptible to bentazone, and contributed by Dr. Genkichi Takeda, the former president of the Japan Plant Breeding Association. This mutant line was a typical japonica variety, and was used to cross with many indica restorer lines to develop restorer lines with an indica genetic background with bentazone lethality, and the same or similar number of days to flowering as CMS female parents. Pedigree and backcross methods were employed in breeding. A study on herbicide dose was first carried out for selecting the best herbicide rate that would let plants show obvious but limited damage by the chemical, and would survive for seed harvest. The herbicide we used was bentazone 25% aqua, made by Jiansu Lulilai Co. Ltd., China. (The normal dosage recommended by the manufacturer for rice fields is 3,000–6,000 mL ha^{-1}.) Experiments showed that at heading stage under 600 mL ha^{-1} of bentazone herbicide, the plants showed no damage. With 900 mL ha^{-1}, plants had minor damage, but recovered in 10 days after herbicide spraying. However, under 1,200–1,800 mL ha^{-1}, the plants showed obvious damage 7 days after spraying, and panicles died after 10 days. When using the herbicide at 2,700–3,600 mL ha^{-1}, very obvious damage was observed in the plants by the fourth day after spraying, and whole plants died 10 days after spraying.

In the breeding process, a dosage of 900 mL ha^{-1} of this herbicide was applied to screen plants with susceptibility at the panicle initiation stage in every breeding generation from the F_2. Selection based on phenotype started from the seventh day after herbicide treatment. Herbicide susceptibility was selected along with other agronomic traits, which followed the same selection criteria as in a general restorer line breeding program. The trait of days to flowering was specifically noted because of required flowering synchronization with female parents. Different CMS lines were planted along with R lines to provide a reference for days to flowering. The selected R lines were single-plant harvested and advanced to the next generation and then pair-testcrossed with various CMS lines to make hybrids for testing on restoration ability, combining ability, and yield (heterosis). Table 1 shows one of the selected R lines used for a herbicide dosage study for bentazone sensitivity.

From testcrosses to different experiments and trials, selected hybrid varieties with high yield potential and acceptable quality were advanced to provincial or national yield trials, as well as for commercial hybrid seed production. Many restorer lines with bentazone lethality in various indica genetic backgrounds and related commercial hybrids have now been developed. One of the hybrids with good performance, Green Rice # 5, has achieved national variety acceptance and is being commercialized. In a farmer's yield trial in 2004, it produced 11.45 t ha^{-1} of grain and outyielded by 1.52 t ha^{-1} the best hybrid check, Shanyou 63, the widest grown hybrid in China, with a 15.3% yield advantage but the same maturity.

In a study of seed production in 2004, two seed production systems, conventional seed production (raw ratio of 1 male:5 females, separated transplanting) and mixed transplanting (seed ratio of 1 male:5 females, mixed transplanting), were contrasted for hybrid seed yield. The results showed that the mixed method produced 2.0 t ha^{-1}

Table 1. Lethal susceptibility of R line 2E06 (Hefei, 2004).

Herbicide dosage (mL ha^{-1})	Fertilized spikelets (%)	Plant survival (%)
0	82.1	98.4
4,500	32.1	46.3
6,000	28.7	17.7
7,500	27.5	6.7
9,000	19.1	1.1
10,500	10.5	0.3
12,000	1.2	0.0

Table 2. Comparison of hybrid seed production methods (Hefei, 2004).

Method	Plants ha^{-1} (× 1,000)	Effective panicles ha^{-1} (× 1,000)	Total spikelets panicle^{-1}	Seed set (%)	1,000-grain weight (g)	Yield (t ha^{-1})
Conventional (C)	18.5	218	192.2	47.0	22	4.7
Mixed planting (M)	20.0	260	199.4	58.9	22	6.7
Increase (M – C)	1.5	42	7.2	11.9	0	2.0
Increase (%)	8.1	19.3	3.5	25.3	0	42.6

more hybrid seeds than the conventional method, with a 42.6% yield increase (Table 2). All yield components in the mixed method had different degrees of increase, but the most significant increase was seed set rate, which was 25% higher than that of the conventional method.

By using molecular marker technology, susceptibility to bentazone lethality was mapped in the rice genome. In the future for line development with bentazone lethality, it will be very helpful to apply molecular marker-assisted selection, which will speed up the selection process and accuracy.

We are also exploring two other potential applications of bentazone lethality in hybrid rice. One is the application in S-line breeding for two-line hybrid rice. It is widely known that sterility in the S line is affected by temperature, that is, a temperature lower than the critical sterility temperature (CST) will turn the S line from sterile to fertile. During the critical stages of two-line hybrid seed production, a sudden temperature change in a few days could become a disaster for a seed company because selfed seeds in the S line would make hybrid seeds unusable. We are working to transfer the lethality into the S line to eliminate selfed seeds in the seedling stage by using seed coat technology or direct spraying of bentazone. Another application is to breed a CMS maintainer line (B line) with susceptibility to bentazone lethality. The lethality can be transferred into an existing B line, and then develop a new line (B') that will have exactly the same genetic background as the original B line, but with bentazone lethality. In parental seed production, a CMS line (A) × B is used to produce more A-line seeds for further parental seed production. However, A × B' is used to provide A-line seeds for A × R hybrid seed production. In A × B' seed production, A and B' are mix-planted and transplanted in a certain ratio. After pollination, bentazone is sprayed to eliminate B' plants; thus, all of the seeds bulk-harvested in the field are A-line seeds used for further A × R seed production. The advantage of B'-line application is to ensure seed purity of the A line, and simplify A × B seed production as well as intellectual property protection because of the small area required for A × B seed increase. A and B' have similar genetic backgrounds and days to flowering, so the heterotic performance of a hybrid will not be affected, and high yield of parental seed production could be obtained because of flowering synchronization.

We are also working to apply the technology of bentazone lethality in hybrid rice in the areas of mixed direct seeding and mechanized operations. The application of this technology will produce hybrid seeds more economically with increased seed yield and purity.

Notes

Authors' address: Green Food Research Institute, Anhui Academy of Agricultural Sciences, Hefei, Anhui 230031, China; email: zhuqisheng0551@163.com.

Agro-management for high yield in hybrid rice seed production

M.P. Pandey, S.R. Dhua, A.K. Gupta, and B.K. Pal

The large-scale adoption of hybrids in India is constrained by the inadequate availability of good-quality seeds and their higher cost. Improved agro-management could help boost hybrid seed yield beyond the current achievable yield of 2.0 t ha^{-1}. As a function of panicles m^{-2}, seeds $panicle^{-1}$, and grain weight, yield can be manipulated by management. Replicated experiments using a randomized factorial block design were conducted during four main crop seasons at the experiment station of GBPUA&T, Pantnagar, on hybrids PSD 1 and PSD 3. Standard cultural and seed production practices to promote outcrossing were adopted. Three factors of nursery management, seed rate, depth of seeding, and nitrogen application method, were combined with two factors of main-field treatments (nitrogen application method and seedling number $hill^{-1}$ at transplanting) to determine the best combination. Further refinement with respect to higher seed rate and higher nitrogen application enhanced yield up to 4.33 t ha^{-1} in PSD 3 (A/R) and 7.8 t ha^{-1} for its restorer. The best agro-technique was 15 g m^{-2} seed rate, seeded at 5-cm depth, and 150 kg ha^{-1} of N in a split application that produced vigorous (taller), robust, multitiller seedlings in the seedbed and, when transplanted, two seedlings $hill^{-1}$ with the application of 210 kg N in splits in the seed production plot. Higher yield was mainly attributed to 2.18 times higher panicles m^{-2} than the control. Implications of the findings are discussed.

Rice is the world's most important food crop and a primary source of about half of the world's population. The world's capacity to sustain a favorable food production–population balance has come under the spotlight in view of continued population growth and a drastic reduction in the growth of cereal production (Brown et al 1996). Various estimates indicate that the world will have to produce 38% more rice by 2030 to satisfy the growing demand without affecting the resource base adversely (Khush 2005). In an Indian context, it is well recognized that the next quantum increase in rice production is possible through the large-scale popularization and adoption of hybrids. Since 1989, the country has released 33 hybrids for cultivation in different situations and regions. India now grows a meager 1.0 million ha area under hybrids.

The slower adoption of hybrids has been constrained by the inadequate availability of good-quality seeds and their higher cost. Available technology in the country is capable of producing 1.5–2.0 t ha^{-1} hybrid seed yield (Pandey et al 1996). Studies in China suggest refining the agro-techniques of seed and pollen parents in hybrid seed production to maximize yield (Peng et al 1998). However, no research data in the country are available on the refinement of agro-techniques for hybrid rice seed production to further enhance seed production. Hence, our experiments were conducted to study (1) the effect of nursery management factors on seedling growth parameters, (2) the tillering pattern of seed and pollen parents, and (3) the effect of management factors in the nursery and field of parental lines of some popular rice hybrids on seed yield and related traits.

Materials and methods

Experimental site and materials

Experiments on agro-management techniques for hybrid seed production of both hybrids were conducted in wet seasons at the experiment station of G.B. Pant University of Agriculture and Technology, Pantnagar, located at the Himalyan foothills at 29°N latitude, 79.3°E longitude, and an altitude of 243.8 m above mean sea level.

The experimental material comprised parental lines of two early-maturing and high-yielding hybrids, Pant Sankar Dhan 1 (PSD 1) (Pal 2003) and Pant Sankar Dhan 3 (PSD 3) (Gupta 2006), released for commercial cultivation under irrigated and transplanted conditions in the plains of Uttar Pradesh and Uttarakhand in northwest India. These hybrids share a common female parent, wild abortive–based cytoplasmic male sterile line UPRI 95-17A flowering at 100 days after seeding (DAS). PSD 1 has a male restorer parent, UPRI 92-133R, flowering (50%) at 95 DAS, whereas PSD 3 has UPRI 93-287R, flowering at 94 DAS.

The restorers (UPRI 92-133R and UPR 93-287R) were studied for two years each with the seed parent (CMS) of the respective hybrids. Single seedings of seed parent UPRI 95-17A were done in the nursery in all four years and the respective restorers were staggered-sown on three dates at a 2-d interval to obtain synchronized flowering between parents. The first seeding of the restorer coincided with the first seeding date of the seed parent.

Raising the nursery and management factors

A nursery field was prepared thoroughly by repeated plowing and uniform leveling. Seedbeds of 2.0 m × 1.2 m for the pollen parent and 4.0 m × 1.2 m for the female parent were prepared separately for each treatment. Application of 1.0 kg N, 0.6 kg P, and 0.4 kg K was made on 100 m^2 of seedbed area. The crop was sprayed with 0.5% Zn SO_4 twice in a nursery at 10 and 20 DAS to overcome the problem of *Khaira* disease (caused by zinc deficiency) as a precautionary measure. Drainage was provided to drain out any excess water. When seedlings were about 2 cm tall, a thin film of water was maintained. All the standard cultural practices were followed and the standard plant protection measures practiced to raise healthy seedlings.

Table 1. Details of nursery management treatments with PSD 3.

Treatment code	Nursery management treatments			
	Seed rate (Pn) (g m–2)	Seeding depth (Dn) (cm)	Nitrogen dose (Nn) (kg ha^{-1})	Nitrogen application (Nnd) method
Vn$_1$	50	5.00	100	Single, basal
Vn$_2$	25	5.00	100	Split (60:30:10)
Vn$_3$	25	5.00	100	Single, basal
Vn$_4$	25	0.50	100	Split (60:30:10)
Vn$_5$	15	5.00	150	Split (60:30:10)
Vn$_6$	25	5.00	150	Split (60:30:10)

For experiments on hybrid seed production of PSD 1, three factors of nursery management, seeding density (25 g and 50 g m^{-2}), seeding depth (5.0 and 0.5 cm), and N-application methods (standard basal and three splits in a ratio of 60:30:10 as basal and at 10 and 20 DAS) each with two levels, were considered in the nursery using 100 kg ha^{-1} nitrogen. All eight possible treatment combinations were attempted in the nursery.

Based on the results obtained with PSD 1 for the first two seasons, further refinement of the nursery management treatments was done and only the best treatment combinations with some new ones were evaluated for hybrid seed experiments for PSD 3. Different seed rates (Pn$_1$—50 g, Pn$_2$—25 g, and Pn$_3$—15 g m^{-2}), seeding depths (Dn$_1$—0.5 cm and Dn$_2$—5.0 cm), and N dose (Nn$_1$—basal and Nn$_2$—splits of N, 60:30:10) were evaluated. Out of all 24 possible combinations, only six specific management combinations in four replications were evaluated in the nursery with the treatment codes of Vn$_1$ (Pn$_1$ Dn$_2$ Nn$_1$d$_1$), Vn$_2$ (Pn$_2$ Dn$_2$ Nn$_2$ Nnd$_1$), Vn$_3$ (Pn$_2$ Dn$_2$ Nn$_2$ Nnd$_1$), Vn$_4$ (Pn$_2$ Dn$_1$ Nn$_2$ Nnd$_1$), Vn$_5$ (Pn$_3$ Dn$_2$ Nn$_2$ Nnd$_2$), and Vn$_6$ (Pn$_2$ Dn$_2$ Nn$_2$ Nnd$_2$). Gross plot size of 10.0 m^2 (5.0 m × 2.0 m) and 6.0 m^2 (3.0 m × 2.0 m) with net plot size of 4.8 m^2 (4.0 m × 1.2 m) and 2.4 m^2 (2.0 m × 1.2 m) for the respective seed and restorer parents were used (Table 1).

Agro-management treatments in the seed production field

The seed and restorer parent lines of hybrid PSD 1 differed in their days to 50% flowering by 5 days and, therefore, were staggered-planted to obtain synchronized flowering. A set of 32 treatment combinations from two levels each of five factors of management (three in the nursery and two in the main field) (Table 2) was evaluated for its effects on hybrid seed yield (A/R) and the yield of the restorer parents and other related characters for two years/seasons. The experiment was laid out in a randomized complete block design with two replications.

For seed production of hybrid PSD 3, agro-management treatments in the nursery and seed production field were modified. Some of the best management treatments

Table 2. Details of nursery and main field management treatments with PSD 1.

Management treatment	Nursery treatments			Main field	
	Seed rate (g m^{-2})	Seeding depth (cm)	N application method	150 kg N in split dose	Seedlings hill^{-1}
T_1	50	0.5	Single basal	Three splits (50:25:25)	1
T_2	50	0.5	Three splits (60:30:10)	Three splits (50:25:25)	1
T_3	50	5.0	Single basal	Three splits (50:25:25)	1
T_4	50	5.0	Three splits (60:30:10)	Three splits (50:25:25)	1
T_5	50	0.5	Single basal	Four splits (30:20:20:30)	1
T_6	50	0.5	Three splits (60:30:10)	Four splits (30:20:20:30)	1
T_7	50	5.0	Single basal	Four splits (30:20:20:30)	1
T_8	50	5.0	Three splits (60:30:10)	Four splits (30:20:20:30)	1
T_9	50	0.5	Single basal	Three splits (50:25:25)	2
T_{10}	50	0.5	Three splits (60:30:10)	Three splits (50:25:25)	2
T_{11}	50	5.0	Single basal	Three splits (50:25:25)	2
T_{12}	50	5.0	Three splits (60:30:10)	Three splits (50:25:25)	2
T_{13}	50	0.5	Single basal	Four splits (30:20:20:30)	2
T_{14}	50	0.5	Three splits (60:30:10)	Four splits (30:20:20:30)	2
T_{15}	50	5.0	Single basal	Four splits (30:20:20:30)	2
T_{16}	50	5.0	Three splits (60:30:10)	Four splits (30:20:20:30)	2
T_{17}	25	0.5	Single basal	Three splits (50:25:25)	1
T_{18}	25	0.5	Three splits (60:30:10)	Three splits (50:25:25)	1
T_{19}	25	5.0	Single basal	Three splits (50:25:25)	1
T_{20}	25	5.0	Three splits (60:30:10)	Three splits (50:25:25)	1
T_{21}	25	0.5	Single basal	Four splits (30:20:20:30)	1
T_{22}	25	0.5	Three splits (60:30:10)	Four splits (30:20:20:30)	1
T_{23}	25	5.0	Single basal	Four splits (30:20:20:30)	1
T_{24}	25	5.0	Three splits (60:30:10)	Four splits (30:20:20:30)	1
T_{25}	25	0.5	Single basal	Three splits (50:25:25)	2
T_{26}	25	0.5	Three splits (60:30:10)	Three splits (50:25:25)	2
T_{27}	25	5.0	Single basal	Three splits (50:25:25)	2
T_{28}	25	5.0	Three splits (60:30:10)	Three splits (50:25:25)	2
T_{29}	25	0.5	Single basal	Four splits (30:20:20:30)	2
T_{30}	25	0.5	Three splits (60:30:10)	Four splits (30:20:20:30)	2
T_{31}	25	5.0	Single basal	Four splits (30:20:20:30)	2
T_{32}	25	5.0	Three splits (60:30:10)	Four splits (30:20:20:30)	2

Table 3. Selected nursery and main-field management treatments in PSD-3.

Field management treatment	Nursery treatments		Nitrogen dose (kg ha^{-1}) and method of application	Main field	
	Seed rate (g m^{-2})	Seedling depth (cm)		Nitrogen dose (kg ha^{-1})	No. of seedlings hill^{-1} transplanted
V_1 (control)	50	5.0	100, basal	120, 3 splits	2
V_2	25	5.0	100, split	150, 4 splits	2
V_3	25	5.0	100, split	150, 4 splits	1
V_4	25	5.0	100, split	180, 4 splits	2
V_5	25	5.0	100, split	180, 4 splits	1
V_6	25	5.0	100, basal	150, 4 splits	2
V_7	25	0.5	100, split	150, 4 splits	2
V_8	15	5.0	150, split	180, 4 splits	2
V_9	15	5.0	150, split	210, 4 splits	1
V_{10}	15	5.0	150, split	210, 4 splits	2
V_{11}	25	5.0	150, split	180, 4 splits	2
V_{12}	25	5.0	150, split	210, 4 splits	1

observed with the production of PSD 1 were attempted together with some new ones to study hybrid seed production of the hybrid PSD 3 and its restorer parent for two years/seasons. Details of 12 selected treatment combinations with standard (V_1) and improved (V_2) controls appear in Table 3.

For isolation, 3-m-wide planting of a *Sesbania rostrata* crop all around the experimental area provided a good physical barrier for the adjoining rice plots. Gross plot size was 5.1 m × 3.0 m using two sets per plot of the seed (UPRI 92-1 > A) and male (UPRI 193-133 R or UPRI 93-287 R) parents transplanted at a ratio of 8:2 for A:R. Basal application of fertilizer P at 90 kg and K at 60 kg ha^{-1} was used. Seedlings were transplanted manually. The plot was rogued for off-type plants regularly at preflowering, flowering, and postflowering. All the recommended operations required for promoting outcrossing in the seed production field such as flag-leaf clipping, GA_3 application (60 g ha^{-1}) twice at 5% panicle emergence on alternate days, and supplementary pollination were done (Pandey et al 1996). Standard practices of harvesting, threshing, and seed drying were followed for the male parents and the hybrids separately.

Data recording

Observations in the nursery were recorded on ten randomly selected seedlings on the 25th day after seeding. Observations on shoot length, root length, and seedling height were recorded in cm with a measuring scale on freshly uprooted seedlings individually. For seedling dry weight, the same seedlings were dried at 80 °C for 24 hours in a hot-air oven followed by cooling inside desiccators, and dry weight was recorded (in g) on a digital Electronic Sartorius Balance made in Germany. The number of tillers per seedling was counted on 10 normal seedlings at the time of transplanting.

Observations in the seed production experiment in the field were recorded on five randomly chosen and tagged plants in each of the two sets of a plot of the female and male parents. Data on individual plants were recorded on days to 50% flowering, plant height, number of tillers hill^{-1}, number of panicles hill^{-1}, panicles m^{-2}, panicle length, spikelets panicle^{-1}, panicle exsertion (%), percent seed set, 1,000-seed weight, seed yield hill^{-1}, and seed yield m^{-2} according to the Standard Evaluation System of IRRI. Observations on number of tillers per plant were also recorded at transplanting and on a periodical basis at 7 days' interval on the female parent up to the 35th day after transplanting to study the pattern of tiller development in seedlings of seed and restorer parents.

The mean data were analyzed as per two-factor RBD analysis of variance (Federer and Raghavarao 1975). The significance of difference among the means of factors and interactions underwent an F test. Critical difference values were calculated whenever the differences were significant. The homogeneity of error mean square due to season was tested by applying an F test (Panse and Sukhatme 1985).

Results and discussion

Effect on seedling growth parameters

Analysis of variance for five seedling growth–related characters in the nursery pooled over two years for the female and male parents for hybrids PSD 1 and PSD 3 (date not given) revealed highly significant differences due to the main factors seeding density, depth of seeding, and nitrogen application methods and some of their interactions.

Effect on parental lines of PSD 1

All the factors studied were significantly influenced by seeding density in the seedbed. The comparative data on the effect of seeding density pooled over years for seed and pollen parents (Table 4) indicate that low seeding density produces vigorous seedlings as also seen from significantly longer shoot (33.18 cm), root (6.26 cm), and seedling (39.44 cm) length and higher seedling dry weight (1.80 g). This also gives more tillers (3.02) per seedling than with higher seeding density. For the restorer parent, similar effects on various seedling growth parameters were recorded. This suggests that a thin plant population density in the raised seedbed produced vigorous and multitillered seedlings for both the restorer and female parents of the hybrid.

For the effect of seeding depth on seedling growth of the parents in the seedbed, results explicitly indicate that deeper seeding influences all the characters significantly and positively. For nitrogen application methods, split application produced healthier seedlings as observed from growth characters for both parents except for root length, for which the split application was on a par with basal N application.

Table 4. Effect of nursery management treatments on seedling growth of seed and restorer parents of PSD 1 pooled over years.

Treatment	Parent	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Dry weight of seedling (g)	Number of tillers seedling^{-1}
Vn_1	Seed	29.56	4.82	34.36	1.21	2.21
	Restorer	32.01	3.68	35.52	1.07	1.15
Vn_2	Seed	33.18	6.26	39.44	1.80	3.02
	Restorer	35.57	4.45	40.02	1.57	1.75
Vn_3	Seed	30.33	5.21	35.53	1.34	2.40
	Restorer	32.63	3.88	36.34	1.21	1.35
Vn_4	Seed	32.41	5.87	38.28	1.67	2.83
	Restorer	34.95	4.26	39.20	1.43	1.55
Vn_5	Seed	30.69	5.38	36.07	1.41	2.48
	Restorer	33.36	3.94	37.04	1.26	1.38
Vn_6	Seed	32.05	5.70	37.73	1.60	2.74
	Restorer	34.31	4.19	38.50	1.38	1.52
CD at 5%	Seed	0.64	0.25	1.16	0.10	0.18
	Restorer	0.78	0.14	1.11	0.05	0.09

Effect on seedling growth parameters in PSD 3. These effects were almost identical to those in PSD 1. The effect of agro-management treatments with further reduced seeding density combined with higher N (150 kg) in the nursery with more splits (Vn_5) produced seedlings with significantly higher root length and seedling height in both the seed and restorer parents and higher seedling dry weight and number of tillers per seedling being on a par in the seed parent, but significantly higher in the restorer parent when compared with moderate/higher seeding densities (Vn_6). The treatment Vn_5 with low seed density (15 g m^{-2}), deep seeding (5.0 cm), and high nitrogen (150 kg ha^{-1}) dose and split application of nitrogen has been the best (Table 5).

The maximum shoot length for the restorer was recorded for treatment Vn_5 (45.51 cm), which is on a par with Vn_6 (41.33 cm) but significantly superior to all treatment combinations and the control, Vn_1 (Table 5). A split application of nitrogen (Vn_2) significantly enhanced the shoot length, root length, and seedling length compared with a basal application (Vn_6) in the seed parent but had no effect on any of the characters in the restorer parent.

Treatments Vn_5 and Vn_6 recorded the maximum tiller number per seedling in both parents. The difference, however, was nonsignificant in the seed parent. Multitillered seedlings were produced due to the deep placement of seeds at sowing with sparse seeding. With our study using hybrids PSD 1 and PSD 3, this is quite evident, which suggests that the raising of vigorous and healthy seedlings with more tillers

Table 5. Effect of nursery management treatments on seedling growth of seed and restorer parents of PSD 3 pooled over years.

Treatment	Parent	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling dry weight (g)	No of tillers at transplanting
Vn_1	Seed	35.20	4.58	39.80	1.29	2.08
	Restorer	36.94	5.39	42.38	1.35	2.36
Vn_2	Seed	38.46	6.27	44.19	1.36	3.13
	Restorer	38.90	6.89	45.41	1.58	3.27
Vn_3	Seed	35.72	5.85	41.86	1.27	2.96
	Restorer	38.52	6.13	45.04	1.55	3.08
Vn_4	Seed	37.49	5.51	43.01	1.29	2.95
	Restorer	36.99	5.70	43.94	1.49	2.90
Vn_5	Seed	42.70	9.12	52.01	1.57	3.39
	Restorer	45.41	9.45	54.77	1.96	3.94
Vn_6	Seed	40.76	8.38	49.11	1.47	3.22
	Restorer	41.33	7.87	49.20	1.77	3.41
CD 5%	Seed	1.94	0.17	1.84	0.12	0.23
	Restorer	4.51	1.36	4.56	0.10	0.20

in the seedbed by sowing parental line seeds very thinly and evenly would help to achieve higher hybrid seed yield. The study also suggests the importance of a low seed rate (15 g m^{-2}) in combination with deep placement of seed and the application of higher nitrogen in splits to produce vigorous multitillered seedlings of parental lines in hybrid seed production. Similar reports on seeding density were also made by Om et al (1997), who revealed a decrease in dry matter production in a hybrid production plot with an increase in seeding density from 20 to 60 g m^{-2} in the nursery.

Pattern of tiller development in parental lines. A highly significant mean square for main effects and interactions (value not given) from pooled data over years for management treatments (T), periods of development (P), and P × T interaction for both hybrids indicates the presence of adequate variation due to these. The effect of agro-management treatments on the development of mean tiller number per hill at different periods during the tillering stage of the seed parent (UPRI 95-17A) of the hybrid PSD 1 in the seed production field is presented in Table 6. Results indicate that the maximum tillers per hill (7.44) was attained with treatment T_{32} at 35 days after transplanting (DAT). This combined low seed rate, deep seeding, and maximum splits of N in the nursery and fields with two seedlings per hill transplanting. This was significantly superior to T_{24} using the same practice as T_{32} but for transplanting of a single seedling per hill.

Table 6. Effect of agro-management treatments (T) on tillering hill^{-1} at varying periods after transplanting of seed parent of PSD 1 (pooled data).[a]

Treatment combination	Mean number of tillers at different periods of development after planting						
	P_0	P_7	P_{14}	P_{21}	P_{28}	P_{35}	Mean
T_1	1.50	2.00	2.80	3.90	4.60	4.70	3.25
T_2	1.60	2.10	2.90	4.00	4.70	4.80	3.35
T_3	1.60	2.10	2.90	4.00	4.70	4.80	3.35
T_4	1.70	2.20	3.00	4.10	4.80	4.90	3.45
T_5	1.70	2.30	3.30	4.50	5.30	5.60	3.78
T_6	1.80	2.40	3.40	4.60	5.40	5.70	3.80
T_7	1.80	2.40	3.40	4.60	5.40	5.70	3.80
T_8	1.90	2.50	3.50	4.70	5.50	5.80	3.98
T_9	2.20	2.90	3.80	5.10	6.00	6.20	4.37
T_{10}	2.30	3.00	3.90	5.20	6.10	6.30	4.47
T_{11}	2.30	3.00	3.90	5.20	6.10	6.30	4.70
T_{12}	2.40	3.10	4.00	5.30	6.20	6.40	4.57
T_{13}	2.40	3.30	4.30	5.50	6.50	6.90	4.82
T_{14}	2.50	3.40	4.40	5.60	6.60	7.00	4.92
T_{15}	2.50	3.40	4.40	5.60	6.60	7.00	4.92
T_{16}	2.60	3.50	4.50	5.70	6.70	7.10	5.02
T_{17}	2.80	3.70	4.70	6.00	7.10	7.30	5.27
T_{18}	2.90	3.80	4.80	6.10	7.20	7.40	5.37
T_{19}	3.00	3.90	4.90	6.20	7.30	7.50	5.47
T_{20}	3.10	4.00	5.00	6.30	7.40	7.60	5.57
T_{21}	2.95	3.95	5.15	6.75	8.05	8.45	5.80
T_{22}	3.05	4.05	5.25	6.85	8.15	8.55	5.98
T_{23}	3.15	4.15	5.35	6.95	8.25	8.65	6.08
T_{24}	3.25	4.25	5.45	7.05	8.35	8.75	6.18
T_{25}	3.40	4.40	5.60	7.20	8.50	9.00	6.35
T_{26}	3.50	4.50	5.70	7.30	8.60	9.10	6.45
T_{27}	3.60	4.60	5.80	7.40	8.70	9.20	6.50
T_{28}	3.70	4.70	5.90	7.50	8.80	9.30	6.65
T_{29}	3.75	4.85	6.35	8.15	9.35	10.15	7.10
T_{30}	3.85	4.95	6.45	8.25	9.45	10.25	7.20
T_{31}	3.95	5.05	6.55	8.35	9.55	10.35	7.30
T_{32}	4.05	5.20	6.70	8.50	9.70	10.50	7.44
Mean	2.69	3.55	4.62	6.01	7.05	7.40	

[a] CD (*P*) at 5%, 0.025; CD (T) at 5%, 0.060; CD (*P* × T) at 5%, 0.140.

Table 7. Effect of agro-management treatments (T) on tillering hill^{-1} at varying periods after transplanting of seed parent of PSD 3 (pooled data).[a]

Treatment combination	Mean number of tillers at different periods of development					
	P_0	P_7	P_{14}	P_{21}	P_{28}	P_{35}
V_1	1.62	2.10	2.77	4.05	5.10	5.25
V_2	4.15	5.32	6.50	8.66	10.10	10.90
V_3	3.20	4.10	5.97	7.05	7.67	8.69
V_4	4.25	5.37	6.45	8.81	10.25	11.10
V_5	3.12	4.05	5.07	7.25	8.63	9.30
V_6	3.05	4.42	6.17	8.50	9.67	10.37
V_7	3.12	4.01	5.10	7.83	9.20	9.45
V_8	5.22	6.87	9.02	11.31	13.40	14.32
V_9	4.12	5.63	6.60	8.55	9.58	10.42
V_{10}	5.55	7.35	9.79	12.51	14.13	14.60
V_{11}	4.90	6.25	8.33	11.25	12.02	13.50
V_{12}	3.35	4.36	6.25	9.43	11.11	11.82
CD 5%	0.26	0.22	0.37	0.21	1.05	0.29

The rate of tiller development on an overall basis increased progressively up to 21 DAT and the rate of increase was maximum between the period from 14 to 21 DAT (1.39), which declined continuously thereafter to attain the maximum tiller number at 35 DAT. At 35 DAT, the seed parent had attained the age of 60 days after sowing and had already reached the panicle initiation (PI) stage. Any new development at PI would therefore only be unproductive tillers and would not contribute to seed yield. A report in the literature on the effect of planting date and nitrogen level in rice hybrids under Madhya Pradesh (India) conditions also indicated an increase in number of tillers up to 60 DAT, with a further decline thereafter. The weight of tiller and dry matter accumulation continued to increase up to 90 DAT. Increasing the level of nitrogen to 150 kg ha^{-1} significantly increased the dry matter accumulation, grain yield, weight of tillers, and tillers per hill (Pandey et al 2001).

Similar observations were recorded for the parental lines of PSD 3 (Table 7). The maximum tillering per hill of 14.55 was observed with the treatment V_{10}, that is, lower seed rate (15 g m^{-2}), deep seeding, a higher dose of nitrogen in three split applications in the nursery, and 210 kg N ha^{-1} in four splits with planting of two seedlings per hill in the hybrid seed plot compared with the control, V_1. Treatment T_8 was also on a par for tillering with T_{10}. This suggests that 180 kg N ha^{-1} in splits is optimum for inducing maximum tillering in the seed parent similar to the seed parent of PSD

1 (150 kg N ha^{-1} in the field) and that the best time for N management for PSD 1 and PSD 3 is from 14 to 21 days for inducing maximum tillering. Part of the N should also be applied at the grain-filling stage to achieve higher hybrid seed production. Similar findings have been reported by Bui et al (1992), Rajarathinam and Balasubramaniyan (1999), and Rajendran and Veeraputhiran (1999).

Effects of agro-management factors on hybrid seed yield and other traits

Results on the mean effects of various management treatments in the nursery and the main seed production plot of seed (A/R) and restorer parents of PSD 1 (Table 8) and PSD 3 (Tables 9 and 10) revealed significant differences among treatments over years for all the characters studied in both parents.

The four treatments (T_{32}, T_{31}, T_{30}, and T_{29}) having seedlings raised with sparse seeding and four split applications of nitrogen in the main field irrespective of seeding depth and nitrogen application method in the nursery and seedlings per hill in the field resulted in earliness for flowering.

Plant height of the parental lines plays an important role in determining the extent of seed set in the hybrids. Plant height was influenced by year variations. Both parents of PSD 3 were slightly taller than the plants in PSD 1. Treatment T_{32} recorded the maximum height in both parents of PSD 1 (Tables 8). Treatment V_9 resulted in maximum plant height with the pollen parent, whereas the treatment combinations of V_6 and V_{11} expressed higher plant height in both the seed (Table 9) and pollen (Table 10) parents of PSD 3.

For seed yield, the average seed yield per hill pooled over seasons varied from 3.81 g (T_1) to 8.86 g (T_{32}) for PSD 1 and from 3.79 g (V_1) to 11.09 g (V_{10}) for PSD 3. The extrapolated values of hybrid seed yield on a per hectare basis reveal a range of 167.42 to 389.62 g, that is, 1,674 to 3,896 kg ha^{-1} for PSD 1, the maximum yield recorded with treatment $T_{32.}$ A further reduction in seed rate in the nursery to 15 g m^{-2} and an increase in nitrogen application in split doses in both the nursery and main field results in hybrid seed yield of 433.81 g, that is, 4,338 kg ha^{-1} (V_{10}) for PSD 3.

The pooled data over seasons for yield and other characters in PSD 1 indicate that T_{32} management has been the best treatment, with hybrid seed yield of 3,896 kg ha^{-1}, followed by T_{31} (3,850 kg ha^{-1}), T_{30} (3,793 kg ha^{-1}), and T_{29} (3,751 kg ha^{-1}), the differences being significant. Treatment T_{32} was significantly superior to T_{31}, which was superior to T_{30}. All other treatments were significantly inferior. It was interesting to note that the top treatments had common crop management factors of sparse seeding in the nursery, split N application in the field, and two seedlings per hill. Treatments T_{32} and T_{30} differed from T_{31} and T_{29} with respect to N application method in the nursery, where the former treatments had a split application of N in the nursery in contrast to the standard application method of N used in treatments T_{31} and T_{29}. There have been identical responses of these management treatments in seed yield per hill and number of panicles m^{-2}. These findings related to the effect of even and sparse seeding in the seedbed are in agreement with Mao et al (1998), who also reported some technological innovations made worldwide to increase hybrid seed yield and decrease the cost

Table 8. Pooled data for yield and other characters of seed parent over years for PSD 1.

Treatment	Days to 50% flowering	Plant height (cm)	Tillers hill^{-1}	Panicles hill^{-1}	Panicles m^{-2}	Panicle length (cm)	Spikelets panicle^{-1}	Panicle exsertion (%)	Seed set (%)	1,000-seed wt. (g)	Seed yield hill^{-1} (g)	Seed yield m^{-2} (g)
T_1	91.2	77.75	4.7	4.50	162.0	22.9	153.3	69.2	31.46	20.56	3.81	167.4
T_2	91.2	77.80	4.8	4.60	165.4	22.0	154.0	69.5	31.61	20.64	3.90	171.4
T_3	91.2	77.80	4.8	4.60	165.7	22.1	154.9	69.9	31.79	20.67	3.90	171.8
T_4	91.2	77.90	4.9	4.70	169.2	22.2	155.5	70.2	31.91	20.81	3.99	175.6
T_5	90.5	78.00	5.7	5.45	196.2	22.4	156.8	70.3	32.18	20.63	4.50	187.8
T_6	90.5	78.10	5.8	5.55	199.8	22.5	157.5	71.1	32.32	20.75	4.58	201.5
T_7	90.5	78.10	5.8	5.55	199.9	22.6	158.3	71.5	32.48	20.75	4.58	201.7
T_8	90.5	78.20	5.9	5.65	203.4	22.7	158.9	71.7	32.61	20.85	4.67	205.5
T_9	91.5	77.45	6.2	6.00	216.0	21.6	151.2	68.2	31.03	20.35	4.95	217.8
T_{10}	91.4	77.55	6.3	6.10	219.6	22.7	151.9	68.6	31.17	20.45	5.19	228.1
T_{11}	91.5	77.55	6.3	6.10	219.7	21.8	152.7	68.9	31.34	20.58	5.19	228.3
T_{12}	91.5	77.65	6.4	6.20	223.2	21.9	153.3	69.2	31.47	20.75	5.28	232.1
T_{13}	90.8	77.70	7.0	6.75	243.0	21.8	152.6	68.9	31.32	20.43	5.74	252.6
T_{14}	90.8	77.80	7.1	6.85	246.6	21.9	153.3	69.2	32.47	20.55	5.82	256.1
T_{15}	90.8	77.80	7.1	6.85	246.7	22.0	154.1	69.6	31.63	20.65	5.83	256.5
T_{16}	90.8	77.90	7.2	6.95	250.2	22.1	154.7	69.8	31.75	20.78	5.91	259.8
T_{17}	90.2	79.00	7.4	7.15	257.5	24.1	168.7	76.1	34.62	21.95	6.08	267.4
T_{18}	90.2	79.10	7.5	7.25	261.0	24.3	170.1	76.8	34.91	22.05	6.16	271.0

Continued on next page

Table 8 continued.

Treatment	Days to 50% flowering	Plant height (cm)	Tillers hill^{-1}	Panicles hill^{-1}	Panicles m^{-2}	Panicle length (cm)	Spikelets panicle^{-1}	Panicle exsertion (%)	Seed set (%)	1,000-seed wt. (g)	Seed yield hill^{-1} (g)	Seed yield m^{-2} (g)
T_{19}	90.2	79.10	7.6	7.35	264.6	24.4	170.8	77.1	35.05	22.02	6.25	274.8
T_{20}	90.2	79.20	7.7	7.45	268.2	24.5	171.5	77.4	35.19	00.08	6.33	278.1
T_{21}	89.5	79.50	8.6	8.35	300.6	24.4	170.5	76.9	34.97	22.15	7.11	312.6
T_{22}	89.5	79.60	8.7	8.45	304.2	24.5	171.5	77.4	35.20	22.29	7.18	315.9
T_{23}	89.5	79.60	8.8	8.55	307.8	24.6	172.2	77.7	35.33	22.23	7.27	319.7
T_{24}	89.5	79.70	8.9	8.65	311.4	24.7	172.9	78.0	35.47	22.02	7.35	323.4
T_{25}	90.5	78.85	9.1	8.80	316.8	23.8	166.6	76.7	34.19	21.88	7.48	328.9
T_{26}	90.5	78.95	9.2	8.90	320.4	24.0	168.0	75.8	34.48	21.95	7.56	332.6
T_{27}	90.5	78.95	9.3	9.00	324.0	24.1	168.4	76.0	34.56	21.99	7.65	336.6
T_{28}	90.5	79.05	9.4	9.10	327.6	24.2	169.1	76.3	34.69	22.05	7.73	340.1
T_{29}	89.8	79.20	10.3	10.05	361.8	24.3	169.8	76.6	34.83	22.12	8.53	375.1
T_{30}	89.8	79.30	10.4	10.15	365.4	24.4	170.8	77.1	35.06	22.15	8.62	379.3
T_{31}	89.8	79.30	10.6	10.30	370.8	24.5	171.5	77.4	35.20	22.19	8.75	385.0
T_{32}	89.8	79.40	10.7	10.45	376.2	24.6	172.2	77.4	35.35	22.25	8.86	389.6
CD at 5%	1.01	0.87	0.15	0.12	6.00	0.51	3.47	1.73	0.71	0.21	0.08	3.38

Table 9. Mean effect of treatments on seed yield and other component characters in the seed parent of PSD 3.

Management treatment combination	Days to 50% flowering	Plant height (cm)	Tillers hill^{-1}	Panicles hill^{-1}	No. of panicles m^{-2}	Pan-icle length (cm)	No of spikelets panicle^{-1}	Percent panicle exsertion	Percent seed set	1,000-seed weight (g)	Seed yield hill^{-1} (g)	Seed yield m^{-2} (g)
V_1	90.7	81.0	5.56	5.20	160.9	21.10	139.2	66.9	31.56	18.75	3.79	164.4
V_2	92.0	81.4	10.79	10.32	201.2	21.78	162.1	72.0	33.55	18.56	9.34	373.4
V_3	92.0	83.4	8.60	9.10	226.3	21.45	171.6	75.6	33.73	19.31	7.13	309.3
V_4	93.7	82.4	11.85	10.83	302.8	22.22	177.3	75.2	34.56	19.36	9.56	387.4
V_5	94.0	83.6	9.75	9.79	283.1	23.01	178.6	75.7	32.60	19.35	8.13	330.8
V_6	92.7	84.5	12.10	9.79	248.7	21.27	181.6	74.0	34.63	18.31	8.27	359.5
V_7	93.0	84.3	11.25	9.42	239.8	21.52	174.5	74.1	34.68	19.26	8.13	356.3
V_8	93.7	84.9	13.96	12.35	300.6	22.65	186.6	75.3	35.23	19.53	10.11	408.4
V_9	94.7	85.9	10.42	11.81	302.4	23.06	179.9	73.7	34.57	20.01	8.65	347.7
V_{10}	93.7	84.4	15.77	13.42	360.1	22.61	195.5	75.3	35.40	19.34	11.09	433.8
V_{11}	94.0	94.9	13.52	12.54	307.7	22.74	195.2	75.2	34.71	19.39	10.52	418.5
V_{12}	94.7	84.3	9.96	11.47	347.5	23.14	182.5	75.8	33.77	20.11	8.30	332.2
CD 5%	0.50	1.82	0.17	0.37	16.71	0.83	5.66	2.87	0.39	0.42	0.20	6.78

Table 10. Mean effect of different treatments on seed yield component characters of the restorer parent of PSD 3.

Management treatment	Days to 50% flowering	Tillers hill^{-1}	Plant height (cm)	Panicles hill^{-1}	Panicle length (cm)	Seed yield hill^{-1} (g)	Seed yield m^{-2} (g)
V_1	93.8	7.67	119.02	7.55	21.77	16.93	591.6
V_2	94.3	11.60	124.02	11.30	22.97	19.75	730.1
V_3	94.5	9.62	123.97	9.54	22.45	23.14	659.7
V_4	94.3	12.67	119.90	12.37	23.42	22.12	758.5
V_5	94.5	11.37	124.37	10.82	23.15	20.53	782.6
V_6	94.0	11.49	124.92	11.27	23.00	21.02	755.7
V_7	95.3	11.56	121.40	11.37	23.70	20.37	731.1
V_8	93.8	14.25	123.35	13.95	24.10	23.67	765.1
V_9	95.0	11.05	126.40	10.77	23.01	22.38	710.4
V_{10}	95.3	14.96	122.77	14.85	24.12	25.33	781.7
V_{11}	94.3	14.37	124.77	14.27	22.58	24.19	724.0
V_{12}	95.3	10.79	122.75	10.65	22.62	21.20	691.2
CD 5%	0.44	0.42	0.41	0.29	0.56	1.03	6.07

of hybrid seed production by raising vigorous and healthy seedlings with more tillers in the seedbed by sowing parental lines sparsely and evenly. The seeding rate in the nursery should be less than 150 kg ha^{-1}.

For super rice hybrid yield, it is very important that the number of productive panicles ha^{-1} in the seed and pollen parent be high. Our study has clearly shown a hybrid seed yield of 3.9 t ha^{-1} with the crop management practices involving 25 g m^{-2} of seed sown deeper by 5.0 cm with a split application of nitrogen in the nursery as well as in the field and transplanting two seedlings per hill. These management practices produced 3.76 million panicles ha^{-1}, with 291.3 million spikelets per hectare, and might have resulted in higher hybrid seed yield due to enhanced pollen load of the male parent, as also reported by Yuan and Fu (1995) and Mao et al (1998).

Enhanced spikelet number ha^{-1} increased the spikelet ratio from 1:2 to 1:2.5 (Yuan and Fu 1995). According to Mao et al (1998), the R line should have 1.2 million and the A line 3.0 to 3.5 million productive panicles ha^{-1}. Rajarathinam and Balasubramaniyan (1999) and Maity and Mishra (2001) a revealed higher yield of hybrid rice with higher doses of N applied in 2–3 splits.

Similar data pooled over two years for hybrid seed yield and other agronomic characters for PSD 3 (Table 10) indicate that treatment V_{10} has the best performance among all other management treatments, with seed yield (A/R) of 4,338 kg ha^{-1}, followed by V_{11} (4,184 kg ha^{-1}) and V_8 (4,094 kg ha^{-1}). Treatment V_{11} was significantly superior to V_8. Compared with the controls, V_{10} recorded 16.2% higher hybrid seed yield than V_2 (3,733 kg ha^{-1}), the best agro-management treatment (T_{32}) with PSD 1 as the improved check, and 163.8% over the traditional control (V_1) recording the lowest yield (1,644 kg ha^{-1}). It was interesting to note that the top three management treatments included a low (15 kg ha^{-1}) to moderate (V_{10}, V_8, V_{11}) seed rate of 25 kg ha^{-1} with higher management in the nursery (all three 150 kg N ha^{-1}) and in the main field (180–210 kg N ha^{-1}) compared with the controls V_1 (standard) and V_2 (improved). A close perusal of the overall results explicitly reveals that the best agro-technology for obtaining maximum hybrid seed yield includes sparse seeding (15 g m^{-2}) area, seeds with deeper seeding (5.0 cm), and the use of 150 kg N ha^{-1} in three splits in the nursery and combined with a high N application (210 kg ha^{-1}) with four splits in the field and two seedlings hill^{-1}. The response of these management treatments on seed yield hill^{-1} is conclusively proved to be due to the synergistic effects of at least two or more major components for seed yield—number of spikelets per panicle, seed set (outcrossing) percentage, and number of panicles hill^{-1}. Thousand-seed weight did not contribute significantly to hybrid seed yield with these management treatments.

Our study also revealed a significant interaction of management treatments with years, indicating a variable performance of management treatments on various characteristics of the seed parent in a hybrid seed production plot.

Agro-management of the restorer parent of the hybrids

The magnitude and extent of variation in mean seed yield m^{-2} in the case of the restorer parents (UPRI 92-133R and UPRI 93-287R) was greater than those of the seed parent. Highly significant variance due to management treatments for seed yield and its related traits revealed the scope of selection of appropriate management techniques for increasing seed yield. A significant interaction of management treatments with seasons for PSD 1 for number of panicles hill^{-1}, seed yield hill^{-1}, and seed yield m^{-2} area indicated that the effect of management treatments was variable in the wet seasons of different years. No management treatment studied was consistent in effect for these traits. This suggests further work involving a number of years and several locations in hybrid seed production areas to obtain results with wider application.

Summarized results on the top-ranking treatments on the basis of performance and consistency for various characters of the seed and male parent appear in Table 11. Treatments V_{16} and V_{15} were the best management techniques for PSD 1 and V_{10} was best for PSD 3 for yield m^{-2} area, seed yield hill^{-1}, panicle length, number of panicles hill^{-1} and number of tillers hill^{-1}, early maturity, and shorter stature. Treatment T_{16} was either on a par with or significantly superior to T_{15}, the next-ranking treatment. For consistency of the treatments over two years, treatment T_{16} was more consistent than T_{15} for yield and the major components of seed yield. Close examination of the results indicated that a maximum yield of 75.90 q ha^{-1} is achievable with treatment T_{16}. This involved managing sparse and deep seeding of the male parent and split N application in both the nursery and the field. This higher yield has been possible due to an increased seed yield hill^{-1}, caused by longer panicle length, a higher number of tillers and productive tillers hill^{-1}, shorter plant height, and early flowering.

Table 11. Top three management treatments with respect to various characters in seed and restorer parents over years for PSD 1 and PSD 3.

Character	Performance of seed parent				Performance of restorer parent			
	1st of PSD 1	2nd of PSD 1	1st of PSD 3	2nd of PSD 3	1st of PSD 1	2nd of PSD 1	1st of PSD 3	2nd of PSD 3
Number of tillers hill^{-1}	T_{32}	T_{31}	V_{10}	V_8	T_{16}	T_{15}	V_{10}	V_{11}, V_8
Days to 50% flowering	T_{21} to T_{24}	T_{29} to T_{32}	V_{12}, V_9	V_{11}, V_5, V_4	T_{15} to T_{16}	T_{11} to T_{14}	V_{12}, V_{10}, V_9, V_7	V_{11}, V_2 to V_6
Panicles hill^{-1}	T_{32}	T_{31}	V_{10}	V_8, V_{11}	T_{16}	T_{15}	V_{10}	V_{11}
Plant height (cm)	T_{24}	T_{22} to T_{23}	V_6 to V_{12}	V_3, V_4	T_{16}	T_{15}	V_9	V_6, V_{11}
Panicle length (cm)	T_{24}	T_{23} to T_{32}	V_{12}, V_9, V_5	V_{11}, V_8, V_4	T_{16}	T_{15}	V_{10}, V_8	V_4, V_7
Seed yield hill^{-1}	T_{32}	T_{31}	V_{10}, V_8	V_{11}, V_4, V_2	T_{16}	T_{15}	V_{10}	V_{11}, V_8
Seed yield m^{-2}	T_{32}	T_{31}	V_{10}, V_8	V_{11}, V_4, V_2	T_{16}	T_{15}	V_5, V_{10}	V_8, V_4, V_6

Conclusions

Four nursery management treatments involving seed rate (50, 25, and 15 g m^{-2}), depth of seeding (0.50 and 5.00 cm), nitrogen dose (100 and 150 kg ha^{-1}), and nitrogen application method (single basal dose, splits) revealed a highly significant effect on various seedling parameters for parental lines of both hybrids. Sparse seeding combined with deep seed placement and split applications of a higher nitrogen dose encourage the development of robust and multitiller seedlings. The responses were identical in both hybrids.

The robust seedlings when transplanted with higher soil fertility produce a maximum rate of tiller development from 14 to 21 DAT and this declines very rapidly thereafter. This suggests the best application of N for maximum tillering during this period.

Pooled analysis of variance revealed significant variances due to agro-management treatments, years, and interaction between treatments and year during individual years and also in the pooled data over environments.

The agro-management treatment of sparse, deep seeding with three split applications of N in the nursery, combined with planting of two seedlings $hill^{-1}$ and split application of nitrogen in the main field (T_{32}), produced a hybrid seed yield of 3.9 t ha^{-1} in PSD 1. With a further reduction in the seeding rate (15 g m^{-2}) and an increase in N application (150 kg ha^{-1}) in the nursery followed by planting of two seedlings $hill^{-1}$ and application of as high as 210 kg ha^{-1} in four split dosages in the main field, V_{10} resulted in a hybrid seed yield of 4.33 t ha^{-1} for PSD 3. The restorer parents of the hybrids also yielded 7.5 t ha^{-1} in treatment T_{32} for PSD 1 and 7.81 t ha^{-1} in treatment V_{10} for PSD 3.

The high-yielding capability of the top agro-management technique was accomplished by a significantly high panicle number $hill^{-1}$ resulting from the planting of vigorous and multitillered seedlings with a higher nitrogen dose to induce more synchronous tillering, a higher number of spikelets per panicle, better panicle exsertion of the seed parent, higher seed set percentage, and higher outcrossing.

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Notes

Authors' address: Central Rice Research Institute, Cuttack 753 006, Orissa, India.

A fast identification system for hybrid seed purity

Zhan Qingcai, Liu Zhixi, Chen Zuwu, Zhu Keyong, Li Zhaohua, and Xiao Zifa

To overcome factors limiting the commercialization of a rapid identification system for hybrid seed purity by using simple sequence repeats (SSRs), this report offers an improved technology for DNA extraction, the PCR amplification process, and gel electrophoresis. For example, the time for DNA extraction, PCR amplification, and gel electrophoresis has been decreased to 2.5 min, 65 min, and 1 h, respectively. Meanwhile, we have reduced the cost, decreased the dosage of dNTP and AmpliTaq Gold DNA enzyme, reused the agarose gel, and abated environmental pollution. Therefore, an accurate, simple, rapid, and low-cost identification system for hybrid seed purity has been established.

Keywords hybrid rice, simple sequence repeats, purity identification, technology optimization

China is the first country in which heterosis of rice has been successfully used in agricultural production. With the expansion of national planting area for hybrid rice year by year, hybrid rice seeds planted had reached nearly 250 million kg annually, reported by Yuan Longping et al (2003), and dissemination of good-quality seeds became increasingly widespread. Consequently, the inspection and supervision of seed quality, largely meaning for authenticity and purity, became more indispensable.

Simple sequence repeat (SSR) molecular markers have many advantages over other categories of markers. These advantages are the large quantity of SSRs, high polymorphism, easy operation, consistent results, and convenient access to primer designing. Thus, they have been widely adopted in approaches to genetic map construction, reported by Panaud and Chen (1995) and McCouch and Teytelinan (2002); germplasm evaluation and genetic diversity research, by Wang Jinhua et al (2005) and Powell (1996); gene orientation and isolation, by Chen Dazhou et al (2002) and Zheng Jingsheng et al (2003); and purity monitoring for varieties of rice seeds, by Zhou Lan and Chen Dianyuan (2005), Li Jingzhao et al (2000), and Su Shunzong et al (2003). Now, many domestic institutes have been undertaking research on seed purity detection of hybrid maize, reported by Guo Jinglun and Wang Bing (1999) and

Table 1. Comparison between the simple DNA extraction method and CTAB method.

Aspect	Kinds of reagent	Maximum samples of each extraction	Time for each extraction	Cost for each sample[a]	Difficulty of the method
CTAB method	>10	24	>5 h	5 yuan	Difficult
Simple method	4	96	2.5 min	0.0025 yuan	Easy

[a] The exchange rate is US$1 = 6.9920 RMB.

Wu Mingsheng et al (2006); hybrid rapeseed, by Marshall and Marchand (1994) and Tang Rong et al (2007); hybrid rice, by Peng Suotang et al (2003) and Lin Jinbo et al (2005); and so on. Nevertheless, because of the high cost, it was difficult to popularize and use this marker technology in production practices. Generally speaking, factors that inhibit the industrialization of SSR marker technology in identifying the purity of hybrid rice seeds include the complex operation of DNA isolation and gel preparation, time-consuming PCR amplification and electrophoresis, low efficiency in dealing with samples, and high costs because of a large amount of dosage for AmpliTaq Gold DNA enzyme, dNTP, and DNA isolation reagents.

The technology of DNA fingerprinting becomes progressively mature, which makes it inevitable for monitoring varietal purity on a molecular level. On the other hand, researchers in molecular detection have been confronted by many problems when identifying the seed purity of more than 200 individuals. These problems consist of how to meet national standards, the feasibility of whether the identification system can be industrialized, and how to lower the cost of field tests in Hainan Province. In this study, a new fast identification system has been improved to solve these problems.

Optimization of a fast identification system

Simplifying a DNA isolation method

As to the problem of time-consuming, costly steps of traditional DNA extraction (Table 1), such as CTAB and SDS methods, a simple method (acid and alkali method) has been modified as follows: the tips of leaves (about 0.5 cm) are cut and put onto a plate, which has 96 wells. After 40 μL of NaOH (0.25 mol L^{-1}) is added and the plate is heated in a boiling water bath for exactly 30 sec, 40 μL of HCl (0.25 mol L^{-1}) and 20 μL of Tris (0.5 mol L^{-1}, pH 8.0, including 0.25% of IGEPAL-CA630) are added for neutralization. Then, the mixture is placed in a boiling water bath for exactly 2 min. After centrifugation, 1 μL of aqueous DNA sample is ready for PCR or is preserved at 4 °C.

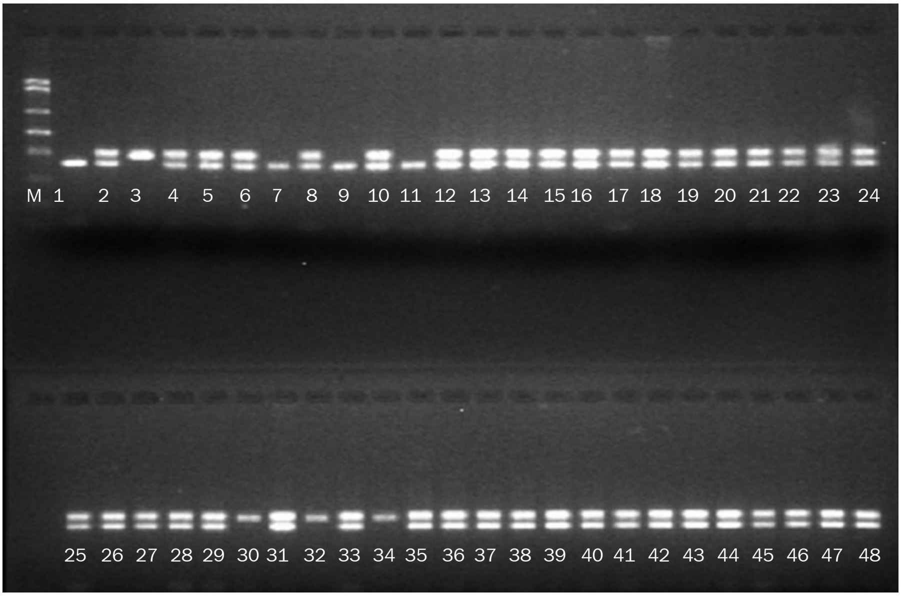

Fig. 1. Identification results for Liangyou Peijiu by primer RM234 (using Adobe Photoshop 7.0.1). M = 100-bp ladder; 1 = Pei'ai 64S; 2 = F_1 hybrid Liangyou Peijiu; 3 = R9311; 1, 2, 3 = standard DNA extracted by CTAB method; 4–48 = DNA extracted by simple method; 7, 9, 11 = male sterile lines; 30, 32, 34 = purposely adulterated restorer lines.

Modifying a PCR amplification system and process

To lower the cost and shorten the time of a fast detection system, hybrid rice combination Liangyou Peijiu and its parental line Pei'ai 64S and *9311* have been tested with primer RM234 to select the most appropriate PCR system and process by using orthogonal experiments (Fig. 1). The optimized protocol is as follows: volume of dNTP is 0.05, 0.1, 0.15, and 0.2 mM, respectively; bulk of the reaction mixture is 5, 10, 15, and 20 µL, respectively; dosage of AmpliTaq Gold DNA enzyme is 0.1, 0.2, 0.5, and 1.5 units, respectively; and different PCR procedures are performed at 94 °C for 4 min, then 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and finally 72 °C for 5 min; or at 94 °C for 4 min, then 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min, and finally 72 °C for 5 min; or 94 °C for 4 min, then 35 cycles of 94 °C for 15 sec, 55 °C for 15 sec, 72 °C for 30 sec, and finally 72 °C for 5 min. According to the excellent outcome, faster speed, and minimal expense, the first-rank volume of the reaction mixture is 10 µL, which consists of 0.2 unit of AmpliTaq Gold DNA enzyme, 0.1 mM dNTP, 0.1 µM primer, 2–5 ng DNA sample, and 1X PCR buffer (including 10 mM Tris-HCl (pH 8.3), 50 mM KCL, 15 mM $MgCL_2$, and 0.01% glutin). The modified PCR procedure has been carried out for predenaturalization at 94 °C for 4 min, 35 cycles of denaturalization at 94 °C for 15 sec, annealing at 55 °C for 15 sec, extension at 72 °C for 30 sec, followed by postextension at 72 °C for 5 min (Table 2). To evaluate the improved reaction system and PCR process, many

Table 2. Comparison between improved procedure and general procedure of SSR-PCR (numbers are minutes).

Reaction process	94 °C Predenaturalization	35 cycles 94 °C Denaturalization	 55 °C Annealing	 72 °C Extension	72 °C Extension	PCR
Normal process	4	1	1	2	5	200
Improved process	4	0.25	0.25	0.5	5	65

materials have been examined for primer selection and purity monitoring, and this is done by using many other primers.

Selection of electrophoresis technique

Considering the complexity of preparation for polyacrylamide gel, the time-consuming electrophoresis process, the severe contamination to the environment, and some other defects, agarose gel is chosen to carry out electrophoresis separation: PCR products with 1.5 µL of 6X loading dye are used for electrophoresis on 4% of Nusieve 3:1 agarose gels, which are stained with ethidium bromide (EB). Other conditions include 5 V cm^{-1} for 100–200 min, and then gels are photographed under UV light using the GelDoc system (Bio-Rad).

Characteristics and effect of a fast identification system

Simple DNA isolation

Two measures have been used to extract total genomic DNA from seedlings of Liangyou Peijiu, that is, CTAB and the simple isolation method. Amplification results of the two methods, using RM234, show that DNA extracted by the simple isolation method wouldn't weaken accuracy and the distinguishing rate.

Compared with CTAB, the simple method decreases extracting time from 5 h to 2.5 minutes and reduces the isolation cost by 2,000 times (excluding the cost of labor). It involves denaturation before extraction and the quality of isolated DNA could meet the needs of a 10 µL PCR system and avoid the use of expensive equipment, such as a high-speed refrigerated centrifuge, and toxic chemical reagents, such as chloroform, isoamyl alcohol, and so on. Therefore, this would lay a solid foundation for commercializing the fast detection technique.

Optimal PCR amplification system and process

The characteristics of the modified PCR system and procedure are as follows: first, the reaction time decreases from 200 min to 65 min; second, the cost decreases sharply by changing the volume of reaction mixtures from 20–25 µL to 10 µL, and reducing the dosage of AmpliTaq Gold DNA enzyme from 0.5–1 to 0.2 unit; third, with the use

Table 3. Comparison between an optimized reaction system and general system in SSR analysis.

Reaction process	Tris-HCl (pH 8.3) (mM)	KCl (mM)	$MgCl_2$ (mM)	Glutin (%)	dNTP (mM)	Primer (μM)	DNA template (ng)	Taq DNA enzyme (unit)	Total (μL)
Normal system	10	50	15	0.01	0.5	0.5	10–20	0.5–1	25
Optimal system	10	50	15	0.01	0.1	0.1	2–5	0.2	10

of agarose gel rather than polyacrylamide gel, the time for electrophoresis declines from 3–3.5 h to 1 h, and pollution caused by the offal is lessened as well (Table 3). As a result, the improved system and process would have a great future for exploring and using a fast identification technique.

Prospects for a fast monitoring system

This study has revealed that the simple DNA isolation method is applied to extract DNA from gramineous crops and dicotyledons. Legible amplifying results for most primers could be obtained by an improved PCR system. Hence, this fast detection technique could be widely used in molecular marker analysis of many agronomic characters and in marker-assisted breeding selection in crops.

In this paper, because we make use of agarose gel rather than polyacrylamide gel, release and contamination caused by the offal are lessened. However, it involves EB, which could cause potential damage to people. Recently, a new-style fluorescent dye has been tested in order to replace the current one. Thereby, a really safe, economical, and nonpolluting technique could come into being in the near future.

With an optimal identification technique, results of seed purity could come out within 2–3 h, and the results would be stable, reliable, and very close to those from field purity tests, as was reported by the Rice Research Institute of Hunan Province (2006). Along with the establishment and perfection of a DNA fingerprinting map of primary hybrid rice in China, the development and application of a DNA isolation kit, PCR kit, and Taqgold enzyme kit, in the near future, purity identification of hybrid rice with SSR markers should become a feasible and practicable technology to replace field tests.

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Authors' address: Laboratory of Biotechnology, Rice Research Institute, Hunan Academy of Agricultural Sciences, Changsha 410125, China.

Physiology and management for high yield and high resource-use efficiency

Yield attributes and nitrogen-use efficiency of "super" hybrid rice

S. Peng, Q. Tang, J. Huang, Y. Zou, K. Cui, Y. Zhang, F. He, R.C. Laza, and R.M. Visperas

China's "super" rice breeding project has developed many F_1 hybrid varieties using a combination of the ideotype approach and intersubspecific heterosis. These hybrid rice varieties have been grown commercially in China since 1998. They produced grain yield of 12 t ha^{-1} in on-farm demonstration fields, 8–15% higher than the hybrid check varieties. We conducted field experiments to compare the grain yield and yield attributes of "super" hybrid rice varieties over a wide range of N rates in Hubei and Hunan in 2004-07. A "super" hybrid rice variety showed higher yield potential than a conventional hybrid rice variety because of its higher biomass production, not harvest index. The higher biomass production was associated with greater leaf area duration. There was no difference between the "super" hybrid rice variety and hybrid check variety in radiation-use efficiency, suggesting that single-leaf photosynthetic rate was not the reason for the high yield potential of the "super" hybrid rice variety. Both the "super" hybrid rice variety and conventional hybrid variety required a minimum total N rate of 120 to 150 kg N ha^{-1} to produce maximum grain yield. There was no clear difference in N-use efficiency between the "super" hybrid rice variety and conventional hybrid variety. A three-line hybrid, SL-8H, has been widely grown in the Philippines since 2003. Morphologically, SL-8H has many similarities with China's "super" hybrid rice. To increase rice yield potential in the tropics, we need to develop new plant type varieties similar to SL-8H with slightly more filled spikelets per panicle.

The recent rice crisis proves again that a sustainable increase in rice production is crucial for food security in the rice-growing countries of Asia (IRRI 2008). An increase in world rice production depends more heavily on higher grain yield today than in the past (Cassman 1999). The development of high-yielding rice varieties is still the main strategy for improving grain yield in many rice-growing countries, although many have realized the importance of agronomic practices in closing the existing yield gap between potential yield and farmers' attainable yield (IRRI 2008).

China has been at the forefront in developing high-yielding varieties using a semidwarf gene in the late 1950s (Huang 2001) and heterosis in the 1970s (Yuan et al 1994). Inspired by IRRI's new plant type breeding program, China established a nationwide mega-project on the development of "super" rice in 1996 in order to raise the national average rice yield to 6.9 t ha^{-1} by 2010 and to 7.5 t ha^{-1} by 2030 (Cheng et al 2007). A "super" rice variety should outyield local widely grown check varieties by 10%, with acceptable grain quality and pest resistance. Another criterion of "super" rice is to produce 100 kg grain ha^{-1} day^{-1} (Yuan 2001). "Super" rice varieties can be developed by breeding inbred and/or hybrid varieties. A "super" hybrid rice breeding program was started in 1998 by Prof. Longping Yuan. In this program, the strategy was to combine an ideotype approach with the use of intersubspecific heterosis (Yuan 2001). The ideotype envisioned has the following morphological traits: moderate tillering capacity (270–300 panicles m^{-2}), heavy (5 g per panicle) and drooping panicles at maturity, and panicle height at about 60% of plant height at maturity (Yuan 2001). The Ministry of Agriculture of China requires that a "super" hybrid rice variety should increase grain yield by 15% in commercial rice production. In 1998-2005, 34 commercially released "super" hybrid rice varieties were grown on a total area of 13.5 million ha and they produced an additional 6.7 million tons of rough rice in China (Cheng et al 2007). They produced grain yield of 12 t ha^{-1} in on-farm demonstration fields, 8–15% higher than the hybrid check varieties (Peng et al 2008). Among these varieties, Liangyoupeijiu was the most popular one because of its high yield and wide adaptability. It was widely grown in 13 provinces in China from 1999 to 2006. We have studied the yield potential and N-use efficiency of Liangyoupeijiu in Hunan and Hubei, respectively. We also studied the yield potential and N-use efficiency of SL8-H, a three-line hybrid rice with plant type similar to that of China's "super" hybrid rice, under the tropical conditions of the Philippines.

Yield attributes of "super" hybrid rice in China

An identical field experiment was conducted in Yong'an and Guidong, Hunan, China, in 2007. A split-plot design was used with N rates as the main plots and varieties as the subplots. The low N rate was 135 kg ha^{-1} in Yong'an and 150 kg ha^{-1} in Guidong. The high N rate was 225 kg ha^{-1} in Yong'an and 250 kg ha^{-1} in Guidong. Basal phosphorus (30 kg P ha^{-1}), potassium (40 kg K ha^{-1}), and zinc (5 kg Zn ha^{-1}) were applied in all plots. The six varieties were Liangyoupeijiu and Liangyou293 ("super" hybrid rice), IIyou838 and Shanyou63 (ordinary hybrid rice), and Yangdao6 and Huanghuazhan (inbred rice). Transplanting was done on 11 June with 20-day-old seedlings in Yong'an and on 21 May with 32-day-old seedlings in Guidong. Hill spacing was 23.3 × 23.3 cm, with two seedlings per hill. Growth analysis was done at key growth stages. Grain yield and yield components were determined at maturity. Solar radiation, canopy light interception, and plant dry weight were measured to calculate radiation-use efficiency (RUE).

The maximum grain yield of 11.7 t ha^{-1} was produced by "super" hybrid rice variety Liangyoupeijiu in Guidong at the N rate of 150 kg ha^{-1} (Table 1). There was no significant difference in grain yield between the two N rates. Grain yield was 17% higher at Guidong than at Yong'an mainly because of lower air temperature at Guidong. The lower air temperature resulted in 15 days' longer growth duration and

Table 1. Grain yield (t ha^{-1}) of "super" hybrid rice, ordinary hybrid rice, and inbred varieties grown in Yong'an and Guidong, Hunan, China, in 2007. Values are the mean of two N rates.

Variety	Yong'an[a]	Guidong
Liangyoupeijiu	9.41 a	11.52 a
Lianyou293	9.16 a	11.38 a
IIyou838	8.14 c	10.21 b
Shanyou63	8.40 bc	9.67 c
Yangdao6	8.74 b	10.40 b
Huanghuazhan	8.62 b	10.29 b

[a] Within a column, means followed by the same letters are not significantly different according to LSD (0.05).

high crop growth rate due to reduced maintenance respiration in Guidong. The two "super" hybrid rice varieties (Liangyoupeijiu and Liangyou293) produced the highest grain yield, followed by the two inbred varieties (Yangdao6 and Huanghuazhan) in both Yong'an and Guidong (Table 1). The two ordinary hybrid varieties (IIyou838 and Shanyou63) had the lowest grain yield. The yield difference among the varietal groups was associated with the difference in biomass production (Table 2). The difference in harvest index was small among the three varietal groups and did not explain their difference in grain yield (data not shown). No single component of yield accounted for the superiority of "super" hybrid rice in grain yield. "Super" hybrid rice and inbred varieties had similar RUE, which was significantly higher than that of ordinary hybrid rice (Table 3).

Katsura et al (2007) compared the grain yield and related traits of Liangyoupeijiu with those of Takanari and Nipponbare in Kyoto, Japan, in 2003 and 2004. Liangyoupeijiu produced significantly higher grain yield than Nipponbare in both years. Liangyoupeijiu had consistently higher grain yield than Takanari, but the difference was statistically insignificant. Liangyoupeijiu achieved a grain yield of 11.8 t ha^{-1} at the N rate of 140 kg ha^{-1} in 2004, which is the highest yield observed under the environmental conditions of Kyoto. The high yield of Liangyoupeijiu was associated with larger leaf area duration before heading, greater biomass accumulation before heading, larger number of grains, and more translocation of carbohydrates from the vegetative organ to the panicle during the grain-filling period. Radiation-use efficiency of the whole growth period did not explain the yield superiority of Liangyoupeijiu.

Table 2. Biomass production (t ha^{-1}) of "super" hybrid rice, ordinary hybrid rice, and inbred varieties grown in Yong'an and Guidong, Hunan, China, in 2007. Values are the mean of two N rates.

Variety	Yong'an[a]	Guidong
Liangyoupeijiu	18.9 a	21.0 a
Lianyou293	18.0 a	20.9 a
IIyou838	15.9 b	18.5 b
Shanyou63	16.3 b	18.7 b
Yangdao6	16.7 b	19.0 b
Huanghuazhan	15.9 b	19.2 b

[a] Within a column, means followed by the same letters are not significantly different according to LSD (0.05).

Table 3. Radiation-use efficiency (g MJ^{-1}) of "super" hybrid rice, ordinary hybrid rice, and inbred varieties grown in Yong'an and Guidong, Hunan, China, in 2007. Values are the mean of two N rates.

Variety	Yong'an[a]	Guidong
Liangyoupeijiu	1.36 ab	1.41 a
Lianyou293	1.35 ab	1.42 a
IIyou838	1.19 b	1.31 b
Shanyou63	1.21 b	1.35 b
Yangdao6	1.36 ab	1.39 ab
Huanghuazhan	1.39 a	1.42 a

[a] Within a column, means followed by the same letters are not significantly different according to LSD (0.05).

Nitrogen-use efficiency of "super" hybrid rice in China

A field experiment was conducted in Xiaonan, Hubei, China, in 2004 and 2005. Treatments were arranged in a split-plot design with N rates as main plots and varieties as subplots. The varieties were two hybrids, Liangyoupeijiu and Shanyou63. Eight and six N treatments were used in 2004 and 2005, respectively. The N rates ranged from 0 to 195 kg ha^{-1} for Liangyoupeijiu and from 0 to 225 kg ha^{-1} for Shanyou63 in 2004. This was increased from 0 to 410 kg ha^{-1} for Liangyoupeijiu and from 0 to 275 kg ha^{-1} for Shanyou63 in 2005. Thirty-day-old seedlings were transplanted on 7 June 2004 and 8 June 2005 at a hill spacing of 20 × 20 cm, with two seedlings per hill. Phosphorus (40 kg P ha^{-1}) and zinc (5 kg Zn ha^{-1}) were applied and incorporated in all subplots 1 day before transplanting. Potassium (100 kg K ha^{-1}) was split equally at basal and panicle initiation.

Growth analysis was done at key growth stages. Grain yield and yield components were determined at maturity. Nitrogen concentrations of straw, rachis, and filled and unfilled spikelets were determined by micro-Kjeldahl digestion, distillation, and titration (Bremner and Mulvaney 1982) to calculate aboveground total N uptake. Nitrogen recovery efficiency and agronomic N-use efficiency were determined based on a comparison of crop performance in treatment plots with and without applied N (Novoa and Loomis 1981). Recovery efficiency was calculated as the ratio of the increase in plant N accumulation at maturity that resulted from N-fertilizer application to the N-fertilizer rate, and agronomic N-use efficiency was calculated as the increase in grain yield per unit of applied N. Internal N-use efficiency was calculated as the ratio of grain yield to total N uptake. Partial factor productivity of applied N was the grain yield per unit N applied.

Grain yield of the two varieties responded to N application in both years (Fig. 1). With the zero-N control, Shanyou63 produced higher grain yield than Liangyoupeijiu. The two varieties required a minimum total N rate of 120 to 150 kg N ha^{-1} to produce maximum grain yield. The difference in maximum grain yield was very small between the two varieties, although Liangyoupeijiu had the potential to produce higher grain yield than Shanyou63. When excessive N was applied (195 to 275 kg N ha^{-1}), grain yield of Liangyoupeijiu was 13% higher than that of Shanyou63 in both years because they responded differently to the high N inputs and Shanyou63 lodged at these high N amounts, but not Liangyoupeijiu.

Liangyoupeijiu had a higher total biomass production than Shanyou63 at high N rates, which was the result of a higher crop growth rate during the flowering and ripening phase in Liangyoupeijiu than in Shanyou63 (Huang et al 2008). The chlorophyll meter readings during the ripening phase suggested that Liangyoupeijiu had slower leaf senescence than Shanyou63 (Huang et al 2008). Yao et al (2000) also observed that Liangyoupeijiu had slower leaf senescence than Shanyou63. Sink size (spikelets per m^2) was about 25% higher in Liangyoupeijiu than in Shanyou63, which was solely due to the larger panicle size (spikelets per panicle) of Liangyoupeijiu than Shanyou63 (Huang et al 2008). When the climatic yield potential was high (high solar radiation and low minimum temperature), the extra sink of Liangyoupeijiu could be

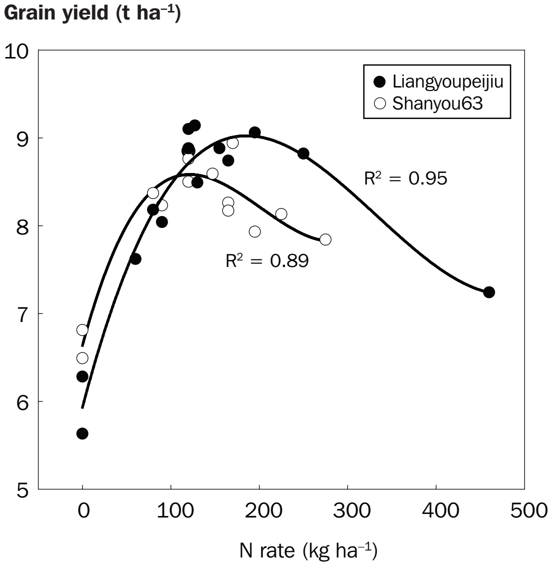

Fig. 1. Grain yield of a "super" hybrid rice (Liangyoupeijiu) and an ordinary hybrid variety (Shanyou63) grown under various total N rates in Xiaonan, Hubei, China, in 2004 and 2005 (modified from Huang et al 2008).

filled and the high yield potential of Liangyoupeijiu could be expressed compared with Shanyou63.

The two varieties did not show a clear difference in fertilizer-N-use efficiency. The two varieties had a similar ability to take up N from indigenous sources (Huang et al 2008). At similar N rates, there was no significant difference in total N uptake or N recovery efficiency and internal N-use efficiency between the two varieties (Table 4). At similar N rates, Liangyoupeijiu generally had higher agronomic N-use efficiency than Shanyou63 and the difference was much greater at excessive N rates than at moderate and high N rates. This difference was partially due to the yield difference between Liangyoupeijiu and Shanyou63 with the zero-N control, because agronomic N-use efficiency was calculated using the "difference" method in this study. The difference in lodging resistance between the two varieties might also contribute to the difference in agronomic N-use efficiency between the two varieties. There was no significant difference in partial factor productivity of applied N between the two varieties (Table 4).

Yield attributes and nitrogen-use efficiency of SL-8H at IRRI

SL-8H is a three-line hybrid rice developed by SL AgriTech Corporation in the Philippines. It has been widely grown in the Philippines since 2003. We conducted a field experiment at the IRRI farm in the dry season of 2005 to compare the yield

Table 4. Nitrogen-use efficiency of a "super" hybrid rice (Liangyoupeijiu) and an ordinary hybrid variety (Shanyou63) grown in Xiaonan, Hubei, China, in 2004 and 2005. Values are the mean of N treatments and two years (modified from Huang et al 2008).

Trait	Liangyoupeijiu[a]	Shanyou63
N recovery efficiency (%)	55.5 a	51.5 a
Internal N-use efficiency (kg kg^{-1})	55.4 a	55.5 a
Agronomic N-use efficiency (kg kg^{-1})	19.0 a	12.9 b
Partial factor productivity of applied N (kg kg^{-1})	66.1 a	61.0 a

[a] Within a row, means followed by the same letters are not significantly different according to LSD (0.05).

Table 5. Yield and yield attributes of SL8-H and IR72 grown at IRRI farm in the dry season of 2005. Total N input was 200 kg ha^{-1}.

Trait	SL8-H[a]	IR72
Spikelets $panicle^{-1}$	138.3 ± 3.0	92.7 ± 8.1
Panicles m^{-2}	321 ± 38	457 ± 37
Grain filling (%)	82.3 ± 2.6	81.9 ± 1.8
Grain weight (mg)	26.1 ± 0.1	22.5 ± 0.2
Plant height (cm)	121.5 ± 4.8	96.0 ± 1.5
Biomass (t ha^{-1})	18.6 ± 2.2	16.7 ± 0.6
Harvest index	0.52 ± 0.01	0.47 ± 0.01
Grain yield (t ha^{-1})	10.62 ± 0.27	8.71 ± 0.32

[a] Mean ± standard deviation.

attributes and N-use efficiency of SL-8H with inbred and hybrid varieties developed at IRRI. A split-plot design was used with N rates as the main plots and varieties as the subplots. The two N rates were 0 and 200 kg ha^{-1}. Six varieties included three inbreds (IR72, PSBRc18, and PSBRc52) and three hybrids (Mestizo1, Mestizo3, and SL-8H). Fourteen-day-old seedlings were transplanted on 5 January at a hill spacing of 20 × 20 cm, with four seedlings per hill. Phosphorus (30 kg P ha^{-1}), potassium (40 kg K ha^{-1}), and zinc (5 kg Zn ha^{-1}) were applied and incorporated in all plots 1 day before transplanting. Growth analysis was done at key growth stages. Grain yield and yield components were determined at maturity. Agronomic N-use efficiency was calculated as the ratio of yield difference between N-treated and N-minus plots over the amount of applied N.

Table 6. Yield and agronomic N-use efficiency of SL8-H and IR72 grown at IRRI farm in the dry season of 2005.[a]

Variety	Yield without N input (t ha^{-1})	Yield with 200 kg N ha^{-1} (t ha^{-1})	Agronomic N-use efficiency (kg kg^{-1})
IR72	4.61 ± 0.32	8.71 ± 0.32	20.5
PSBRc18	4.87 ± 0.05	9.11 ± 0.86	21.2
PSBRc52	4.62 ± 0.21	8.44 ± 0.16	19.1
Mestizo1	4.66 ± 0.55	9.51 ± 0.15	24.3
Mestizo3	4.57 ± 0.30	9.63 ± 0.31	25.3
SL-8H	4.99 ± 0.17	10.62 ± 0.27	28.1

[a]Mean ± standard deviation.

SL-8H is very different in plant type compared with most indica inbred varieties that have been widely grown under tropical conditions. The plant type of IR72 is a typical example of these indica inbred varieties. SL-8H has lower tillering capacity, larger panicle size, and taller plants than indica inbred varieties. In the dry season of 2005, SL-8H produced a grain yield of 10.62 t ha^{-1}, 22% higher than IR72 (Table 5). This high grain yield was attributed equally to biomass production and harvest index. SL-8H produced more spikelets per panicle and fewer panicles per m^2 than IR72 (Table 5). There was no significant difference in grain-filling percentage between the two varieties, although the grain weight of SL-8H was 16% higher than that of IR72. SL-8H was 25 cm taller than IR72 at maturity, but SL-8H was more lodging resistant than IR72 due to a greater diameter of lower internodes (Islam et al 2007).

Unlike the "super" hybrid rice in China, SL-8H did not produce lower grain yield than indica inbred varieties or other hybrid rice varieties when N was not applied (Table 6). When N was applied, SL-8H had 12% higher grain yield than Mestizo1, another popular hybrid rice variety in the Philippines. Agronomic N-use efficiency of SL-8H was the highest among the six test varieties (Table 6). More importantly, the high agronomic N-use efficiency of SL-8H was due to higher grain yield when N was applied but not due to lower grain yield of the zero-N control, which was the case for the "super" hybrid rice variety grown in Xiaonan, Hubei, China (Table 4 and Fig. 1).

Morphologically, SL-8H has many similarities with China's "super" hybrid rice. SL-8H has partially overcome several problems that crop physiologists at IRRI are facing: (1) poor grain filling as a result of large panicle size, (2) low harvest index due to tall plants, (3) insufficient panicle number due to low tillering ability, and (4) lodging susceptibility as a result of taller plants.

Conclusions

"Super" hybrid rice has the following common traits: large and heavy panicles, few tillers, sturdy stems, tall stature, and efficient translocation. It has higher rice yield potential than inbreds and ordinary hybrids. Its higher yield is mainly the result of higher biomass, but high harvest index is also responsible for the high yield in the tropics. High intercepted solar radiation due to greater leaf area duration contributed to the higher biomass. Photosynthetic rate or radiation-use efficiency does not explain the difference in biomass. There is no significant difference in N-use efficiency, except for high agronomic N-use efficiency in "super" hybrid rice. To increase rice yield potential in the tropics, we need to develop new plant type varieties similar to SL-8H with slightly more filled spikelets per panicle.

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Notes

Authors' addresses: S. Peng, R.C. Laza, and R.M. Visperas, International Rice Research Institute; Q. Tang, Y. Zou, and Y. Zhang, Hunan Agricultural University; J. Huang, K. Cui, and F. He, Huazhong Agricultural University.

Physiological characteristics of hybrid rice and cultivation techniques for high yield

Zhu Defeng, Lin Xianqing, Zhang Yuping, and Chen Huizhe

Planting area covered by hybrid rice in China represents more than 55% of the total national rice-planting area. It yields about 20% more than inbred varieties in farmers' fields and contributes to the continuous increase in rice yield in China.

Experimental results indicate that hybrids have higher tillering capacity, more roots, more leaf area and biomass, more large panicles, and heavier panicle grain weight than inbreds. Hybrids have a yield advantage over inbreds mainly because of their large panicles and heavy panicle grain weight. Compared with improved inbreds, hybrids have more biomass, resulting in higher yield.

Based on the characteristics of growth and yield components for hybrid rice, integrated cultivation techniques of hybrid rice for high yield mainly involve raising strong seedlings by reducing seed rate per unit, transplanting seedlings sparsely, properly increasing potassium fertilizer application and percentage of nitrogen fertilizer application in the late stage, and practicing intermittent water management. An optimum population and ideal plant type of hybrid rice are established through basic establishment of seedling number and management of fertilizer and water.

Keywords: hybrid rice, physiology, cultivation technology, germination, tiller, root, high yield

Rice is a staple food crop in China, playing an important role in food security because 60% of China's population lives on rice. So, China focuses on increasing rice yield by improving varieties and developing cultivation technology. Two breakthroughs in rice production technology have resulted in a continual increase in rice yield for several decades in China. The first one was semidwarf varieties. Since the end of the 1950s, before the Green Revolution in tropical areas, China shifted from traditional tall varieties to semidwarf rice varieties. Semidwarf varieties were widely adopted in farmers' fields with supporting cultivation technology developed according to the crop's characteristics. That allowed a big increase in rice yield in China. The second rice production technology breakthrough was hybrid rice. Hybrid rice in China has been grown commercially since 1976. Being supported by special cultivation technology, hybrid rice has maintained a yield increase since then (Cheng et al 2004, 2007). Experiments and statistics indicate that hybrid rice gives about a 20% yield advantage over improved inbred varieties (Yuan and Wu 2004). In recent years, good progress has been made in super rice varieties since the initiation of the super rice program in 1996. Several super hybrids and inbred varieties have been released and used in farmers' fields (Cheng et al 2007). Now, planting area of hybrid rice represents more than 55% of the total national rice-growing area (Yuan and Wu 2004). With improvements in the yield and quality of hybrid rice, and the development of japonica hybrid rice, two-line hybrid rice, and super hybrids, the planting area of hybrid rice will increase (Cheng et al 2007, Yuan and Wu 2004).

The important experience with the wide adoption of hybrid rice in China is the combination of hybrid rice and its supporting cultivation technology. In the initial period of the application of hybrid rice, farmers could not find any yield advantage of hybrid rice over improved inbreds if farmers practiced traditional cultivation technology such as improved inbreds, a high seed rate in seedbeds, high hill density per unit with more seedlings, deep floodwater irrigation, and unsuitable fertilizer application, and others. After understanding the characteristics of hybrid rice, supporting cultivation technology for hybrid rice was developed. Thus, hybrid rice could show its yield advantage over inbreds in farmers' fields.

In recent years, Chinese paddy fields and rice-growing area have steadily decreased because of urbanization and industrial development, agricultural planting structure adjustments, and a shift from a double-cropping system to a single-cropping system. Agricultural labor transfer, water limitations, soil fertility degradation, and the rising price of chemical fertilizer are limitations and challenges to increasing rice yield further. Although a lot of new rice inbred varieties and hybrids have been released, rice yield has stagnated in recent years. We also found in the survey a large yield gap in hybrids between varietal yield potential and farmer yield, among farmers, and between yield guided by experts and farmer yield. We need to close the exploitable gap between yield potential and average farm yield to increase the yield of hybrids. So, it is important to practice supporting cultivation technology according to the characteristics of hybrids to exploit their yield potential.

Response of germination to seed soaking time

Different kinds of rice varieties show various rates of seed water absorption under the same temperature. The rate of indica inbreds (Zhongzao 21) and indica hybrids (Xieyou 9308) is faster than that of japonica varieties (Xiushui 63). The saturated ratio of seed water absorption in indica inbreds is much higher than that of indica hybrids and japonica inbreds (Fig. 1).

The ratio of seed water absorption required to reach a certain ratio of seed germination depends on the kind of variety. When Xieyou 9308 reached 18% of seed water absorption on a seed weight basis, Zhongzao 21 reached 21.7% and Xiushui 63 only 14.3% at 6 hours of seed soaking. The germination rate of Xieyou 9308, Zhongzao 21, and Xiushui 63 is 96.5%, 73.0%, and 38.7%, respectively. Experimental

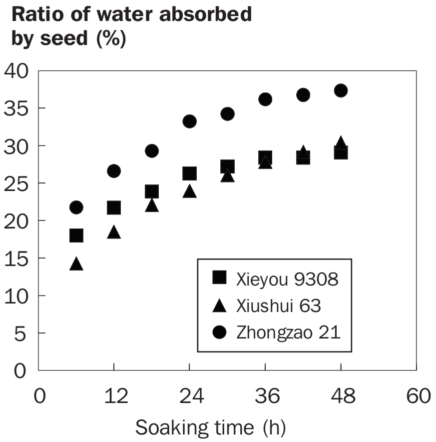

Fig. 1. Comparison of effect of soaking time on ratio of water absorbed among hybrids (Xieyou 9308), japonica inbreds (Xiushui 63), and indica inbreds (Zhongzao 21) under conditions of 24 °C.

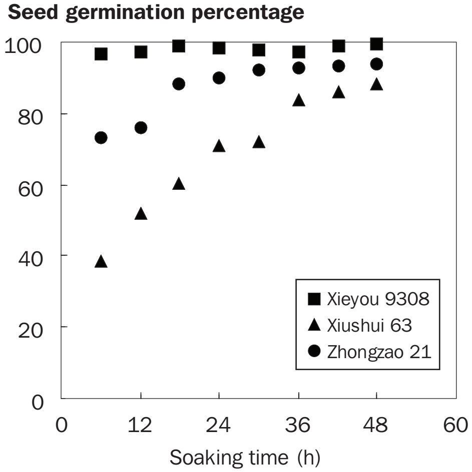

Fig. 2. Comparison of effect of soaking time on germination percentage among hybrids (Xieyou 9308), japonica inbreds (Xiushui 63), and indica inbreds (Zhongzao 21).

results indicated, under conditions of 24 °C, that seed soaking time required for normal germination is 12 h for Xieyou 9308, 36 h for Zhongzao 21, and 48 h for Xiushui 63, respectively (Fig. 2). That means that seed soaking time required for hybrids is shorter than for indica inbreds and much shorter than for japonica inbreds. This characteristic of hybrid seed may be related to the loose closure of the lemma and platea of hybrid

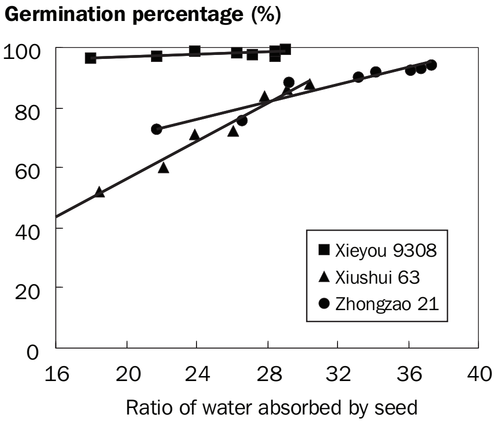

Fig. 3. Comparison of relation of ratio of water absorbed by seed to germination percentage among hybrids (Xieyou 9308), japonica inbreds (Xiushui 63), and indica inbreds (Zhongzao 21).

seed. Experiments also indicate that too long a soaking time for hybrid seed also results in a low rate of seedling establishment and makes seedlings weak.

Seed germination rate is positively related to the ratio of water absorbed by seed in different kinds of rice varieties. Hybrids can reach a normal germination rate when the seed absorbs 20% of the water on a seed weight basis. However, indica inbreds and japonica inbreds must absorb 36% and 30% of water, respectively, on a seed weight basis to reach a normal germination rate (Fig. 3).

Tillering capacity

When hybrid rice started to be released for commercial use, it was observed that all hybrids had an advantage in tilling capacity over inbred cultivars. However, some inbred cultivars released in recent years also have higher tillering capacity. Also, some hybrids with large panicles, such as heavy panicle-type hybrid Xieyou 9308, have the same tillering capacity as inbred cultivar Zhe 156 (Table 1). In general, hybrids have stronger tillering capacity than inbred cultivars. A comparison of tiller number per plant between hybrids and inbreds in experiments with a super rice variety in 2000 indicates that hybrids have stronger tillering capacity than inbred varieties (Fig. 4).

Root growth

The hybrid growth advantage can also be found in root growth (Zhu et al 2003). Hybrid Liangyoupeijiu, widely used in China, has a significant advantage in root density in the top soil layer and a small advantage in root density in the deep soil layer over inbred Tesanai 2. Hybrid Xieyou 9308 has a significant advantage in root density from the top soil to deep soil layer over inbred Tesanai 2 (Fig. 5). That makes Xieyou

Table 1. Comparison of tillering capacity between hybrids and inbreds at 25 days after sowing.

Type	Variety	Tillers (no. seedling^{-1})	Relative percentage (%)
Hybrid	Liangyoupeijiu	1.42 a	187
	Teyouming 86	1.36 ab	180
	Shanyou 63	1.24 b	163
	Xieyou 9308	0.83 c	110
Inbred	Zhe 156	0.76 c	100

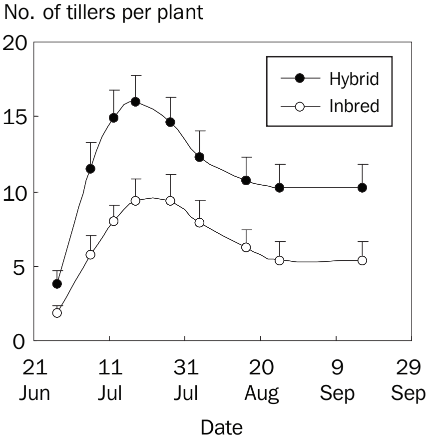

Fig. 4. Comparison of tiller number per plant between hybrids and inbreds in 2000.

9308 tolerant of leaf senescence (Zhu et al 2000) and also promotes the formation of heavy panicles (Zhu et al 2001). The hybrid root growth advantage may be related to tillering capacity and above-ground growth advantage.

Biomass production

Hybrids have more leaf area per hill and per unit area, which contributes to more biomass production than with inbred varieties (Xie 1997). Experimental results also indicate that hybrid rice produces more biomass than inbreds after heading. Research indicates that biomass production after flowering is positively related to grain yield in rice. One reason for the higher biomass production of hybrids is the higher photosynthetic rate of upper leaves in hybrids and a lower decrease in leaf photosynthetic rate after flowering (Fig. 6). The photosynthetic rate of the flag leaf

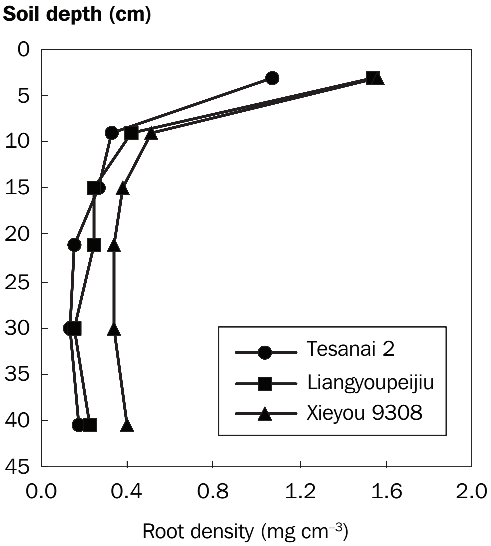

Fig. 5. Root density in hybrids (Liangyoupeijiu and Xieyou 9308) and inbreds (Tesanai 2).

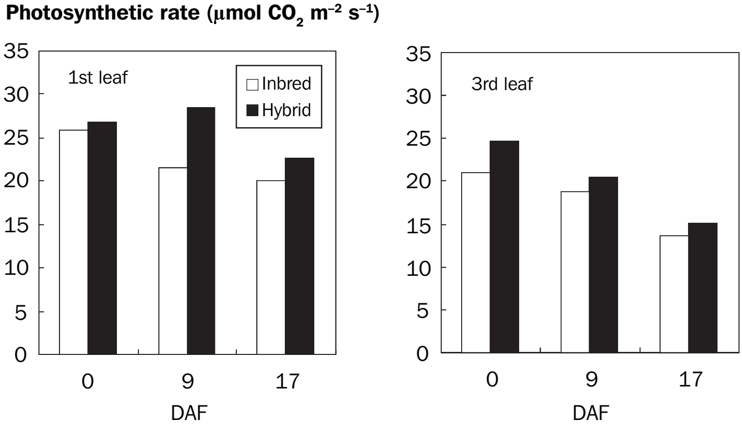

Fig. 6. Photosynthetic rate of flag leaf (first leaf countdown) and third leaf in hybrids and inbreds at days after flowering (DAF).

in hybrids is 4%, 32%, and 12%, respectively, higher than that in inbreds at 0, 9, and 17 days after flowering and that of the third leaf in hybrids is 17%, 8%, and 10%, respectively, higher than that in inbreds at 0, 9, and 17 days after flowering. The photosynthetic rate of the flag leaf and the third leaf in hybrids at 17 days after flowering decreases by 17% and 41%, respectively, whereas in inbreds it decreases by 22% and 47%, respectively (Fig. 6).

Table 2. Contribution of biomass and harvest index to yield increase in the evolution of rice varieties from the 1940s to 1990s in China.

Period	Variety type	Biomass ($t\ ha^{-1}$)	Harvest index	Yield ($t\ ha^{-1}$)
1940s-'60s	Tall	11.04	0.385	4.25
1960s-'80s	Semidwarf	11.82	0.545	6.44
1970s-'90s	Hybrid	15.03	0.545	8.19

Yield and its components

Several research efforts on the contribution of biomass and harvest index to yield increases in the improvement in the evolution of rice varieties in China have been made (Zhu et al 2003). These indicate that yield increases in rice through the evolution of tall varieties to semidwarf varieties mainly result from the increase in harvest index, whereas the yield advantage of hybrids is attributed to the increase in biomass compared with semidwarf varieties (Table 2).

In recent years, japonica hybrid breeding made great progress in China. Some japonica hybrids grown in the reaches of the Yangtze River and the northeast area had a good yield advantage over local inbred cultivars. The yield of japonica hybrids and inbred varieties in a single japonica variety test at 10 locations in the reaches of the Yangtze River from 2005 to 2007 was analyzed. Results indicated that japonica hybrids gave 5% to 22% more yield than japonica inbred cultivars in various years. Japonica hybrids had fewer panicles and larger panicles than japonica inbred varieties. Therefore, the increase in grain number per panicle in japonica hybrids resulted in a 46.9% average yield increase over japonica inbred varieties from 2005 to 2007. The decrease in panicle number per unit area in japonica hybrids resulted in a 27.8% yield decrease over japonica inbred varieties from 2005 to 2007 (Fig. 7). Variation in grain weight between hybrids and inbreds has a relatively small influence on yield. The yield increase in japonica hybrids mainly results from the increase in grain number per panicle compared with japonica inbred varieties.

Integrated management technology for hybrid rice

Seedling preparation

One of the advantages of hybrid rice over inbreds is tillering capacity. To exploit the tillering advantage of hybrid rice, it is important to promote early tillers in the lower tiller position of the rice plant. So, strong seedlings with more tillers should be prepared for higher yield of hybrids. To prepare strong seedlings, seed selection, a low seed rate, and proper fertilizer and water management are practiced. When hybrid seeds are put in pure water, about 9–10% of the seeds will float. Those floating seeds have a low germination and seedling establishment rate (Table 3). The seedlings from these

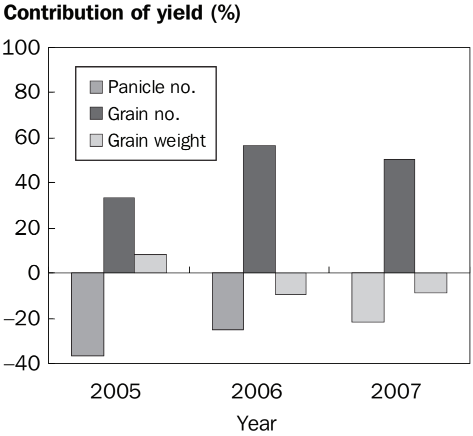

Fig. 7. Contribution of yield components to yield increase of japonica hybrid rice compared with japonica inbred rice.

Table 3. Effect of seed quality on germination and seedling establishment.

Hybrid	Item	Floating seeds	Sinking seeds	Total
Liangyoupeijiu	Grain (%)	8.7	91.4	100
	Germination rate (%)	34.6	97.5	92.1
	Seedling establishment rate (%)	30.7	74.6	70.8
Xieyou 7954	Grain (%)	9.8	90.1	100
	Germination rate (%)	66.3	96.6	93.5
	Seedling establishment rate (%)	42.1	77.4	73.9

are usually weak. For higher yield, only heavy seeds are sown. In hybrid seedling preparation, seed soaking time is usually 12 to 24 hours because there is a difference in seed soaking time of hybrid rice from inbreds. For hybrid rice, some hybrids also need to use a chemical regulator, such as MET, to retard seedling height and promote tiller emergence (Table 4).

Seed amount is generally 12 to 15 g m^{-2} at 25 to 30 days of seedling age and 25 to 30 g of the seed weight of hybrids. With the increase in seed rate per unit, tiller number per seedling will decrease (Table 4).

Sparsely transplanting and population regulation

In China, with the shift from the double-rice cropping to single-rice cropping system and inbreds to hybrids, the density of plants transplanted per unit area decreased. In traditional rice cultivation technology with improved inbreds, 45 hills were transplanted

Table 4. Effect of seed rate of hybrids and a chemical regulator (MET) in seedbed on tiller emergence at 7-leaf stage.

Variety	Treatment	Seed rate (kg 667 m^{-2})				
		5	10	15	25	40
Ilyou 7954	MET	5.4	5.4	3.8	3.9	3.4
	Check	4.7	3.7	2.8	3.3	1.8
Nei2you6	MET	5.0	3.3	2.9	2.8	1.5
	Check	3.7	2.9	2.5	1.9	1.0

Table 5. Comparison of yield (t ha^{-1}) of hybrid combinations in different plant densities.[a]

Density (hills m^{-2})	2002		2003	
	Zhongyou 6	Liangyoupeijiu	Ilyou 7954	Liangyoupeijiu
19.5	6.37 b	7.36 b	10.66 b	8.18 b
16.5	6.69 a	7.81 a	11.30 a	8.81 a
13.5	6.16 b	7.32 b	9.82 c	8.05 b
10.5	5.11 c	6.33 c	8.11 d	7.77 c
7.5	4.86 d	6.43 c	7.20 e	7.68 c

[a] Letters after numbers indicate significance at the 5% level.

with several seedlings in late rice in the double-cropping system. Even in single rice with improved inbred cultivars, 25 to 30 hills were transplanted with several seedlings just a decade ago. Now, in most hybrid rice in a single rice season, only about 15 hills are transplanted with 1 or 2 seedlings per hill. A low density of 1 hill per unit with 1 or 2 seedlings per hill is a main planting method for hybrids (Zou et al 2003).

This is because the characteristics of hybrids are different from those of inbred cultivars. Hybrids have a taller plant, stronger tillering capacity, and large canopy with more leaf area and biomass production. Too high a density of plants per unit area results in a yield decrease. Experiments showed that yield for four hybrids decreased with more than 16.5 hills per square meter, but increased when plant density per unit was lower than 16.5 hills per square meter (Table 5).

Suitable maximum tiller number is also important for high yield. A reasonable maximum for indica hybrids is 1.3 to 1.4 times the final target panicles. In farmers' fields with hybrids, maximum tiller number is controlled through drainage and the regulation of nitrogen fertilizer application at a certain stage.

Table 6. Effects of water management on seedling growth in seedbed.[a]

Water depth	Tillers (no. seedling^{-1})	Plant height (cm)	Root weight (g 10 seedlings^{-1})	Ratio of root to above-ground part
Wet	5.6 a	28.4 c	1.89 a	0.34 a
2 cm	4.9 b	28.0 c	1.72 ab	0.32 a
4 cm	4.0 b	32.4 b	0.90 c	0.17 b

[a]Letters after numbers indicate significance at the 5% level.

Table 7. Comparison of root number and weight of two hybrids at panicle initiation stage between deep floodwater (6 cm) and intermittent irrigation.

Hybrid	Irrigation	Root number (no. hill^{-1})	Root weight (g hill^{-1})
Liangyoupeijiu	Intermittent irrigation	780	2.3
	Deep floodwater	719	2.0
IIyou 7954	Intermittent irrigation	671	2.3
	Deep floodwater	597	2.1

Intermittent irrigation

Water depth influences rice growth, leaf shape, and canopy architecture (Lin et al 2005). In seedbeds, research (Chen et al 2005) indicated that tiller number per seedling and root weight decrease with an increase in water depth (Table 6). Seedlings raised under deep water are tall, and need more time to recover growth because of severe transplanting shock. That also influences the emergence of early tillers from the low tiller position.

Hybrids Liangyoupeijiu and IIyou 7954 grow under two kinds of irrigation practices: deep floodwater and intermittent irrigation. Results indicate that rice plants under intermittent irrigation have more roots and root weight per hill than under deep floodwater (Table 7). Under intermittent irrigation, the plants have more tillers per hill and more erect leaves than under deep floodwater. Plants with erect leaves will improve canopy photosynthesis and be resistant to lodging.

Shallow and intermittent irrigation are widely practiced as a key item in supporting cultivation technology for hybrids (Zhu et al 2007, Zhang et al 2007).

Precision fertilization

The fertilizer requirement for hybrid rice is basically determined according to the supply of nitrogen, potassium, and phosphorus in soil and the target yield. There is not much difference in agronomic efficiency and physiological efficiency of nitrogen

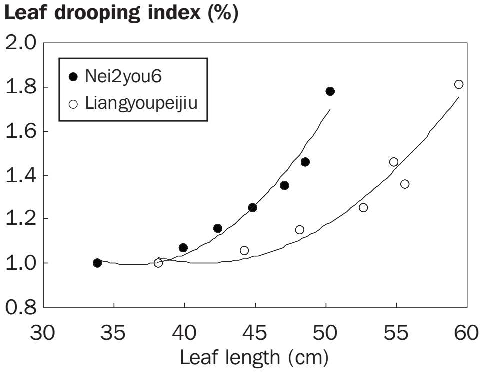

Fig. 8. Relation of leaf length and leaf drooping index in two rice hybrids.

between hybrid rice and inbreds. This mainly depends on the yield difference and variety. But, most experiments indicate that hybrid rice needs more potassium than inbreds.

In general, phosphorus fertilizer is applied just before transplanting to incorporate it with soil during plowing and harrowing. Potassium fertilizer is applied two times, as basal fertilizer and at 1 week after transplanting or at the panicle initiation stage. For high yield of hybrid rice, three split applications of nitrogen fertilizer are recommended: a basic fertilizer application before transplanting, a first dressing of fertilizer at 1 week after transplanting, and a second dressing of fertilizer at panicle initiation (Zhu et al 2002, 2007). The second topdressing of N fertilizer is important for the formation of panicles. Spikelet number per panicle will increase by 10–20% with this application of N fertilizer compared with a check without N application at panicle initiation. The application amount of N fertilizer at that stage strongly depends on variety type and plant growth. If too much N fertilizer is applied at that time, the leaves of hybrids, especially indica hybrids, will become long and droopy (Fig. 8). That will result in more diseases and lodging, and decease yield.

Pest control

Hybrid resistance to pests is combination dependence. Pest control is mainly done according to the incidence of diseases and insects at special locations. But, experiments show that intermittent irrigation and low plant density can reduce the occurrence of sheath blight because of low humidity in the plant population.

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Notes

Authors' address: State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 31006, China.

Approaches and practices for cultivating nitrogen-saving and high-yielding super hybrid rice

Ma Guohui

This paper deals with the conflicts of rice production in China in the past 50 years between a continuous increase in both rice production and nitrogen consumption. Super high-yielding rice cultivation occurs with a large quantity of nitrogen and excessive nitrogen application along with low rice profits and serious environmental pollution. Researchers first put forward the new concept of nitrogen-saving and high-efficiency cultivation (NSHEC) of super hybrid rice and further discussed three approaches for NSHEC of super hybrid rice, using genetic dominance to tolerate low nitrogen or have high efficiency, and optimizing the techniques of nitrogen management and applying new fertilizer products, such as slow-release urea. Based on field experiments with super hybrid rice, research results indicated that significant differences exist in nitrogen use between super rice hybrids, types of fertilizer, along with cropping techniques. I optimized the techniques of NSHEC for super hybrid rice for yield of 12 t ha^{-1}.

The invention and application of hybrid rice helped to simultaneously improve both rice yield per unit area and rice production on a large scale in China, allowing China to use 9% of the world's arable land to feed 22% of the world's population. However, China also consumes 27% of the world's nitrogen resources. As with other food crops, the foundation for substantial yield improvement is partially the double increase in the amount of fertilizer application along with progress in breeding technology. From 1960 to 2000, the amount of nitrogen consumption increased 6.4 times in the world, while increasing in China by 43.8 times, seven times the world average growth rate. Therefore, agricultural experts believed that, "in the 20th century, 50% of the increased crop yields worldwide came from fertilizers." Although this point of view may be somewhat biased, it has also given us an important hint that it is one issue of super high-yielding rice cultivation that must be resolved to achieve the goal of hybrid rice being environmentally friendly, resource-saving, high-yielding, and having high profit for farmers along with meeting China's growing food demand.

The continuous increase in rice yield and excessive consumption of N resources

China has been consuming 37% of the world's total nitrogen quantity on only 20% of the world's rice area, and a high nitrogen application per unit area and lower efficiency of N use are two distinct characteristics of rice production. On the one hand, rice yield has increased continuously. In the 1950s, the average rice yield was only 1.5 t ha^{-1}; in 1999, it increased rapidly and stabilized at 6.1 t ha^{-1} on 31.28 million hectares in China. On the other hand, in recent years, rice area in China has declined continuously. However, total N consumption has risen continuously. In 1999, rice area compared with area in 1978 declined by about 10%, for a total area of about 3.13 million hectares. But, over the past 15 years, nitrogen consumption has increased year by year and total N consumption around the country increased rapidly up to 21.809 million tons in 1999 from 12.049 million tons in 1985, an increase of 81%. In the same period, the consumption of N fertilizer for agriculture in Hunan Province of central China also increased by 65.9%. Along with the rapid growth of China's agricultural nitrogen use, rice production rose with higher N use and a lower efficiency of N use.

According to a sample survey, the rates of nitrogen absorption and use in China are currently 30–35% lower than the world's average: agronomic efficiency of N use in rice declined from 15–20 kg kg^{-1} nitrogen in the 1950s to 7.5 kg kg^{-1} N in the 1990s. The national average amount of N application is 226 kg ha^{-1} in single cropping of rice and 185 kg ha^{-1} in double cropping of rice, of which 33.6% of the households applied more than 250 kg ha^{-1} of N and one-third of the rice farmers made excess N application (China Agricultural University, unpublished data). The amount of N application in China is far higher than the world average N application (of 90 kg ha^{-1}, FAO 2002). What is worrisome about this is that about 60% of China's farmland application of chemical fertilizers goes into the surrounding environment, along with the lower efficiency of N use and excessive consumption of resources. A lot of N fertilizer losses have led directly and indirectly to a series of adverse environmental effects. It is estimated that more than 10 million tons of chemical fertilizers are lost annually in Chinese rice cultivation, which aggravates the regional environment.

Nitrogen applications for high-yielding cultivation and proposed nitrogen-saving techniques

Along with the achievements of a super-high-yield rice breeding program in China and the application of super hybrid rice on a large scale, the amount of nitrogen application in rice increased greatly under high-yielding and super-high-yielding culture, which caused great concern. Ye Huabin (2003), Luo Jianjun et al (2005), and Xin-ming Ruan et al (2005) showed in their experiments (Table 1) that the highest yield was 8.9–11.32 t ha^{-1} under much higher nitrogen at 300 kg ha^{-1}. Even variety Wujin 9728 had a maximum yield of only 8.98 t ha^{-1} under 375 kg ha^{-1} N application (Tao Feng et al 2006). That indicates that nitrogen-saving cultivation techniques are not seriously dealt with in research and extension on high-yielding cultivation in China.

Table 1. Yield response with nitrogen application in different nitrogen experiments.

Variety	Highest yield (t ha^{-1})	Purity of N applied (kg ha^{-1})	Data originated from
Wujin 9728	8.98	375.0	Tao Feng et al (2006)
9746	11.32	300.0	Ye Huabin et al (2003)
Wuxianggen 14	8.93	300.0	Jian-Jun Luo et al (2005)
Xieyou 9019	8.90	300.0	Xin-Ming Ruan (2005)
Xianyou 63	9.54	170.1	Liu Lijun (2005a,b)
9915	9.34	141.5	Liu Lijun (2005a,b)
Liangyou 293	12.14	231.0	Demonstration plots
Liangyoupeijiu	12.26	255.0	Demonstration plots

Fortunately, nitrogen-saving techniques in high-yielding rice cultivation resulted in high-yield, efficient, and environment-friendly cropping. Liu Lijun's study (2005, Table 1) achieved high yield of 9.34 t ha^{-1} under lower nitrogen of 141.5 kg ha^{-1}. Ma Guohui (2005, 2006) studied a package of techniques for saving nitrogen and reducing pollution in rice cultivation based on humus fertilizer. His research also showed good control with fertilizer application of 142.5–187.5 kg ha^{-1} and super high yield for the cultivation of super hybrid rice through balanced fertilizer and new products with components designed for lower nitrogen, suitable phosphorus, and higher potassium. With nitrogen-saving techniques, super hybrid rice demonstration plots had a high yield of 12 t ha^{-1}, with a relatively lower amount of N at 231–255 kg ha^{-1}, which indicates that it is possible for super hybrid rice to achieve super high yield and highly efficient nitrogen savings.

Supporting cultivation technology featuring nitrogen savings, high yield, and high efficiency

In 1996, the Chinese government launched a super rice program for feeding its growing population and significant progress has been made. For instance, super hybrid rice achieved a yield of 10.5 t ha^{-1} in 2000 and 12 t ha^{-1} in 2004 on large areas (at least 6.7 ha), which realized the target yield within the first and second phases set by the Chinese Ministry of Agriculture. Therefore, Yuan Longping, the father of hybrid rice, recently proposed a new target of 13.5 t ha^{-1} for the third phase. Facing the new target, the choice should be made between following super-high-yield culture with super-high nitrogen application simultaneously or seeking a new way to investigate techniques such as super-high-yielding culture based on nitrogen savings. If the first choice is made, how much nitrogen would be needed to achieve a yield of 13.5 t ha^{-1}? And, what are the bad effects of high-nitrogen cultivation?

Moreover, super hybrid rice in the test area has shown a yield potential of 12 t ha^{-1} in Hunan Province but the average yield of single-cropping rice in Hunan was

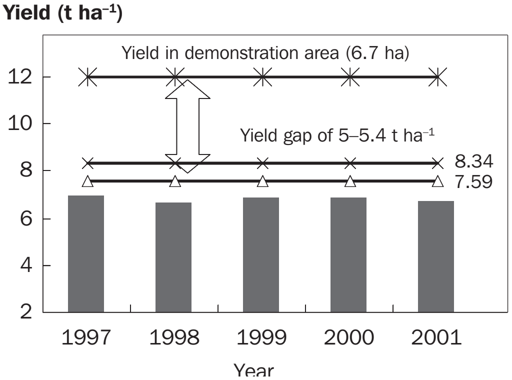

Fig. 1. Average yield (in a demonstration area of 6.7 ha) of single-cropping rice from 1997 to 2001 in Hunan Province.

only 6.84 t ha^{-1} in 1997-2001. There is a large yield gap of 5.16 t ha^{-1} (Fig. 1). We cannot overcome this gap even using the new varieties of super hybrid rice by focusing much more on nitrogen application. But, we can gradually reduce the yield gap step by step by developing adaptable techniques for super hybrid rice. For instance, we can set a first target of increasing average yield by 0.75–1.5 t ha^{-1} to 7.59–8.34 t ha^{-1} by studying the corresponding nitrogen-saving and high-yielding cultivation techniques. It thus may be possible to achieve our objectives and accelerate the development and application of super hybrid rice.

Cultivating super hybrid rice with nitrogen savings and high yield

More and more nitrogen is applied in China's rice production than before, especially for high-yielding cultivation, which leads to increased cost, serious pest damage, and worse grain quality, but also results in serious pollution. Higher nitrogen application is mainly due to seeking high yield as well as lower efficiency of nitrogen use. Therefore, approaches for obtaining nitrogen savings and high yield could be followed in three aspects: good varieties (genotypes), advanced fertilizer management (management techniques), and modern fertilizer varieties based on advanced techniques such as slow-release fertilizer (materialized technology).

Genetic potential

The genetic potential of genotypes means that yield and agronomic nitrogen-use efficiency should be key indicators for breeding programs in two ways. New varieties selected should perform not only with lower nitrogen toleration but also have higher yield and wide adaptation so that less nitrogen will be needed in large-scale rice production.

Management techniques

Nitrogen supply from indigenous sources is seldom sufficient for high rice yield so that techniques and skills for supplemental nitrogen management have been developed for higher yield and farmers' profit. Achieving technical potential means adopting different advanced techniques and skills for fertilizer management, such as NPK balanced fertilization and site-specific nutrient management (SSMN). In general, these advanced techniques of fertilizer management will help farmers obtain higher yield with less fertilizer application.

Materialized technology

Materialized technology means combining special techniques into special kinds of fertilizers, such as component fertilizer with lower N, suitable P, and higher K based on balanced fertilization and sulfur-coated urea (SCU) and coated component fertilizer (CCF) based on slow-release techniques. It is especially a foundation for achieving nitrogen savings and high yield in the future, targeting efficient improvement of N use as SCU and CCF, thus combining technology in products.

Nitrogen savings and high-yielding cultivation

Differences between genotypes in nitrogen response of super hybrid rice

We dealt with 13 genotypes of super hybrid rice to study the differences in nitrogen response of yield based on experiments under nitrogen stress (two treatments with 180 kg ha^{-1} N and 90 kg ha^{-1} N). Our experiments indicated that 13 super hybrid rice genotypes could be divided into two kinds of N-response types, namely, the lower-nitrogen-tolerant or high efficient-nitrogen-absorption type variety and the nitrogen-favoring type variety (Table 2). The first type could increase yield by 0.04–0.61% (Zhun S/1243 and 58S/2469) or decrease yield by 7.11–10.97% (C-liangyou 343 and Long liangyou No. 1) in 2006, but not significantly under nitrogen stress. Especially Zhun S/1243 yielded 3.2% more under nitrogen stress in 2007. This means that the varieties could obtain high yield even under lower N application or some variety might efficiently absorb nitrogen. The second type of varieties would significantly decrease yield by 8.64–15.14% (58S/0293 and Peiai 64s/1243) or very significantly decrease yield by 19.57–36.79% (Liangyoupeijiu and 58S/747) under nitrogen stress. This means that the varieties could obtain super high yield only under higher N application. In other words, these varieties do not show super high yield potential under lower N application.

Based on the results (Table 2), further experiments dealing with the nitrogen response of yield in treatments with N0 (no nitrogen application, check), N1 (N application at 90 kg ha^{-1}), and N2 (nitrogen application at 180 kg ha^{-1}) were conducted at Changsha, China, in 2007. The results showed (Fig. 2) that the lower-nitrogen-tolerant type varieties ZhunS/1243 and C-Liangyou343 from N0 to N1 had a stronger nitrogen response than the nitrogen-favoring type, Liangyoupeijiu, while there was almost no nitrogen response in yield (C-liangyou343) from N1 to N2, even with a negative effect

Table 2. Yield differences between different super hybrid rice genotypes under different N application (2006-07, Changsha, China).

Year	Hybrid combination	Yield (t ha^{-1})		N1 to N2[a] ±(%)	Type
		N1	N2		
2006	Zhun S/1243	10.272	10.268	0.04	A
	58S/2469	9.400	9.343	0.61	
	Long liangyou No. 1	9.044	9.687	–7.11	
	C-liangyou 343	9.303	10.147	–9.07	
	58s/3218	9.270	10.287	–10.97	
	1161S/2469	8.447*	9.177*	–8.64	B
	Anlong3S/Aixun 6	8.592*	9.726*	–13.20	
	Peiai64S.1243	8.495*	9.777*	–15.09	
	Y-liangyou No. 1	9.012*	10.376*	–15.14	
	C-liangyou 87	9.238**	11.046**	–19.57	
	Liangyoupeijiu	8.237**	10.140**	–23.10	
	Keyou 21	7.623**	9.483**	–24.40	
	58S/747	8.140**	11.135**	–36.79	
2007	Zhun S/1243	11.299	10.937	3.20	A
	C-liangyou 343	10.855	11.070	–1.98	
	Zhun S/187	10.472	10.766	–2.81	
	58S/2469	10.277*	11.290*	–9.86	B
	Guang S-1/P117	10.486*	11.069*	–5.56	
	Liangyoupeijiu	9.182*	10.388*	–13.13	

[a] N1 and N2 stand for the treatments with nitrogen purity of 90 kg ha^{-1} and 180 kg ha^{-1}, respectively. * and ** mean significance at $P<0.05$ and $P<0.01$, respectively.

(Zhun S/1243). In other words, the nitrogen-favoring type varieties should obtain high yield only with much higher nitrogen application. Therefore, lower-nitrogen-tolerant type varieties of super hybrid rice should be highly valued by plant breeders.

Nitrogen-saving effects through the application of humus fertilizer

Much research and many demonstrations have shown that some kinds of humus fertilizer, such as so-called Black Fertilizer (BF), have obvious effects of nitrogen savings (NS) in high-yielding rice cultivation. Quality hybrid rice varieties such as Fengyou 299 and T-you207 have been used in our experiments with six treatments and three replications: No BF + NS 25%, BF+ NS 25%, No BF + NS 15%, BF + NS 15%, No BF + Full N, and NF + Full N (here, No BF means applied no black fertilizer, NS 15% or 25% means minus 15% or 25% nitrogen of total amount of full nitrogen applica-

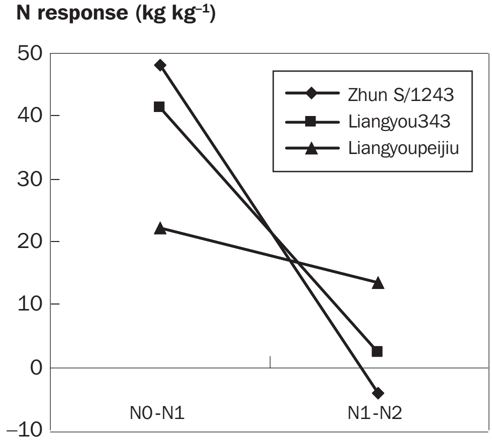

Fig. 2. Differences in nitrogen response between genotypes of super hybrid rice.

tion, and Full N means applying pure nitrogen at 180 kg ha^{-1}) in 2004 and 2005. The results showed (Table 2) that two hybrid rice varieties tested showed almost the same trends, that applied black fertilizer treatments (BF) under nitrogen-saving conditions (minus 15% to 25% of total nitrogen, NS 15% and NS 25%) significantly increased the yield over treatments with no applied black fertilizer (No BF) by the analysis of variance. Under 15% nitrogen savings, BF application yielded almost the same as the full nitrogen treatment (180 kg ha^{-1}), or even slightly higher under higher N conditions of full nitrogen treatment, but there was no obvious difference between applied BF or no BF under higher N conditions of full nitrogen treatment. Further analysis showed, under nitrogen-saving conditions (pure nitrogen at 153 kg ha^{-1} and 225 kg ha^{-1}), that the application of black fertilizer could increase effective panicles by 2.9–6.3% and produce 2–9.3% more spikelets per panicle and 0.5–2.9% more filled spikelets.

Nitrogen-saving effects through balanced fertilization

Following the characteristic of fertilizer absorption of super hybrid rice, we have developed a kind of technical materialized component fertilizer—lower N, suitable P, and higher K (12% N, 14% P, and 14% K)—based on balanced fertilization to adapt super high-yield hybrid rice cultivation. Our experiments have shown that this technical materialized component fertilizer could obtain more stable high yield under a lower amount of N (187.5 kg ha^{-1}), yielding 9.9–14.8% more than conventional fertilization with 15% less N fertilizer.

The results of an experiment with Liangyou 293, a super hybrid rice variety, shows (Table 3) that, under high nitrogen (treatment A7, total N = 206.25 kg ha^{-1}, total NPK = 497.25 kg ha^{-1}), it yielded a maximum of 10.41 t ha^{-1}, although this output is not the best and the ratio of output to cost is only 1:9.02 in the high-yielding rice-cropping area of Lilin in Hunan Province. Yield decreased by 0.6% only when

Table 3. Effects of nitrogen-savings of humus black fertilizer in quality hybrid rice.

Variety	Treatments	Pure nitrogen (kg ha^{-1})	Effective spikelets		Filled spikelets per panicle	Seed-setting rate (%)	1,000-grain weight (g)	Yield (t ha^{-1})	
			(Panicles m^{-2})	(per panicle)				Theoretical	Paddy[a]
Fengyou299	No BF + NS 25%	135	204.4	122.4	109.5	89.4	29.5	6.60	6.61 Cc
	BF + NS 25%	135	212.8	133.8	123.9	92.6	29.8	7.86	6.95 Bb
	No BF + NS 15%	153	214.0	139.4	121.7	87.3	29.3	7.63	6.92 Bb
	BF + NS 15%	153	227.5	136.9	126.0	92.0	28.8	8.26	7.30 Aa
	No BF + Full N	180	230.4	128.8	116.6	90.5	29.2	7.84	7.25 Aa
	NF + Full N	180	225.9	125.4	112.1	89.4	28.8	7.29	7.24 Aa
T-you207	No BF + NS 25%	135	233.8	124.1	113.8	91.7	26.7	7.11	6.76 Cc
	BF + NS 25%	135	240.6	128.2	121.3	94.6	26.5	7.73	7.08 Bb
	No BF + NS 15%	153	242.2	131.8	126.1	95.7	25.8	7.88	7.11 Bb
	BF + NS15%	153	254.4	134.5	128.4	95.5	26.7	8.73	7.47 Aa
	No BF + Full N	180	250.5	135.2	128.6	95.1	25.4	8.18	7.45 Aa
	NF + Full N	180	244.2	130.0	125.1	96.2	25.9	7.91	7.41 Aa

[a] $F_{Fengyou299} = 73.39$, $F_{T\text{-}you207} = 126.46$, $F_{0.05,\ 1.0} = 3.33$, $F_{0.01,\ 10} = 5.64$, $SE_{Fengyou299} = 2.087$, $SE_{T\text{-}you207} = 1.658$.

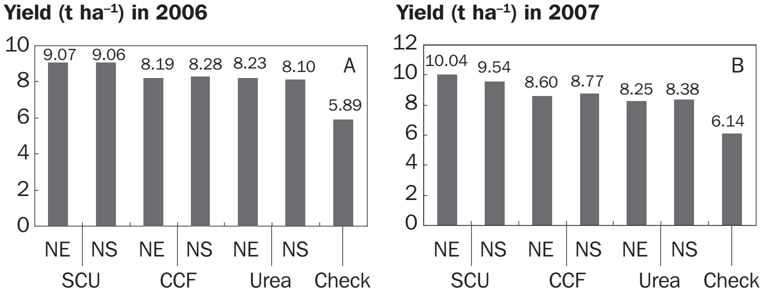

Fig. 3. Yield differences between slow-release fertilizer and nitrogen-saving treatments (NS = 135 kg ha^{-1}) and equal nitrogen (NE = 180 kg ha^{-1}) in 2006 (A) and 2007 (B). Note: SCU is sulfur-coated urea, CCF is coated component fertilizer, urea is a common nitrogen product, and the check is no N application.

minus 10% total nitrogen (A6, total N = 187.5 kg ha^{-1}) was used compared with the nitrogen amount of A7. In addition, with minus 30.9% of A7 total nitrogen amount (A3, total N = 142.5 kg ha^{-1}), the treatment A3 yielded only 7.76% less. The amount of nitrogen application even declined by 39.6% from A7 (A1, total N = 124.5 kg ha^{-1}), and yield decreased by only 12.1%. That result indicated that the application of this technical materialized component fertilizer could help obtain balanced fertilization nitrogen-savings, a lower total amount of nitrogen, high yield, and more profit.

The experiment also showed that Liangyou 293 did not always produce high yield under higher nitrogen application in districts such as Xiangtan and Xiangxiang in Hunan, which have medium-high yield in rice because of relatively poor soil conditions (Table 4). There were no significant differences in yield between treatments A1–A7 in Xiangxiang and Hunan, and A3 yielded higher than other treatments with only 142.5 kg ha^{-1} of total N application, 31.6% less than that of A6. Even with minus 50.6% of total N amount (A1), it yielded almost the same as A6. This indicates that the amount of nitrogen could be 135–180 kg ha^{-1} to achieve stable yield for super hybrid rice in medium-yield rice-cropping areas. In fact, the excessive nitrogen is not helpful for obtaining high yield, and a waste of nitrogen deteriorates the environment of the paddy field.

The nitrogen-saving effect of slow-release urea

The yield differences between slow-release fertilizers and nitrogen-saving treatment. Two kinds of slow-release fertilizer, SCU (sulfur-coated urea) and CCF (coated component fertilizer), were studied with common urea and no nitrogen application (CK) in two treatments, NE and NS (NE is equal N treatment at 180 kg ha^{-1}; NS is N-saving treatment at 135 kg ha^{-1}) in standing plots/fields of a 2-year continuous experiment in Changsha, China, in 2006 and 2007. The results (Fig. 3) showed that (1) SCU yielded much more than CCF and urea by 8.8–26.1%; (2) there was no significant difference between NE and NS for any kind of fertilizer, which indicates that the nitrogen-saving technique could be valuable in high-yielding cultivation.

Table 4. The effect of component fertilizer characterizing lower N, suitable P, and high K in 2004 and 2005 (variety: Liangyou 293, kg ha^{-1}).

Location	Treatment	A1	A2	A3	A4	A5	A6	A7	A8
	Total N	124.5	106.5	142.5	123.75	141.75	187.5	206.25	0
	Total NPK	334.5	274.5	394.5	291.75	351.75	440.1	497.25	0
Liling, Hunan	Yield	9.15	8.40	9.66	8.80	9.60	10.35	10.41	5.85
	Output to input	13.60	10.21	9.92	12.40	11.17	9.68	9.02	
Xiangxiang, Hunan	Yield	9.57	9.11	9.83	9.35	9.59	9.64	9.18	7.85
	Output to input	11.63	13.54	10.10	13.18	11.15	9.01	7.96	
Xiangtan, Hunan	Yield	8.51	8.36	8.95	8.39	8.78	8.99	8.78	6.18
	Output to input	10.33	12.41	9.20	11.82	10.21	8.47	8.92	

Effect on the activity of nitrate reductase (NR). Nitrate reductase is the key enzyme in the metabolism of nitrogen assimilation, as its activity can directly reflect the ability of plants to absorb nitrogen. We observed several results (Fig. 4): (1) Nitrate reductase activities of the rice leaf increased gradually from tillering stage to heading stage. (2) There were no significant differences at the end of the tillering stage between N applications, which indicated that SCU has a capacity to control and slowly release nitrogen at the early stage. (3) Up to booting stage, nitrate reductase activities increased to show higher nitrogen metabolism. Moreover, it is not conducive

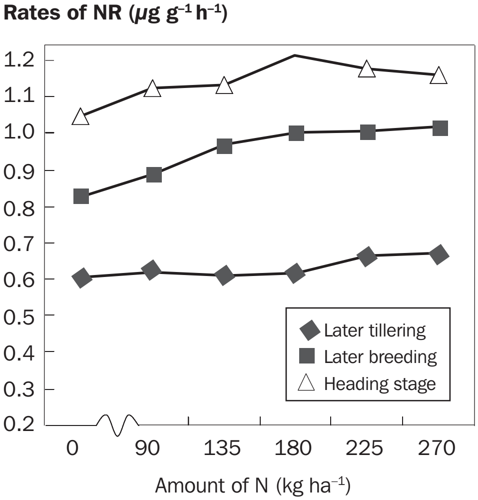

Fig. 4. Activities of NR in rice leaves at different nitrogen levels.

to transform rice from nitrogen metabolism to carbon metabolism. (4) After heading, NR activities are higher than in tillering stage and booting stage and NR activities increase when nitrogen increases. The value of NR was highest at 180 kg ha^{-1}, then decreased but was still high with an increase in N application. This means that SCU can steadily supply nitrogen at the middle and late stage, which accords basically with the demand trends of N absorption for the high-yielding cultivation of super hybrid rice, but this needs further research.

The effects of SCU nitrogen-saving cultivation on diseases and pests. The effects on diseases and pests by applying SCU nitrogen-saving cultivation have been observed (Table 5) and there is a positive correlation of the damage caused by the main rice pests and diseases with the amount of nitrogen: the more nitrogen, the more serious the damage. Whether the leaf-folding rate by leaffolder, number of planthoppers per 100 hills, or the susceptible plant rate and susceptible hill rate of sheath blight, they were higher as the amount of N increased, and there is a deadly turnaround point of pest damage from 90 to 135 kg ha^{-1}. Therefore, nitrogen-saving cultivation apparently reduces harmful pests for super hybrid rice cultivation.

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Table 5. The relationship between different N amounts and damage by main pests and diseases.

Amount of N (kg ha^{-1})	Leaf-folding rate by leaffolder (%)	Number of planthoppers per 100 hills	Sheath blight	
			Ratio of susceptible plants (%)	Ratio of susceptible hills (%)
0	0.89	60	0	0
90	4.44	440	8	20
135	8.22	1,640	16	20
180	10.44	1,620	27	40
225	15.33	2,180	35	40
270	26.00	2,480	45	40

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Notes

Author's address: China National Hybrid Rice R&D Center, Changsha 410125, Hunan, China.

Biomass accumulation and sink regulation in hybrid rice: consequences for breeding programs and crop management

Tanguy Lafarge, Crisanta Bueno, Estela Pasuquin, and Bancha Wiangsamut

The higher crop performance of rice hybrids over elite inbreds has been related in many studies to higher biomass accumulation and higher harvest index. Dissecting crop growth should reveal the basic traits underlying high hybrid performance and should have substantial implications for breeding programs and crop management practices. In a recent analysis in which hybrids and inbreds with the same crop duration were compared, higher biomass accumulation and more efficient biomass partitioning were observed during the entire crop growth and promising plant variables such as organ growth rate, specific culm length, sink strength index, and unfilled grain dry matter were identified. The same features were also observed in this paper when crop duration of hybrids was shorter than that of inbreds: in particular, organ growth rate and sink strength index were highlighted as key traits underlying hybrid performance. In addition, earlier cessation in tillering emergence observed with hybrids versus inbreds was another likely component supporting higher partitioning efficiency through an earlier increase in partitioning to culm. The variation in crop performance over distinct crop establishment practices had the same pattern for both plant types: (1) transplanting younger seedlings increased the grain yield of both hybrids and inbreds because of lower plant competition in the nursery; (2) adaptation to direct-seeding conditions was not observed among the studied genotypes possibly because of a lack of clump plasticity in response to uneven plant arrangement; and (3) the window for favorable sowing dates was the same for both plant types, highlighting the positive effect of cool temperature during the vegetative phase and high radiation during grain filling. Apart from nitrogen requirement, higher for hybrids because of higher biomass, the best cultural practices for inbreds in irrigated rice may be the same for hybrids. Improving these practices should reduce the yield gap in farmers' fields and promising traits underlying hybrid performance should help to improve breeding programs for yield potential.

Increasing global average rice yields by 12% over the next 10 years is of high priority in order to assure that rice production will meet the demand of a population that is projected to increase from 6 billion to 9 billion by 2050 (United Nations 2005). Rice demand is indeed increasing by around 5 million tons each year, which means that in 10 years the world will need to produce 50 million tons more than it does now. In the major rice-growing countries of Asia, however, yield growth over the past 5 to 6 years has been almost nil (IRRI 2008). And, cultivated land area for rice is decreasing because of ongoing economic development and the expanding use of land for biofuel and cash-crop production. One option with which rice science can have substantial impact in farmers' fields is to increase yield potential in irrigated areas. Superiority in grain yield of the best hybrids over elite inbreds has been reported in many studies in which standard heterosis observed under favorable conditions in breeders' fields was about 20% to 25% (Virmani et al 1982, Peng et al 2003). Identifying the traits of the hybrid phenotype leading to this higher performance would greatly help breeders in selecting their parental lines and improving their screening protocols in field trials and would have substantial impact on yield potential, for both hybrid rice and inbred lines.

Analyses aiming at comparing yield performance between the best hybrids and inbreds focused on yield and simple supporting growth and agronomic traits. Most analyses reported a higher crop growth rate during the vegetative phase and higher harvest index at maturity as the major factors supporting the greater yield of hybrids (Laza et al 2003, Katsura et al 2007). Some highlighted higher biomass accumulation during grain filling (Peng et al 1999). Others pointed out particular yield components as relevant traits (Ponnuthurai et al 1984, Yang et al 2007), whereas searching for desirable values of yield components appears irrational due to the great compensation among yield components and the diverse plant growth strategies underlying them. Although such traits appear accessible enough to be used easily in breeders' fields, by being strongly integrated, they may hide some more mechanical and basic processes that could improve the efficiency of breeding programs if included in the process. Conducting analyses that go deeper than these previous comparative studies and examine underlying traits supporting the major growth characteristics of interest should generate more targeted breeding activities relying on more relevant and less integrated traits.

Reducing the yield gap in farmers' fields is another key activity for which much benefit can be achieved in irrigated areas (IRRI 2008). This includes fields grown with both elite inbreds and hybrid rice. Considering the higher performance of hybrid rice, it is relevant to wonder whether crop management for hybrid rice should be different from that for inbreds. This inquiry is tackled in light of the key growth traits supporting the higher performance of hybrid rice and identified in this analysis. The relevance of promoting distinct practices to manage hybrids and inbreds in the field with respect to their growth characteristics was examined here by establishing several field trials designed to include a wide range of conditions and to minimize confounding effects.

Traits underlying hybrid rice performance

Previous studies aiming at understanding the factors involved in the higher performance of hybrids over inbreds did not consider the crop duration of these genotypes when comparing their ability to accumulate dry matter and the factors underlying growth and yield formation processes (Peng et al 1998, Laza et al 2001). Other studies clearly pointed out the large effect of distinct crop duration on final yield (Katsura et al 2007, Yang et al 2007). In a recent analysis in which high-yielding hybrids and inbreds with the same phenology were compared, Bueno and Lafarge (2009) reported that a higher accumulation of dry matter with hybrids was observed during the entire crop growth from sowing to maturity: the crop growth rate of hybrids was higher during each of the three crop phases, vegetative, reproductive, and ripening, as was the blade growth rate during the vegetative phase, the stem growth rate during the reproductive phase, and the panicle growth rate during the ripening phase. In addition, Lafarge and Bueno (2009) reported that more efficient dry matter partitioning among plant organs was also measured during each of the three crop phases: partitioning to culm at the end of the vegetative phase and at the beginning of the reproductive phase for hybrids was favored to the detriment of that to blade and sheath, partitioning to panicle at the end of the reproductive phase and at the beginning of the ripening phase was favored to the detriment of partitioning to the culm, and remobilization from the culm to the panicle during the ripening phase was higher for hybrids. Sink strength index (SSI) as an improved indicator compared with harvest index was then used to account for the efficiency of dry matter partitioning by integrating stem (or culm) vigor with the weight of the panicle. The value of SSI at maturity was substantially higher for hybrids than for inbreds whatever cropping season was considered, wet or dry. A series of key traits accounting for higher biomass accumulation and more efficient partitioning strategy, underlying the better hybrid rice performance of genotypes with the same crop duration, was clearly identified. It is then relevant to wonder whether or not the same kind of characteristics and traits would account for this if the crop duration of hybrids were shorter than that of inbreds.

Site description and crop management

This study was conducted at the lowland research farm of the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines (14°11′N, 121°15′E, 21 m elevation), and the data presented here were collected in the 2007 dry season (DS). The soil was classified as Andaqueptic Haplaquolls with particle size distribution of 58% clay, 33% silt, and 9% sand. Pregerminated seeds of seven rice genotypes, four hybrids, IR79118H (H6), IR68284H (H12), IR81958H (H13), and IR82386H (H14), and three elite inbreds, PSBRc80 (I4), IR76999-52-1-3-2 (I11), and IR77186-122-2-2-3 (I13), from IRRI breeding programs with high genetic diversity were sown in seedling trays at 3,000 seeds m^{-2}. Twelve-day-old (4-leaf stage) seedlings were pulled out from the trays and transplanted in 4 replications at hill spacing of 20 × 20 cm with one seedling per hill.

Phosphorus (50 kg P ha^{-1}), potassium (50 kg K ha^{-1}), and zinc (5 kg Zn ha^{-1}) were applied and incorporated in all plots a day before transplanting. Nitrogen was applied in the form of urea according to weekly SPAD readings (Peng et al 1996): 180 kg N ha^{-1} was applied in five splits (40 kg N ha^{-1} as basal, 20 kg at mid-tillering, 50 kg at maximum tillering, 40 kg at panicle initiation, and 30 kg at booting) in all plots. Fertilization was then managed optimally to avoid any deficiency and toxicity, as confirmed a posteriori with laboratory analyses. Seedlings in the nursery were grown under saturated moisture conditions to field capacity and the plants in the main field were maintained in flooded conditions from 2 days after transplanting until 10 days before physiological maturity. Weeds and pests were controlled by pesticide application and hand weeding was done when required.

Plant measurements

Panicle initiation (PI) was determined by observing the meristem under a binocular microscope. Flowering (Fl) was determined when an average of 50% of spikelets per panicle of half of the main tillers had exserted their anthers. Maturity was determined when 95% of the spikelets of the whole plot had turned from green to yellow. The seven genotypes under study were characterized with contrasting duration of each phenological phase: on average, hybrids reached panicle initiation 2 days earlier than inbreds (49 vs. 51 days after sowing, DAS), reached flowering 4 days earlier (75 vs. 79 DAS), and reached maturity 5 days earlier (106 vs. 111 DAS).

Total green and dead tillers were counted systematically from weekly plant samples and plants were separated into green leaf blades, dead leaf blades, leaf sheaths, culms (internodes and nodes), and panicles (including juvenile ones when present). Culm length was measured, the area of the green blades was determined using a leaf area meter, and leaf area index (LAI) was calculated. Dry matter of each entity, green and dead leaf blades, leaf sheaths, culms, and panicles, was determined by weighing the material after drying during 72 h in an oven at 70 °C. Stem dry matter was calculated as the sum of leaf sheath and culm dry matter. Specific leaf area (SLA, cm^2 g^{-1}) was calculated by dividing leaf blade area by the corresponding leaf blade dry matter. Total leaf blade dry matter was calculated as the sum of green and dead leaf blade dry matter and shoot dry matter as the sum of the dry matter of leaf blades, stems, and panicles. Crop growth rate (CGR, g m^{-2} d^{-1}) was calculated based on changes in shoot biomass accumulation per unit ground area over a time interval. Blade (BdGR), stem (StGR), and panicle growth rate (PaGR) were determined following the same procedure. Specific culm length (SCL, cm g^{-1}) was calculated by dividing the total culm length by the corresponding culm dry matter. Sink strength index (culm-based SSI), designed to integrate culm vigor with the weight of the panicle, was calculated as the product of panicle dry weight and specific culm length. At maturity, total tiller number with respect to productive and unproductive tillers, leaf blades, leaf sheaths, culm and panicle dry matter, total filled and unfilled grain weights, dry weight of 1,000 filled and unfilled grains, and, consequently, filled and unfilled grain number per panicle were determined. Stem length was measured from the top root to the highest collar and specific stem length (SSL) was computed as the ratio of stem length to its

Table 1. Grain yield (GY), biomass production at physiological maturity (PM), harvest index (HI), panicle number (PanNB), filled grain number (FiGrNB), and 1,000-seed weight for H6 (IR79118H), H12 (IR68284H), H13 (IR81958H), H14 (IR82386H), I4 (PSBRc80), I11 (IR76999-52-1-3-2), and I13 (IR77186-122-2-2-3) in the 2007 dry season.

Genotype	GY[a] (t ha^{-1})	Biomass at PM (g m^{-2})	HI	PanNB (m^{-2})	FiGrNB (pan^{-1})	1,000-seed wt (g)
Hybrid						
H6	10.80	2,093	0.54	310	145	23.97
H12	10.76	2,598	0.46	303	131	27.13
H13	10.70	2,149	0.52	290	134	27.48
H14	11.18	1,954	0.52	328	135	25.11
Mean	**10.86 a**	**2,205 a**	**0.51 a**	**308 b**	**136 a**	**25.92 a**
Inbred						
I4	10.06	1,904	0.52	338	124	23.98
I11	10.18	2,243	0.44	406	106	23.69
I13	9.86	1,905	0.51	369	121	22.05
Mean	**10.03 b**	**2,017 a**	**0.49 a**	**371 a**	**117 b**	**23.24 b**
LSD (0.05)	0.22	202	0.03	18.9	6.1	1.17

[a] Within columns, means of hybrids and inbreds followed by the same letter are not significantly different at the 5% level. LSD values are for the comparison of 8 genotypes.

corresponding stem dry matter. Sink strength index (SSI-stem based) was the product of panicle dry matter and specific stem length. Harvest index (HI) was computed as filled grain dry matter divided by shoot dry matter. Grain yield was calculated at 14% moisture content from the total filled grain dry matter of a 5-m^2 area. Data were analyzed following analysis of variance (SAS 1995) and means were compared based on the least significant difference test (LSD) at the 0.05 level of significance. Standard error was computed for each sampling date plotted in the graphs.

Results

Average grain yield of hybrids was significantly higher than that of inbreds by almost 1 t ha^{-1} even though the crop duration of inbreds was longer by 5 days (Table 1). Average shoot biomass and harvest index were not significantly different between hybrids and inbreds. The significantly lower number of panicles per unit area of hybrids (308 vs. 371) was compensated for by the higher number of filled grains per panicle (136 vs. 117). Overall, the number of total grains per unit area and the fertility rate were similar for both plant types (Table 2). The main component supporting the higher grain yield of hybrids was the individual grain size, for which 1,000 filled grain dry matter of hybrids, 25.9 g, was significantly higher than that of inbreds, 23.2 g (Table 1). The

Table 2. Total grain number (ToGrNB), grain-filling rate (Fi%), stem length (StL), specific stem length (SSL), and sink strength index (stem-based SSI) for H6 (IR79118H), H12 (IR68284H), H13 (IR81958H), H14 (IR82386H), I4 (PSBRc80), I11 (IR76999-52-1-3-2), and I13 (IR77186-122-2-2-3) in the 2007 dry season. The least significant difference test (LSD) at the 0.05 probability level is indicated.

Genotype	ToGrNB[a] (m^{-2})	Fi%	StL (cm)	SSL (cm g^{-1})	Stem-based SSI (g cm g^{-1})
Hybrid					
H6	62,490	0.80	87.7	50.6	213
H12	55,241	0.72	89.2	51.1	210
H13	48,396	0.72	82.3	46.9	198
H14	63,142	0.68	78.5	66.9	220
Mean	**57,317 a**	**0.73 a**	**82.7 a**	**54.0 b**	**210 a**
Inbred					
I4	57,274	0.77	82.0	63.1	201
I11	61,014	0.75	79.2	60.7	156
I13	59,698	0.73	76.4	65.6	159
Mean	**59,329 a**	**0.75 a**	**79.2 b**	**61.9 a**	**172 b**
LSD (0.05)	4,457	0.04	3.7	5.5	17.9

[a] Within columns, means of hybrids and inbreds followed by the same letter are not significantly different at the 5% level. LSD values are for the comparison of 8 genotypes.

value of specific stem length (SSL), corresponding to the reverse of stem vigor, was significantly lower for hybrids (54.0 vs. 63.1 cm g^{-1}), whereas stem length of hybrids was significantly higher (Table 2). Despite less vigorous stems, hybrid plants were able to bear heavier panicles. Hence, SSI, which was designed to account for the efficiency of dry matter partitioning more accurately than harvest index, was significantly higher for hybrids (210 g cm g^{-1}, stem-based SSI) than for inbreds (172 g cm g^{-1}).

Tillering and specific leaf area (SLA) dynamics of hybrids and inbreds were similar until the maximum tillering stage (Figs. 1A and 1C), whereas, at that time, values of leaf area index (LAI) of hybrids were significantly higher than those of inbreds, with values measured at 45 DAS of 2.3 vs. 1.7, respectively (Fig. 1B). This would indicate higher individual leaf area for hybrids. Tiller emergence of hybrids stopped earlier (Fig. 1A) and maximum tillering was then lower (540 tillers m^{-2}) than that of inbreds (610 tillers m^{-2}). Since about the same difference in tiller number was observed at maturity, values of tillering efficiency (productive to maximum tiller

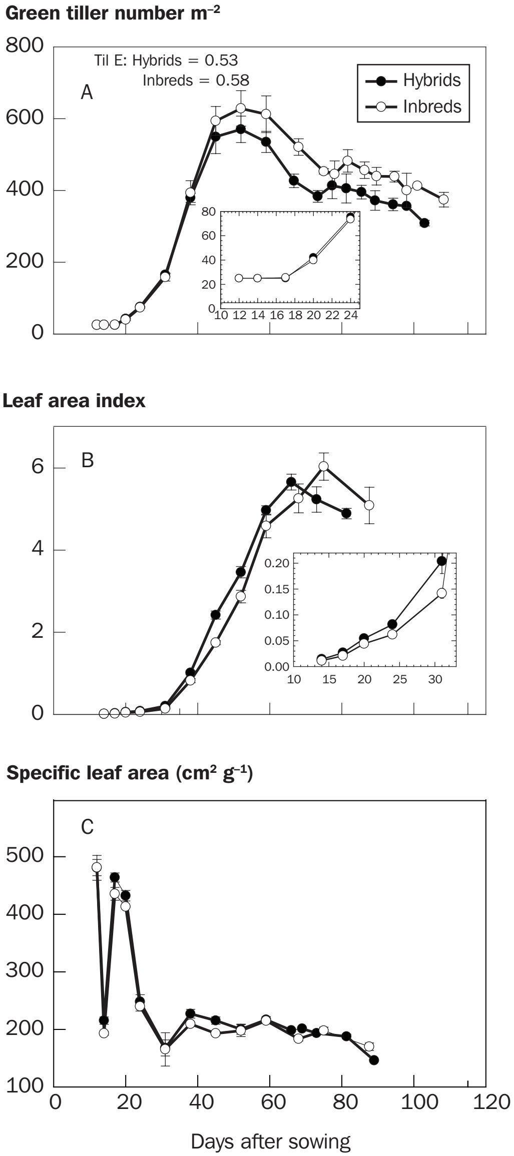

Fig. 1. Changes with time in green tiller number m^{-2} (A and inset), leaf area index (B and inset), and specific leaf area, cm^2 g^{-1} (C), of an average of 4 hybrids and 3 inbreds in the 2007 dry season. In A, values of tillering efficiency are indicated for hybrids and inbreds. Vertical lines represent the standard error of the mean of four replicates.

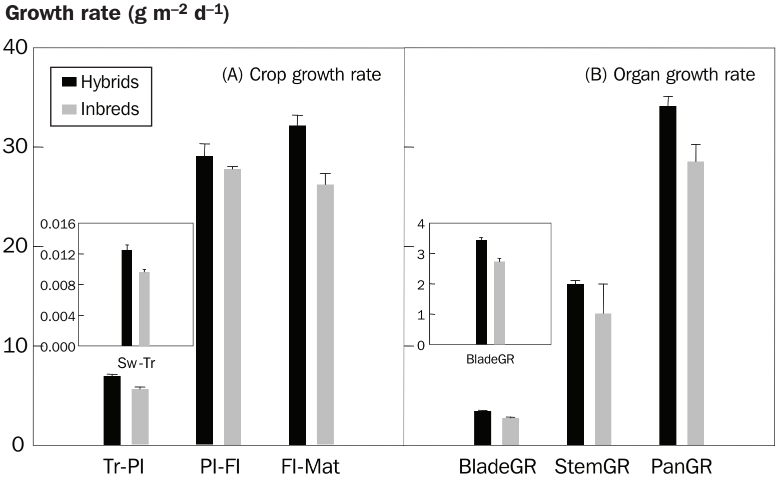

Fig. 2. Crop growth rate from sowing (Sw) to transplanting (Tr) (inset in A), from Tr to panicle initiation (PI), from PI to flowering (Fl), from Fl to maturity (A), and blade growth rate (BladeGR) from transplanting to maximum tillering, stem growth rate (StemGR) from maximum tillering to flowering, and panicle growth rate (PanGR) from flowering to harvest (B and inset) of an average of 4 hybrids and 3 inbreds in the 2007 dry season. Vertical lines represent the standard error of the mean of four replicates.

number) were similar for both plant types. A strong and transient decrease in SLA of both plant types was observed right after transplanting (Fig. 1C).

Crop growth rate (CGR) was substantially higher for hybrids than for inbreds during each crop phase (Fig. 2A) and even during the early time spent in the nursery (Fig. 2A inset). During each of these phases, the growth of the key organ was also faster for hybrids than for inbreds (Fig. 2b): the blade growth rate of hybrids was higher during the vegetative phase, stem growth rate was appreciably higher during the reproductive phase, and panicle growth rate was higher during the ripening phase. Sink strength index was also calculated with respect to culm characteristics (Fig. 3) in order to remove any side effect introduced in stem-based SSI by potential variability of sheath senescence across plant types. From shortly after panicle initiation when it was first calculated, culm-based SSI of hybrids increased faster than that of inbreds and the difference kept on increasing until maturity. The values at maturity were significantly higher for hybrids (398 g cm g^{-1}) than for inbreds (closer to 259 g cm g^{-1}, Fig. 3), and this confirmed the significant difference observed between values of stem-based SSI (Table 2).

Discussion

A yield advantage of hybrid rice over elite inbreds was observed here even if the crop duration of hybrids was substantially shorter than that of inbreds, by an average of 5 days with each growth phase affected, and even though the lowest yield of inbreds studied here was as high as 9.8 t ha^{-1}. The same features that finally resulted in a

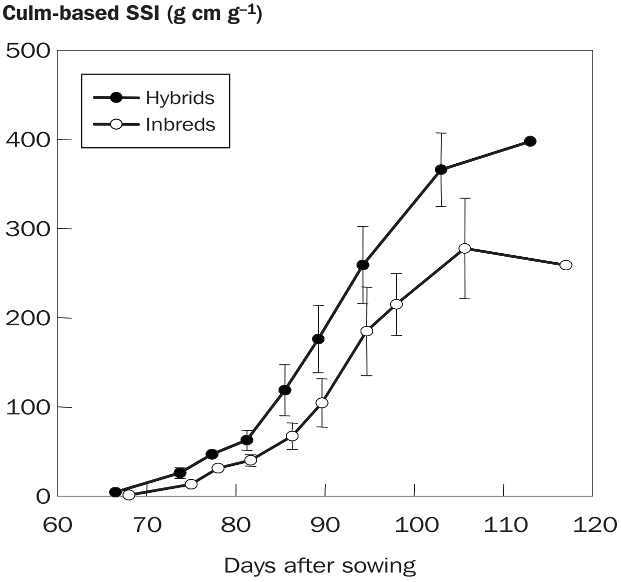

Fig. 3. Changes with time in culm-based sink strength index of hybrids and inbreds in the 2007 dry season. Vertical lines represent the standard error of the mean of four replicates.

yield advantage of hybrids over inbreds with the same crop duration were reported here (Bueno and Lafarge 2009, Lafarge and Bueno 2009). First, biomass accumulation of hybrids was faster than that of inbreds during each growth phase despite the variation in phase duration. In particular, hybrids were characterized by a higher crop growth rate in each phenological phase, and also a higher growth rate of the key organ of each phase. The gap in biomass accumulation between plant types was smaller, however, than that between plant types with the same crop duration. Second, more efficient partitioning of biomass among plant organs was observed with hybrids as sink strength index from panicle initiation onward was significantly higher than that of inbreds. The difference between plant types with stem-based SSI at maturity was even stronger in this study than that between plant types with the same crop duration, whereas that of harvest index was weak. This confirmed the use of SSI as a better factor than harvest index when accounting for plant ability to partition its biomass efficiently (Hay 1995). Interestingly, tiller emergence of hybrids stopped earlier than that of inbreds, at a similar LAI value close to 3. This early cessation of tillering was also observed when comparing plant types with the same crop duration during the wet season, but not during the dry season (Bueno and Lafarge 2009). This event could be one of the processes improving the overall efficiency of hybrids by inducing stronger biomass allocation to the culm at an earlier stage. A lower tiller production generated by an earlier cessation of tiller emergence rather than by a slower rate of tiller emergence as it was designed with the new plant type (NPT) (Dingkuhn et al

1991) could be a promising trait to include in breeding programs. This would also improve tillering efficiency and might reduce dry matter loss of nonproductive tillers (Berry et al 2003). Even though cessation of tillering is likely related to the status of the internal resources of the plant, it is still unclear how this event is controlled and whether the value of the red to far-red ratio inside the canopy is involved (Ballaré and Casal 2000). A high crop growth rate during the late reproductive phase, reported by others (Sheehy et al 2001, Horie et al 2003) as a key factor governing yield potential through the formation of sink size, was not substantially higher here with hybrids. Sink size was also similar between hybrids and inbreds in this study, in which higher grain yield of hybrids was supported by higher individual grain size. This is a rare case in which the higher performance of hybrids was not the consequence of higher sink size (Khush et al 1998, Yang et al 2007): this may be due to the longer crop duration of inbreds, which was compensated for by higher tiller production and higher panicle density at maturity. This indicates that higher sink size does not seem to be an absolute requirement for higher yield potential, in contrast to many studies (Horie et al 2003, Laza et al 2004). This supports the conclusion of Lafarge and Bueno (2009), who suggested that biomass accumulation and sink partitioning were the key variables for yield potential. With its better ability to account for the partitioning efficiency of the plant, SSI appears to be a promising tool to use for screening at heading and maturity in breeders' fields.

The general features of crop development of high-yielding hybrids and inbreds were similar at the early stage although crop growth was slightly higher with hybrids. (1) The early dynamics of tillering and LAI between plant types were alike, as were the dynamics of leaf number on the main tiller and plant height (Bueno and Lafarge 2009). (2) The values of SLA dropped dramatically for both plant types right after transplanting for a very short period of time. The higher yield potential of hybrids was due to an accumulation of improved elementary processes over time rather than to a remarkable and substantial phenotypic change. These observations do not argue for the adaptation of crop establishment practices to the specific characteristics of hybrid rice. This issue was tackled in a series of experiments presented below.

Relevant crop establishment practices for maximizing hybrid potential

The need to adapt crop management practices to hybrid rice characteristics has already been examined, but in very few studies. In particular, in comparison with the N requirement of inbreds, Peng et al (1998, 2003) reported that the N requirement of hybrids was higher to match with their higher biomass accumulation and this could be accounted for by adding 20 kg N ha^{-1} at the booting stage. No clear assessment has been conducted, however, on crop establishment strategies.

Nursery management

Seedling age at transplanting varied substantially in farmers' fields and, in most cases, farmers did transplanting with seedlings not younger than 20 days and sometimes as old as 40 days (De Datta 1981, Singh and Singh 1999). Overall results of previous studies were quite inconsistent and did not really identify proper guidelines for improved nursery management in order to reduce yield gaps in irrigated fields (Chandra and Manna 1988, Khatun et al 2002). In addition, high seedling vigor, as a response of transplanting young seedlings, had been reported to increase tiller production, but also tiller senescence, and that was assumed to have a negative effect on final crop performance through higher respiration and biomass loss (Schnier et al 1990, San-oh et al 2004). Recently, a study focused on the effect of contrasting nursery management on crop growth and final grain yield and yield attributes in transplanted rice (see details in Pasuquin et al 2008). One high-yielding hybrid, IR75217H (H1), and an elite inbred, IR72 (I1), were sown during the dry season and the wet season in four kinds of nursery, a classic wet-bed, a dapog, an improved mat nursery, and seedling trays, all set up at 3,000 seeds m^{-2} to minimize confounding effects. Seedlings were then transplanted at 7, 14, and 21 days after sowing (which corresponded to the 3-leaf, 5-leaf, and 7-leaf stage) in the main field with one seedling per hill at 20 × 20-cm spacing. Irrigation and fertilization were managed optimally in order to maintain favorable conditions. Plants were sampled weekly, live tillers were counted, and the area of green blades of all plants was measured using a leaf area meter. Grain yield and tillering efficiency (ratio of productive tillers to maximum tillers) were determined at maturity.

Tiller emergence and subsequent leaf area production were delayed in the dry season as seedling age at transplanting increased, which also delayed the accumulation of shoot dry matter (Fig. 4). These features applied to both inbreds (Fig. 4A and C) and hybrids (Fig. 4B and D) and to both the dry and wet seasons (only the data from the dry season are presented here). In addition, the different kinds of nursery did not cause any significant difference in final crop performance (see Pasuquin et al 2008). Grain yield increased significantly in response to younger seedling age at transplanting in both the dry and wet season and even up to 1 t ha^{-1} in the dry season (Table 3). At the same time, tillering efficiency (TilE) decreased significantly, which indicates that high tiller production at the early stage was more crucial for high yield than low tiller senescence. These effects of seedling age on final crop performance were similar for both genotypes and did not highlight any specific requirement in nursery management with respect to plant type. These findings supported the transplanting of young seedlings as a promising technique for both plant types to reduce the yield gap. The relevance of tillering efficiency as a breeding trait used to increase yield potential as was done with the NPT was even questioned (Dingkuhn et al 1991).

Direct seeding and clumping

Crop growth of high-yielding hybrids and inbreds was similar at the early stage (as observed with tillering, LAI, and SLA) and was affected similarly by a range in nursery management (contrasting nursery types and seedling age at transplanting). Among these high-yielding genotypes, those adapted to uneven plant arrangements in the field (broadcasting and row seeding in comparison with transplanting) and to low hill density in transplanted fields should have the ability to adapt their plant stand to the distribution of incoming radiation in order to maximize access to light (Khush et al 1998). This ability was evaluated by setting up a transplanted field experiment in which

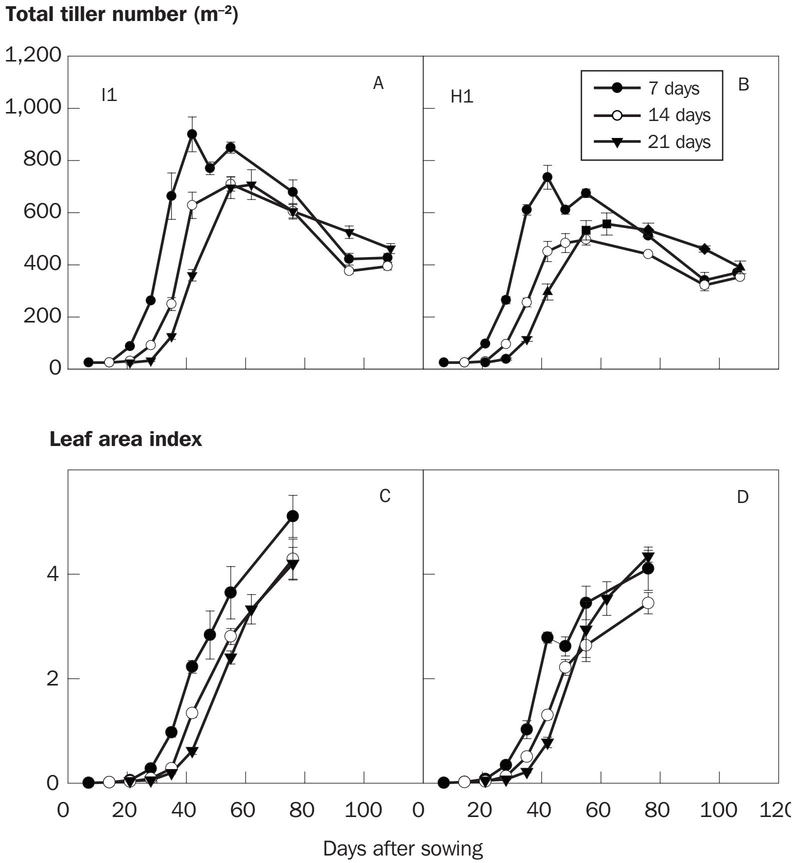

Fig. 4. Changes in total tiller number m^{-2} (A and B) and leaf area index (C and D) of I1 (IR72) and H1 (IR75217H) as affected by seedling age at transplanting in the 2003 dry season. Vertical lines represent the standard error of the mean of four replicates (adapted from Pasuquin et al 2008).

hill spacing was fixed at 30 cm between rows and 10 cm between hills inside the row: free space around each hill was thus variable, with plant competition expected to be three times greater inside rows than across rows. Six high-yielding hybrids and inbreds, IR8 (I0), IR72 (I1), IR64 (I2), IR75217H (H1), IR78386H (H5), and IR79118H (H6), were sown on 2 January 2006 in seedling trays and 4-leaf-stage (10-day-old) seedlings were transplanted at 30 × 10-cm hill spacing with a single seedling per hill. Irrigation and fertilization were managed optimally in order to maintain favorable conditions throughout crop growth. Any clump (plant) could be represented as a reverse cone with the plant stubble as the tip of the cone, and the virtual area of the horizontal section delimited by the outside tillers of the plant at the height of the highest collar as the base area of the cone. The diameters of this section both in the direction of the 30-cm

Table 3. Variation in grain yield (t ha^{-1}) and in tillering efficiency (TilE) with respect to seedling age at transplanting for I1 (IR72) and H1 (IR75217H) during the dry and wet seasons of 2003 on the IRRI farm.

Dry season				Wet season			
Genotype	Seedling age at transplanting (d)	Yield (t ha^{-1})	TilE	Genotype	Seedling age at transplanting (d)	Yield (t ha^{-1})	TilE
I1	7	6.99 a	0.47 b	I1	7	5.32 a	0.48 b
I1	14	6.34 a	0.56 ab	I1	14	5.14 b	0.50 b
I1	21	6.06 b	0.59 a	I1	21	5.18 b	0.57 a
H1	7	7.75 a	0.50 b	H1	7	6.62 a	0.59 b
H1	14	6.98 b	0.68 a	H1	14	6.02 b	0.61 b
H1	21	6.97 b	0.61 ab	H1	21	5.89 b	0.82 a

[a]Within a column for each genotype, means followed by the same letter are not significantly different at the 5% level. Source: Pasuquin et al (2008).

hill spacing (across rows) and in the direction of the 10-cm hill spacing (between hills inside the row) were measured weekly with a simple ruler between the two extreme tillers of the clump in each of the two directions.

For all genotypes under study, the clump diameter in the 10-cm spacing direction increased with time from 6 to 13 cm until about 50 DAS, and then again up to 19 cm from 70 to 85 DAS (Fig. 5A). No significant difference in clump diameter dynamics was observed across genotypes. It is notable that this clump diameter reached the size of the distance between hills (10 cm) as early as 35 DAS, whereas no genotype was able to adapt to competition by minimizing its diameter. The clump diameter in the 30-cm spacing direction also increased with time for all genotypes up to 16 cm measured at 50 DAS, and then again up to 23 cm from 65 to 85 DAS (Fig. 5B). This clump diameter, however, had not yet reached the size of the distance between rows (30 cm) at 85 DAS. No significant difference was observed in the ability of each of these genotypes to expand their tiller growth preferably in the 30-cm spacing direction despite the large plasticity in culm structure (Islam et al 2007) and in culm growth in response to light availability in plants (Ballaré et al 1989). In fact, the difference between both diameters was minimal for all plants, whereas the hill spacing in both directions was 3 times different.

None of the plants observed here seemed to have a better ability to modify their clump morphology and to arrange their tiller angle so that leaf area would be preferably assigned to surrounding empty spaces under uneven plant distribution and contrasting plant competition. This indicates that the trend of variation in crop performance with contrasting crop establishment techniques (arrangements) would be similar across genotypes, as long as all other crop management practices were the same. This was evaluated when comparing the crop performance of the hybrid Magat (H4) with IR72

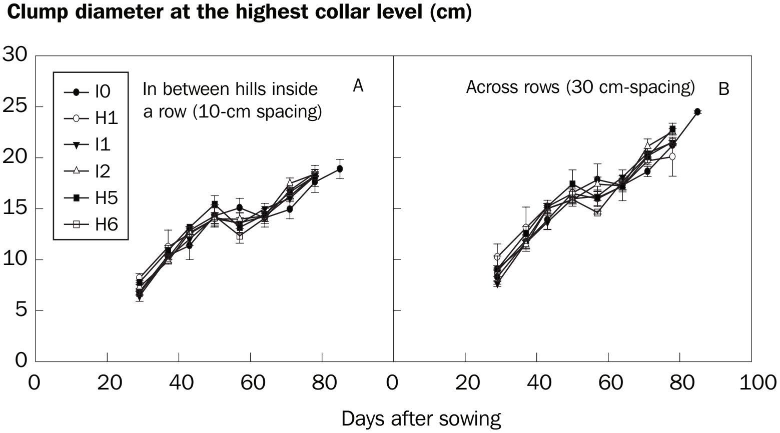

Fig. 5. Changes in clump diameter of the hill at the highest collar level (cm) between hills inside the row in a 10-cm hill spacing (A) and across rows in a 30-cm hill spacing (B) of I0 (IR8), H1 (IR75217H), I1 (IR72), I2 (IR64), H5 (IR78386H), and H6 (IR79118H) during the dry season of 2006. Vertical lines represent the standard error of the mean of four replicates.

(I1) and PSBRc80 (I4) grown in transplanted fields at 100 plants m^{-2} (TP100) and in row-seeded fields at 80 kg seeds ha^{-1} (SR80, 320 seeds m^{-2}). Seeds were sown at IRRI on the same day, 28 June 2004, in both the nursery and the row-seeded field to avoid any contrasting effect of weather conditions. The trend in grain yield variation with crop establishment was similar across genotypes, with grain yield being higher by more than 1 t ha^{-1} in SR80 than in TP100 (Table 4). To avoid any confounding effect of contrasting plant density on crop growth, another experiment was set up in which transplanting density was 150 plants m^{-2} (TP150, hill spacing of 20 × 20 cm with 3 seedlings per hill) and seed rate for direct seeding was 200 seeds m^{-2} (SB50, 50 kg seeds ha^{-1}), with a germination rate of 75–80%. Seeds of one hybrid, IR75217H (H1), and two inbreds, IR72 (I1) and PSBRc82 (I3), were sown on 14 June 2005 at IRRI in both the nursery and the broadcast field and 10-day-old seedlings were transplanted. Grain yield in TP150 was significantly higher than in SB50 for all genotypes (Table 5). Plant arrangement and density were also compared in direct-seeded fields for two genotypes, H1 and I1, using three establishment techniques, hill sowing (HS), row seeding (SR), and broadcasting (SB), and two contrasting seed rates, 25 and 50 kg seeds ha^{-1} for hybrids and 50 and 100 kg seeds ha^{-1} for inbreds. All seeds were sown on 15 January 2004 at PhilRice, Philippines. There was an appreciable difference in grain yield of H1 compared with I1 (Table 6); however, the variation in grain yield across establishment techniques was nonsignificant for both genotypes as it was for variation across seed rate. These series of results confirmed that none of the hybrids and inbreds tested here were adapted to uneven plant arrangement canopies and this emphasized the strong need to develop breeding programs aiming at selecting rice genotypes suitable for direct-seeding conditions.

Table 4. Variation in grain yield (t ha^{-1}) with respect to crop establishment practices for I4 (PSBRc80), I1 (IR72), and H4 (Magat) during the wet season of 2004 on the IRRI farm.

Genotype	Treatment	Yield (t ha^{-1})
I4	TP100	6.89 b
I4	SR80	8.65 a
I1	TP100	6.60 b
I1	SR80	7.84 a
H4	TP100	6.93 b
H4	SR80	8.04 ab

[a] Within a column for each genotype, means followed by the same letter are not significantly different at the 5% level.

Table 5. Variation in grain yield (t ha^{-1}) with respect to crop establishment practices for H1 (IR75217H), I1 (IR72), and I3 (PSBRc82) during the wet season of 2005 on the IRRI farm.

Genotype	Treatment	Yield (t ha^{-1})
H1	TP150	7.68 a
H1	SB50	5.90 b
I1	TP150	6.41 a
I1	SB50	5.66 b
I3	TP150	6.89 a
I3	SB50	6.10 b

[a] Within a column for each genotype, means followed by the same letter are not significantly different at the 5% level.

Table 6. Variation in grain yield (t ha^{-1}) with respect to crop establishment practices for H1 (IR75217H) and I1 (IR72) during the dry season of 2004 on the PhilRice farm.

Genotype	Treatment	Yield (t ha^{-1})
H1	HS50	8.25 a
H1	SB25	8.19 a
H1	SB50	7.63 a
H1	SR25	8.03 a
H1	SR50	7.71 a
I1	HS50	6.70 a
I1	SB50	6.72 a
I1	SB100	6.85 a
I1	SR50	6.71 a
I1	SR100	7.08 a

[a] Within a column for each genotype, means followed by the same letter are not significantly different at the 5% level.

Sowing date and effects of weather conditions

Weather conditions basically drive biomass accumulation through light interception (supply) and plant growth through local temperature (demand). Although the crop performance of hybrids and inbreds is different under given weather conditions, some of these conditions could create more variation in the respective performance than others (Sheehy et al 2001, Peng et al 2003). The ability of one genotype to accumulate or partition biomass may be affected by given weather conditions differently than others (Ainsworth et al 2004) and may create discrepancies in the pattern of variation of their performance over time. This could signify that some sowing dates are not recommendable for particular genotypes. Two hybrids, IR75217H (H1) and IR78386H (H5), and two inbreds, IR72 (I1) and IR64 (I2), were sown over nine contrasting dates every second week from 19 December 2005 (TP1) until 10 April 2006 (TP9) in seedling trays and 4-leaf-stage (10-day-old) seedlings were transplanted at 20 × 20-cm hill spacing. Irrigation and fertilization were managed optimally to maintain favorable conditions throughout crop growth. Solar radiation and daily minimum and maximum temperature were collected from the IRRI lowland agro-meteorological station within a 500-m radius from the experimental field. Grain yield was calculated at maturity, with 14% moisture content.

The pattern of variation in grain yield with sowing date was similar across genotypes (Fig. 6A), even though the grain yield of hybrids (from 7 to 10 t ha^{-1} for

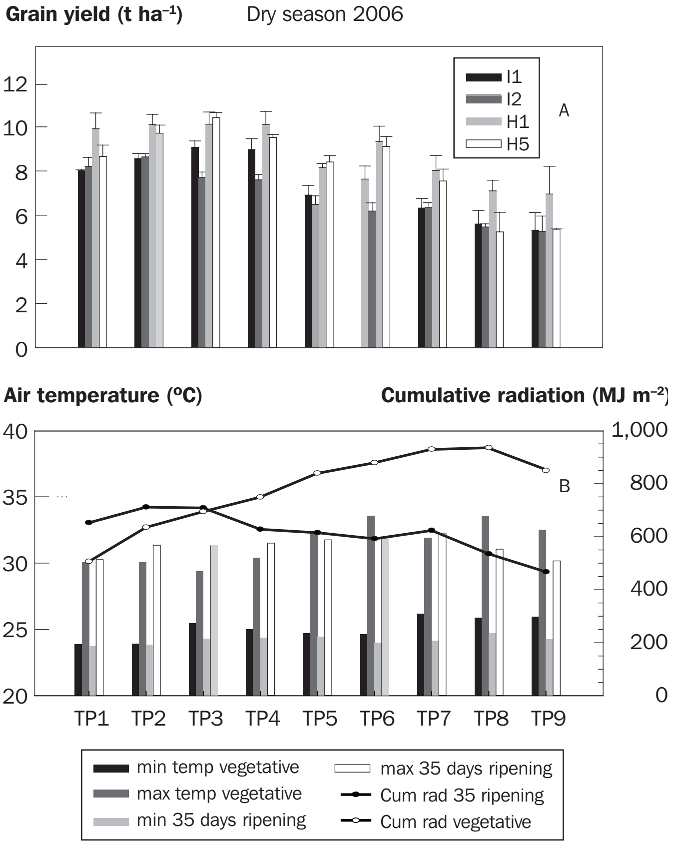

Fig 6. Grain yield of I1 (IR72), I2 (IR64), H1 (IR75217H), and H5 (IR78386H) as affected by transplanting (TP) dates (A) and changes in average minimal and maximal temperature, and in cumulative radiation, during the vegetative and ripening phases of each crop (B). Vertical lines represent the standard error of the mean of four replicates.

H1) was substantially higher than that of inbreds (from 5.5 to 8.5 t ha^{-1} for I2): yields of three genotypes increased slightly from TP1 to TP3 and the highest yields were calculated with TP3 for three genotypes, but with TP2 for I2. Yields of all genotypes then decreased from TP3 to TP9, despite a transient increase with TP6, except for I2, and the lowest values were observed for TP8 to TP9 (Fig. 6A). It is noteworthy that I2 was partially affected by tungro during crop growth. The grain-filling rate of late sowings was most probably limited by the reduction in cumulative daily radiation that decreased from 700 MJ m^{-2} for early sowings to 500 MJ m^{-2} or less for late sowings (Fig. 6B). The minimum and maximum temperatures did not vary substantially across growing periods. During the vegetative phase, even though cumulative daily radiation increased from 500 MJ m^{-2} for early sowings to more than 900 MJ m^{-2} for late sowings, minimum temperature increased from about 24 to 26 °C and maximum temperature from 30 to 33 °C with delayed sowings (Fig. 6B). This increase in temperature might have increased biomass losses (higher night temperature) through respiration (Peng et al 2003) and reduced crop growth (higher day temperature) because of detrimental values for organ elongation rate (Lafarge, unpublished). In addition, climatic demand was higher with late sowings because of the combination of higher day temperature and lower relative humidity (data not presented), which may have reduced stomatal aperture. These weather conditions were contrasting enough to provide a significant response of crop performance across sowing dates: they were more favorable for early sowings, when lower temperature during the vegetative phase favored leaf area expansion and high radiation during the ripening phase favored grain filling. However, the similarity of the pattern of variation in crop performance across treatments observed here between hybrid and inbred genotypes suggested that favorable weather conditions for IRRI inbreds are also favorable for IRRI hybrids.

Conclusions

The superiority of high-yielding hybrids over inbreds bred at IRRI was supported by a better ability of the hybrids for both biomass accumulation and efficiency in biomass partitioning. This was observed during the overall crop cycle, not only for genotypes with the same phenology and crop duration but also in the case of crop duration of elite inbreds, which is substantially longer than that of hybrids. Peng et al (1998, 2003) reported a higher N demand of hybrids in order to meet their higher yield potential, leading to contrasting N management for hybrids and inbreds. It is highly probable that this higher demand is the consequence of the higher biomass accumulation during crop growth and the higher biomass remobilization during grain filling as reported in this paper. Although other management practices must be sound in order to obtain the best performance of hybrids and inbreds, establishment practices can be similar for both despite the contrast in their overall crop performance, and improved practices adapted to inbreds (Horie et al 2003**)** should also be applied to hybrids. Low seedling age at transplanting appeared to be an easy and promising technique to reduce any yield gap. This needs to be implemented together with optional management practices to overcome a possible increase in pest occurrence such as weeds and snails. The window for the optimum sowing date in the field conditions at IRRI appeared to be relatively large, about 1 month long, outside of which crop performance was gradually affected, whereas hill density did not seem to have a significant effect, thus supporting a general effort to reduce seed rates, particularly under direct-seeding conditions. In the same way, none of the high-yielding hybrids and inbreds evaluated here appeared to be adapted intentionally to direct-seeding conditions, such as having a particular ability to adapt their clump architecture to the surrounding uneven canopy. All genotypes evaluated here were bred under transplanted conditions and therefore were not expected to be characterized by a direct-seeding adaptation pattern. In most cases, the best genotypes selected under transplanted conditions are also the same grown under direct-seeding conditions. Developing a breeding program entirely devoted to uneven plant arrangements in the field should have a significant impact on the delivery of promising genotypes suitable to direct-seeding conditions. Some valuable germplasm expressing promising traits could be precursors of an enhanced breeding program for yield potential and for uneven plant arrangement.

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Authors' addresses: Tanguy Lafarge, Crisanta Bueno, Estela Pasuquin, Bancha Wiangsamut, Crop and Environmental Sciences Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines; Tanguy Lafarge, Centre International de Recherche Agronomique pour le Développement, UPR Adaptation Agroécologique et Innovation Variétale, Montpellier, F-34398, France; Estela Pasuquin, School of Agriculture and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW, Australia 2678; email: tanguy.lafarge@cirad.fr.

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An analysis of the yield gap of hybrid rice in farmers' fields

Zhang Yuping, Zhu Defeng, Chen Huizhe, and Lin Xianqing

Planting area of hybrid rice in China has been increasing since the 1970s. Now, it covers more than 50% of the total national rice-planting area. Hybrid rice gives about 20% more yield than inbred rice in farmers' fields to contribute to the continuous increase in the yield of rice in China. However, a yield gap of hybrid rice is common among farmers' fields and demonstration fields guided by rice experts in hybrid rice-growing regions. This study describes the main factors that result in a yield gap of hybrid rice in farmers' fields for the development of corresponding management practices.

More than 100 farmers' fields planted with hybrid rice with various yields in early rice, late rice, and single rice were targeted for a survey. Yield and its components and a series of management practices were investigated. Through comparison and analysis, results are as follows. (1) The yield gap mostly ranges from 10% to 50% between farmers' fields in the same location, season, and hybrid combination. (2) For yield components, the yield gap mainly results from panicle size and spikelet fertility. However, in some transplanted hybrid rice, insufficient panicle number also results in lower yield due to insufficient basic seedlings, poor seedlings, and less growth in the early stage. And, in direct-seeded hybrid rice, too many panicles caused by a high seed rate is the main factor reducing yield. (3) For management practices, the main factors causing small panicles of hybrid rice are poor seedlings resulting from a high seed rate, incorrect water and fertilizer management, unsuitable pest control, and longer seedling duration in the seedbed. In the main field, hybrid rice overgrows in the early stage. That results in poor plant type, which causes low spikelet fertility and small panicles due to strong competition.

In addition, optimum population and ideal plant type of hybrid rice are important for bridging the yield gap among farmers' fields.

Keywords: hybrid rice, yield gap, yield components, cultivation technology

Rice is China's most important food crop. Rice varietal improvement during the 1950s in China led to the release of semidwarf indica, nitrogen-responsive, and lodging-resistant varieties available for commercial use in farmers' fields (Cheng et al 2007). In the 1960s, the release of semidwarf rice varieties in tropical regions caused the Green Revolution in many rice-producing countries, which enabled global rice production to meet the demand of the world's increasing population (Yuan Longping and Wu Xiaojin 2004).

At the end of the 1970s, China started to release hybrid rice for commercial use. Hybrid rice varieties have a yield advantage of about 15–20% over improved inbred varieties. Hybrid rice now covers about 50% of the total national rice-planting area in China. It is also used outside of China (Yuan Longping and Wu Xiaojin 2004). Semidwarf inbred varieties and hybrid rice achieved a continuous increase in yield in China from the 1960s to 1990s. The successful experience with semidwarf varieties and hybrid applications in farmers' fields indicated that a new generation of rice varieties needs supporting cultivation technology to obtain potential yield in farmers' fields.

With the progress of rice breeding technology in recent decades, the number of rice varieties, especially hybrids, released and used in farmers' fields increased and varietal renewal has been accelerating every year (Yang Shihua et al 2006). Unfortunately, from the end of the 1990s, average national rice yield stagnated. Though new varieties and hybrids have high yield potential and also perform much better than old ones in experimental fields, farmers' yield does not increase with new varieties and hybrids (Zhang Yuping et al 2006, 2008, Fang Fuping et al 2004). It seems that yield potential of new varieties and hybrids is not exploited at the farmer level. With new varieties and hybrids and newly developed supporting cultivation technology, rice yield in demonstration fields guided by experts is very high in different locations in the main rice-growing area (Zhu 2004, Cheng et al 2007, Yuan and Wu 2004). There are big differences in yield among rice fields and farmers even with the same hybrids (Chen et al 2004, Cheng Zaiquan et al 1997, Fu Haowei et al 2006). That yield gap implies that there is a possibility to raise average yield if we can overcome the yield gap and practice suitable supporting cultivation technology with hybrid rice.

Therefore, we try to analyze the yield gap and find factors that explain it through a survey of yield components and cultivation technologies under different yields in farmers' fields. According to the limitations to yield and factors causing a yield gap, suitable rice production technology is proposed to bridge the exploitable yield gap.

Materials and methods

Survey

A survey of the rice yield gap was done in the main rice seasons, early rice and late rice in the double-cropping rice system and single rice, in Zhejiang Province in 2007 and 2008. Three locations (Qujiang, Yuyao, and Ruian) for early rice and for late rice, and four locations (Tiantai, Fuyang, Xinchang, and Xianju) for single rice were selected. In each location and rice season, widely used hybrids were selected for the survey.

Survey fields with different yield under the same plant type and the same hybrid were determined after the evaluation of rice growth and yield and interviews with field owners in each season. Then, three representative sample locations for transplanted rice or 10 sample locations for direct-seeded rice and seedling-throwing rice in each survey field were selected after visual evaluation of the representative yield of survey fields for measuring yield and yield components.

Measurement of yield and its components

About 40 to 60 hills for transplanted rice and a 0.6 m × 0.6 m area for direct-seeded rice and seedling-throwing rice were determined to measure yield and its components. Before the rice harvest, plant density was measured and panicle number counted in the area. After that, rice in the area was harvested and threshed. At the same time, four representative hills or six representative plants were taken according to average panicle number per hill or plant, plant growth, and plant height near the sample area for estimating spikelet fertility. Spikelet fertility is used as an estimate at one of the sample locations (sites). Yield of the harvested area was weighed after cleaning and air drying. A grain sample is taken from the harvested yield to measure grain weight. Yield was calculated on the basis of 14% grain moisture content. Filled grain number at the sample location is calculated based on yield, panicle number, and grain weight.

Data analysis

Data were analyzed using MS Excel. The contribution of yield components to a yield increase or decrease between different yields is calculated according to the method developed by Zhu (2003).

Results

Yield gap in different rice seasons

Late rice. Yield and its components were measured using the same methodology. Also, all panicles are counted, grain weight is taken from harvested yield, and filled grain number per panicle is estimated based on those parameters from the same sample. So, real harvest yield is equal to theoretical yield. That makes it realistic to analyze and compare the contribution of yield components to yield.

In the late rice season, 65 sample sites were taken with three hybrids, Zhongyou 838, II You 8220, and Yongyou 1.

From the yield distribution frequencies of late rice (Fig. 1), the survey location rice yield was minimum at 4.18 t ha^{-1} and the maximum was 10.65 t ha^{-1}, with differences in yield of 6.47 t ha^{-1}. Production is distributed, ranging from 6.00 to 8.25 t, accounting for 76.9% of the surveyed area.

Zhongyou 838 yielded the highest, at 7.31 t ha^{-1}, and next was II You 8220 (6.60 t ha^{-1}), and last was Yongyou 1 (6.06 t ha^{-1}) (Table 1). The difference between the highest yield and the lowest yield among varieties was 1.25 t ha^{-1}.

The cause analysis and technical countermeasures of the late-rice yield gap showed that, when comparing Zhongyou 838 and II 8220, the yield increase mainly

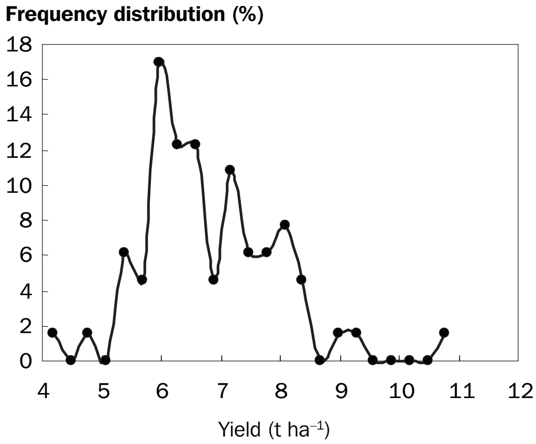

Fig. 1. Yield frequency distribution of late rice.

Table 1. Late-rice yield and yield components (the average values of sampling locations).

Variety	Density ($\times 10^4$ ha^{-1})	Yield (t ha^{-1})	Grains per panicle (no.)	1,000-grain weight (g)	Average panicles per hill	Effective panicles (no.$\times 10^4$ ha^{-1})
Zhongyou 838	19.35	7.31	98.0	30.7	12.8	16.43
Ilyou 8220	19.65	6.60	94.5	27.6	13.0	17.08
Yongyou 1	24.00	6.06	86.5	27.7	11.0	17.14

came from grain weight increasing, reaching 102%, but reaching 35% by grain number increasing, reaching –37.3% by effective panicles increasing. When comparing Zhongyou 838 and Yongyou 1, the yield increase mainly came from grain weight increasing, too, reaching 55%, but reaching 67.3% by grain number increasing, and –22.9% by effective panicles increasing. So, for these three late-rice varieties, the yield gap mainly came from grain number and grain weight. When effective panicles surpassed 2.55 million, the output was mainly associated with grain number per panicle and the correlation coefficient was 0.8534 (Fig. 3). To ensure continuous increases in rice production, it is necessary to reduce differences in yield among different farmers by ensuring effective panicles and improved large panicles. We need to choose suitable varieties and a density of 210,000–240,000, through a rational application of fertilizer and water management, to improve spikelet rate and increase grain number. If the yield of throwing-seedling Ning 03-88 reaches 6.75 t ha^{-1}, effective panicles need to reach 3.45 million and grain number per panicle should surpass 65.

Single rice. In the single-rice season, 22 sample sites were used with one hybrid, Liangyoupeijiu. The yield gap of Liangyoupeijiu at two locations in a single season

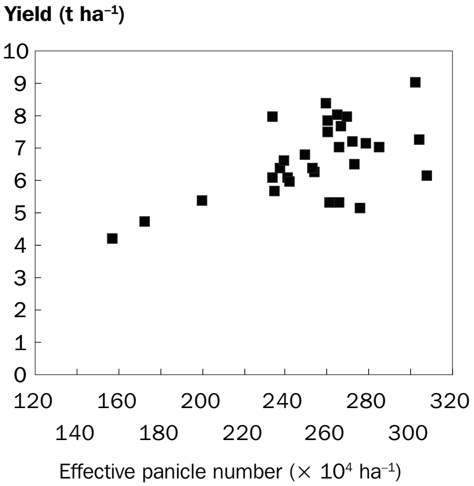

Fig. 2. Relation between panicle number and yield in late rice.

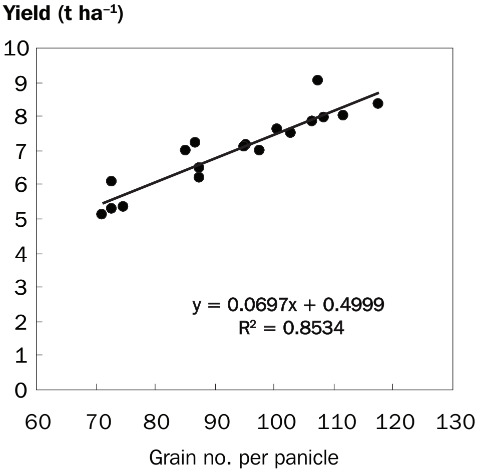

Fig. 3. Relation between grain number and yield in late rice.

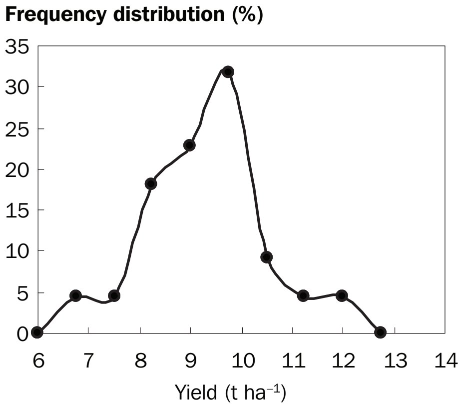

Fig. 4. Yield frequency distribution of Liangyoupeijiu (22 sampling locations).

Table 2. Yield and its components for hybrid Liangyoupeijiu in different fields.

Item	Yield (t ha^{-1})	Panicles (no. m^{-2})	Grains (no. $panicle^{-1}$)	1,000-grain weight (g)
Minimum	6.0	205.3	113.2	25.3
Maximum	12.1	287.5	187.8	26.2
Difference	6.1	82.2	74.6	0.9
Average	8.9	246.3	140.6	25.8
S.D.	1.3	22.0	18.1	0.2

is 6.05 t ha^{-1}. Some 85% of the yield is in the range of 7.6 to 10.3 t ha^{-1} (Fig. 4). For yield, the difference in panicle number per m^2 ranges from 205.3 to 287.5, filled grain number per panicle from 113.2 to 187.8, and 1,000-grain weight from 25.3 to 26.2 g. The CV of panicle number, grain number, and grain weight is 22.0%, 18.1%, and 0.2%, respectively (Table 2). For yield, grain weight is more stable and more variation comes from panicle number per unit and filled grain number per panicle.

When panicle number per m^2 is less than 240 and yield is also relatively low, yield is positively related to panicle number. After that, no relationship between both exists. However, filled grain number is positively related to yield within the range of filled grain number (Fig. 5).

Average yield components were calculated by yield class after grouping the samples based on the yield range (Table 3). Grain weight does not vary much among three yield classes. From the low-yield class to middle-yield class, panicle number and grain number increase 10.8% and 14.3%, respectively. From the middle-yield class to high-yield class, panicle number and grain number increase 3.5% and 17.6%,

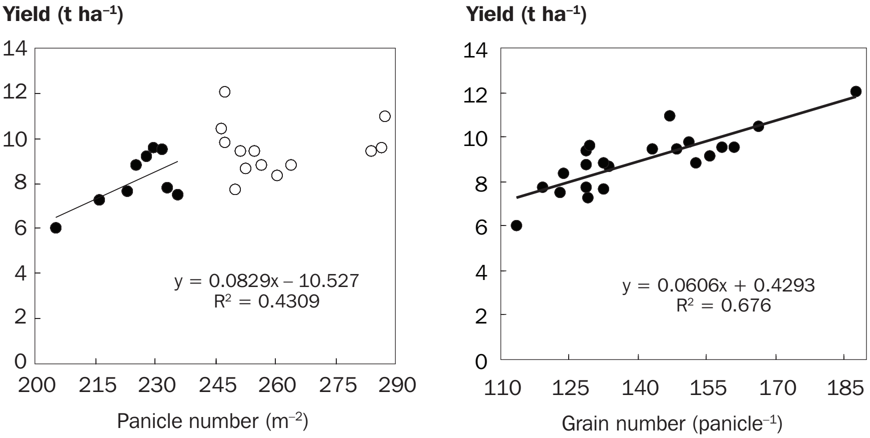

Fig. 5. Relation of panicle number and grain number to yield in Liangyoupeijiu.

Table 3. Average yield and its components in different yield classes of Liangyoupeijiu.

Yield range (t ha^{-1})	Yield class	Average yield (t ha^{-1})	Panicles (no. m^{-2})	Grains (no. panicle^{-1})	1,000-grain weight (g)
<8.0	Low	7.32	227.22	124.32	25.91
8.0–10.0	Middle	9.19	251.75	142.08	25.69
>10.0	High	11.16	260.56	167.12	25.64

respectively. This indicates that, at low yield, yield increases come mainly through an increase in panicle number per unit and grain number per panicle. However, at middle yield, yield increases mainly come through an increase in grain number per panicle. The contribution of yield components to a yield increase or decrease was calculated based on data in Table 2. Results indicate that, when yield increases by 1.87 t ha^{-1} from the low-yield class to middle-yield class, 45% of this yield increase is from an increase in panicles and 59% from an increase in grain number. When yield increases by 1.97 t ha^{-1} from the middle-yield class to high-yield class, only 18% of this yield increase is from an increase in panicles and 83% from an increase in grain number.

Yield gap in different planting methods

Seedling throwing and transplanting. Yield in transplanted rice ranged from 6.36 to 7.11 t ha^{-1} (Table 4), an increase of 0.75 t ha^{-1}, and the contribution of total effective panicles and grain was 32.2% and 67.8%, respectively. Improving panicles and grain helped to increase yield. Rice yield increased from 7.11 to 7.83 t ha^{-1}, and production increased 0.72 t. The cause was mainly increased grain weight, whose contribution was 70.2%. Rice yield also increased from 7.83 to 8.61 t ha^{-1} because of an increase in

Table 4. A comparison between yield and yield components of different planting patterns.

Planting pattern	Yield (t ha^{-1})	Yield gap (t ha^{-1})	Increase in panicles to promote yield		Increase in spikelets and grain to promote yield	
			t	%	t	%
Transplanting	6.36–7.11	0.75	0.24	32.2	0.51	67.8
	7.11–7.83	0.72	0.21	29.8	0.51	70.2
	7.83–8.61	0.78	0.50	63.6	0.29	36.4
Seedling throwing	5.84–6.42	0.58	0.38	64.0	0.20	35.1
	6.42–7.15	0.73	0.50	68.4	0.23	31.6
	7.15–7.94	0.79	0.11	14.0	0.68	86.0

effective panicles, whose contribution was 63.6%. Therefore, to improve the yield of transplanted rice and reduce the yield gap, it is necessary to mainly rely on increased grain weight and effective panicles.

Fields with throwing seedlings generally yielded more with a higher number of effective panicles. When yield was low, the crop needed more effective panicles to increase yield, but for middle to high yield, the crop needed to increase grain number.

Direct seeding and transplanting. In a survey of transplanted single rice at 76 sampling locations, there were 19 locations with yield of 6.75 to 7.50 t ha^{-1}, 20 locations with yield of 7.50 to 8.25 t ha^{-1}, 30 locations with yield of 8.25 to 9 .75 t ha^{-1}, and only 7 locations with yield above 9.75 t ha^{-1}. The yield difference mainly comes from grain number and effective panicles. Direct seeding of single rice surveyed at 72 sampling locations showed 10 locations with yield of 6.75 to 7.50 t ha^{-1}, 24 locations with yield of 7.50 to 8.25 t ha^{-1}, 34 locations with yield of 8.25 to 9.75 t ha^{-1}, and only 4 locations with yield of above 9.75 t ha^{-1}. The yield of direct-seeded rice ranged from 8.25 to 9.75 t ha^{-1} (Table 5), with differences mainly attributed to the number of effective panicles.

Conclusions and discussion

For transplanted late rice, the main difference in yield comes from grain number and grain weight. For throwing-seedling rice, the main difference in yield comes from the number of panicles, followed by grain number per panicle. For transplanted single rice, the average density is about 165,000, the main difference coming from panicle number per hill and grain number. But the number of effective panicles was the first condition for guaranteeing the yield of direct-seeded rice.

To obtain the target yield for different planting patterns, we need to take different technical countermeasures according to the varietal type. We need to investigate

Table 5. Single-rice yield and yield components of different planting patterns.

Planting pattern	Yield (t ha^{-1})	Sampling location (no.)	Lowest panicle density (×10^{4})	Maximum panicle density (×10^{4})	Average panicle density (×10^{4})	Grains per panicle (no.)	1,000-grain weight (g)	Average no. of panicles per hill
Transplanting	6.75–7.50	19	0.5	1.2	1.0	125.5	27.3	15.0
	7.50–8.25	20	0.9	1.6	1.1	129.0	26.9	14.5
	8.25–9.00	15	0.5	1.2	1.0	138.7	26.0	16.3
	9.00–9.75	15	0.7	1.4	1.0	148.5	26.3	16.2
	9.75–10.50	5	0.6	1.1	0.9	159.5	26.2	18.2
	>10.50	2	1.0	1.2	1.1	167.4	25.9	16.0
Direct seeding	6.75–7.50	10	24.91	39.81	33.39	93.1	24.1	
	7.50–8.25	24	23.52	46.85	33.35	100.1	24.6	
	8.25–9.00	16	24.35	43.43	28.45	126.5	24.8	
	9.00–9.75	18	25.28	57.50	36.86	108.6	24.9	
	9.75–10.50	4	34.44	35.65	35.05	119.7	24.5	

the difference caused by fertilizer, water, and pesticide, and so on. We can increase the survey area and main varieties, according to variety and planting pattern, and we can take feasible measures to narrow the differences in yield and achieve the target output.

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Authors' address: China National Rice Research Institute, Hangzhou, Zhejiang 31006, China.

Hybrid rice economics, public-private partnerships, and intellectual property management

Determining the economic value and viability of a rice hybrid

Robin D. Andrews and Fangming Xie

A basic economic framework is presented for determining and quantifying the value and economic viability of a rice hybrid. The methodology can be applied across different countries, currencies, and cultivation systems. The focus is on the fundamental characteristics of hybrid rice and how economic value is created. The ways in which economically viable hybrids differ in attributes and how these attributes contribute to overall product value are described and illustrated. The principles, practices, and approaches involved in measuring and sharing the added value of a hybrid (between researchers, developers, seed companies, farmers, and other participants) are described. Examples of the use of the economic model are presented together with the impact of grain price and crop input costs on product viability.

The breeding and successful introduction of a new rice hybrid involves the evaluation of large amounts of data. Large-scale trials in both seed and grain production are conducted toward the end of the breeding and product development process. Information is accumulated on yields, seeding rates, agronomic practices, and crop input costs.

In this paper, a methodology is described for assembling this late-stage information. The economic value of the potential new hybrid is compared with one or more existing viable products and a seed price is established that will benefit all the parties involved.

The methodology can be used for any country, currency, and cultivation system. The primary objective is to avoid the launch of nonviable hybrids. A secondary objective is to set a fair and competitive seed price.

Core principles and basic method

Seed production, grain production, and grain sale are first considered to exist within a single economic entity or unit. An economic unit is visualized that grows the seed in one season and then plants the seed in the next season to produce and sell grain. Since the growing and planting of the seed occurs within the one entity, it is not necessary, in this first step, to set a seed price.

The economics of a new hybrid are compared with that of an established variety (or an established hybrid) within this single economic unit. The term "system-wide" is used to describe the comparisons made in the first step. The economic advantage (i.e., the net of the costs and benefits) for the new line over the established product is determined. The net advantage is called the *added value*.

In the second step, the economic entity is divided into a seed production unit and a farming unit. The farming unit purchases seed from the seed production unit. A hybrid seed price is set which, in essence, divides the added value between the two entities. The added value can be shared in different proportions between the seed unit and the farming unit by setting different seed prices. The added value sharing must be to the benefit of each entity; otherwise, the product will not be viable.

Additional entities, such as a breeding unit that sells parental seed to the seed unit, could also be created. In that case, the added value is divided into three shares. For simplicity here, only two entities are created: one is the seed producer and the other is the farmer.

Economic measurements

All costs and benefits are expressed on a *per grain hectare* basis. Costs at the seed production stage, which might initially be collected and stated *per seed hectare* or *per kilogram of seed,* are converted to a *per grain hectare* basis by using the seed production yields and the rate of seeding of the hybrid seed in the grain field.

Costs and benefits are probably measured in a local currency (e.g., yuan, yen, dollar, euro). These are converted to a *grain yield equivalent in kg per grain hectare* by dividing by the nominal local price of rice grain of a standard quality. Each cost or benefit is then stated and easily visualized in terms of a quantity of grain harvested from a farmer's field. This approach simplifies comparisons between countries and cultivation systems. The nominal grain price is always stated when presenting data in this way.

Step 1—added value

Table 1 shows a system-wide comparison of the economics of a hybrid compared with that of an established variety where the nominal grain price is \$200 t^{-1}. There are no other significant differences between the two products, such as maturity or propensity to lodge, which might influence the comparison at this stage.

Line (i) in Table 1 shows the grain yield. Line (ii) shows the impact of grain quality on grain prices. In this example, the hybrid quality is above that of the standard grain and the hybrid grain sells at a premium. The varietal quality is also better than that of the standard grain but below that of the hybrid. The combination of lines (i) and (ii), shown on the bottom line of the table, provides the revenue per hectare for each product expressed in kilograms of standard quality grain per grain hectare.

Line (iii) shows the variable production costs of the hybrid seed and varietal seed. Although the costs are shown on a per grain hectare basis, it should be emphasized

Table 1. Step 1. Example 1—hybrid added value, relative to a variety – kg per grain ha – grain $200 t^{-1}.

Item	Hybrid	Variety	Difference
(i) Grain yield	9,000	7,480	1,520
(ii) Grain price	50	10	40
(iii) Seed cost	(460)	(111)	(349)
(iv) Grain input cost	(6,275)	(6,475)	200
Hybrid added value			1,411
Note: Revenue (i) + (ii)	9,050	7,490	1,560

Example 1. Grain at $200 t^{-1}.

that these are the *costs* to the seed production unit, not the *prices* to a farming unit. These separate units and the seed price are not established until Step 2.

Line (iv) shows the variable input costs for grain production, excluding seed cost. It is not necessary on this line to include all of the costs involved in grain production. One can simply include those input cost items (perhaps fertilizer or harvesting and drying costs), which differ between the hybrid and the variety. The second and third columns in the table are not totaled since some input costs may not be included. The focus is on the difference between the hybrid and the established variety, which is shown in the fourth column. The sum of these differences is the *added value*.

The *added value* in Table 1 is 1,411 kg per hectare. This is the economic difference between the hybrid and the variety in a single economic entity that produces seed and grain and sells the grain.

Step 2—sharing the added value

The single economic entity and the added value are now divided into two, as shown in Figure 1. In this example, 67% of the added value goes to the farmer and 33% to the seed unit (or seed company).

The chart and the relative length of the bars shown in Figure 1 are created from the data in Table 1 in the following way:

- 33% of 1,411 = 466 kg ha^{-1} is the hybrid seed margin (to seed company).
- 466 + the hybrid seed cost of 460 = 926 kg ha^{-1} and is the hybrid seed price.
- The hybrid seed premium for the farmer is 926 – 111 = 815 kg ha^{-1}. It is assumed that the varietal seed price is the same as the varietal seed cost.
- The farmer's revenue gain is 1,560 plus lower crop input costs of 200, giving 1,760 kg ha^{-1}.
- 1,760 – hybrid seed premium of 815 = 945 kg ha^{-1} net gain to the farmer.
- 945 kg ha^{-1} is the farmer's 67% share of the added value.

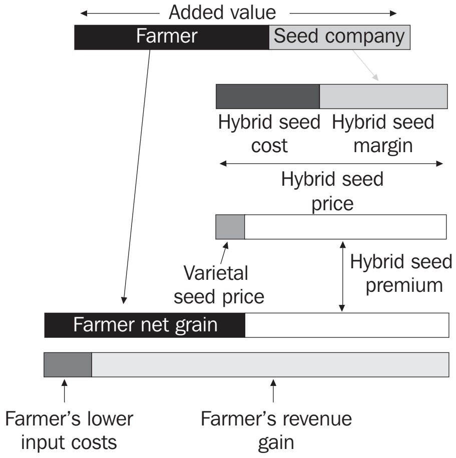


Fig. 1. Step 2. Sharing the added value.

Table 2 shows how changing the share of the added value given to each entity changes the seed price to the farmer and the hybrid seed margin for the seed producer.

For the hybrid to be viable from the farmer's perspective, the farmer's share of the added value must be sufficient to cover performance variability. This becomes an issue if the added value is low and the seed price is high.

For the hybrid to be viable from the seed producer's perspective, the producer's share of the added value (the hybrid seed margin) must be sufficient to cover research and development costs, administration costs, royalties paid (if any), sales and distribution costs, the cost of capital, and profit.

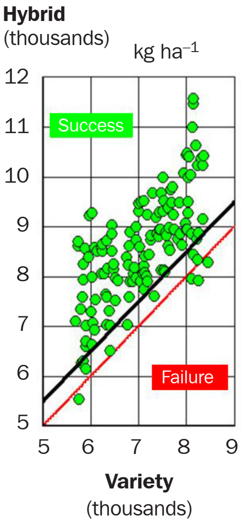
An alternative sequence for setting the seed price is to use the data in Step 1 in conjunction with multilocation field trial data to determine a seed price such that the net gain to the farmer is positive in, say, 95% of the field trials. With this performance and at this price, we can assume that the product is viable from the farmer's perspective. The price, determined in this manner, establishes the percentage sharing of the added value and the hybrid seed margin for the seed producer. One can then assess whether this margin is sufficient from the seed producer's perspective. The technique is illustrated and explained in Figure 2.

In practice, the setting of the hybrid seed price has to take into account the price being charged for competing hybrid seed products. The added value of the competing products needs to be known and a commercial decision made whether to charge the farmer more or less for any additional value provided by the new product. If the new product provides less added value than an existing product, the seed company might decide to charge a lower price, still launch the product, accept a lower profit margin, and provide the farmer with a competitive value.

Table 2. Step 2. Hybrid seed changes as share of added value changes.

Farmer	Seed producer	Farmer advantage	Seed producer margin = X	Hybrid seed price X + Y	Seed producer's margin
Share of HAV	Share of HAV	kg grain^{-1}	kg grain^{-1}	kg grain^{-1}	% of price
33%	67%	466	945	1,405	67%
50%	50%	706	706	1,166	61%
67%	33%	945	466	926	50%

HAV = hybrid added value. Hybrid added value from Example 1. Grain at \$200 t^{-1}.



Y-axis is revenue per ha of hybrid

Each point is one field trial

X-axis is revenue per ha of variety

Red line is hybrid result = variety result

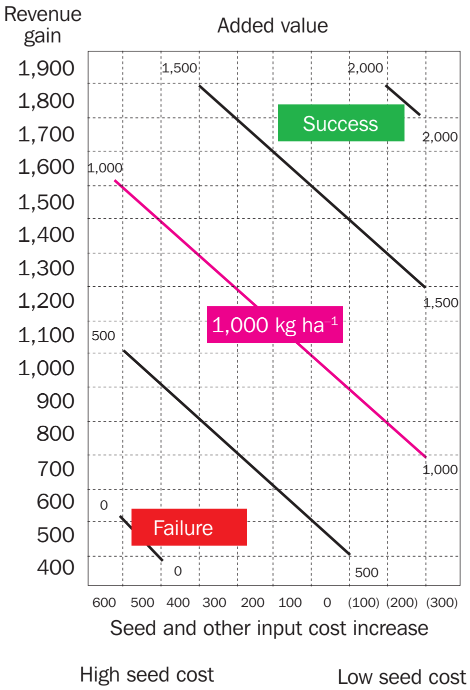
Black line is displaced vertically from red line by an amount equal to proposed hybrid seed premium net of any other grain input cost change.

Points below black line show failure of performance to cover proposed seed price. The higher the seed premium, the higher the black line.

Fig. 2. Field trials revenue per hectare chart (kg ha^{-1}).

It is useful after Step 1 to visualize the added value and the balance between the components of added value by using the chart shown in Figure 3. One point is placed on the chart for each comparison of a hybrid with a variety.

In the next two sections, we will show how the methodology described in this paper facilitates the calculation and the visualization of the impact on hybrid viability of changes in factors such as grain price and hybrid seeding rate.



Y-axis is revenue gain, with yield and grain price combined.

X-axis is grain cost change, with seed and input costs combined.

Sloping lines are constant added value.

Successful hybrids are at top right.

Unsuccessful hybrids are at bottom left.

Fig. 3. Added value component chart (kg ha^{-1}).

Impact of grain price changes

Table 3 uses the data in Table 1, which were based on a nominal standard grain price of \$200 t^{-1}, and restates it for a price of \$400 t^{-1}. The restatement assumes that only the grain price changes.

Comparing Table 1 and Table 3, we see that the doubling of the grain price has no impact on the revenue per hectare when expressed on a grain yield equivalent basis. This assumes that the grain price discounts or price premiums for quality remain a percentage of the grain price.

The seed cost and grain input costs are halved when expressed on a grain yield equivalent basis as the grain price doubles. This results in an increase in added value from 1,411 to 1,486 kg per hectare. In dollar currency terms, the added value has increased from \$282 per hectare to \$594 per hectare.

If the sharing of added value was left unchanged as grain cost doubled (33% to the seed producer and 67% to the farmer), the seed price would decrease from 926 kg ha^{-1} to 720 kg ha^{-1}. In dollar currency, the seed price would increase from \$185 to \$288 per hectare and the farmer's share of the added value would increase from \$189 to \$398 per hectare.

Table 3. Step 1. Example 2—hybrid added value, relative to a variety – kg per grain ha – grain \$400 t ha^{-1}.

Item	Hybrid	Variety	Difference
(i) Grain yield	9,000	7,480	1,520
(ii) Grain price	50	10	40
(iii) Seed cost	(230)	(56)	(174)
(iv) Grain input cost	(3,138)	(3,238)	100
Hybrid added value	1,486		
Note: At \$200 t–1 the HAV is 1,411			
Note: Revenue (i) + (ii)	9,050	7,490	1,560

In Table 1 with grain at \$200 t^{-1}, the hybrid seed cost premium at 349 kg ha^{-1} was 25% of the added value. In Table 3 with grain at \$400 t^{-1} , the hybrid seed cost premium at \$174 kg ha^{-1} was only 12% of the added value. This illustrates how a higher grain price increases the likelihood that a hybrid, even one with a high seed cost, will be economically viable.

Impact of hybrid seeding rate changes

Table 4 uses the data in Table 1, and restates it for a halving of the seeding rate in the grain field. The restatement assumes that only the seeding rate changes.

Comparing Table 1 and Table 4, we see that the halving of the seeding rate halves the hybrid seed cost, expressed on a grain yield equivalent basis, from 460 to 230 kg ha^{-1}. This savings of 230 kg ha^{-1} results in an increase in added value from 1,411 to 1,641 kg ha^{-1}.

If the sharing of the added value was unchanged, with 33% to the seed producer and 67% to the farmer, the seed price would decrease from 926 to 772 kg ha^{-1} and the value of the farmer's share of the added value would increase from 945 to 1,099 kg ha^{-1}. The value of the seed company's share of the added value increases from 466 to 542 kg ha^{-1}, which is 70% of the seed price.

Seeding rate in the hybrid grain field is obviously critical to the viability of a hybrid and the methodology clearly shows the impact.

Seed production costs

The use of this methodology by breeders requires an understanding of the costs of hybrid seed production in Step 1. These are less readily available than hybrid and varietal grain production costs and are often proprietary to the hybrid seed companies. However, breeders do conduct experiments to determine whether new parental lines have outcrossing rates and hybrid seed yields competitive with existing commercial

Table 4. Step 1. Example 3—hybrid added value, 50% lower seedling rate – kg per grain – grain $200 t^{-1}.

Item	Hybrid	Variety	Difference
(i) Grain yield	9,000	7,480	1,520
(ii) Grain price	50	10	40
(iii) Seed cost	(230)	(111)	(119)
(iv) Grain input cost	(6,275)	(6,475)	200
Hybrid added value	1,641		
Note: At the higher seedling rate, the HAV is 1,411			
Note: Revenue (i) + (ii)	9,050	7,490	1,560

Example. Grain at $200 t^{-1}.

lines. This information can be used to estimate relative hybrid seed production costs between a proposed new hybrid and an existing commercial hybrid. *The Little Book on Hybrid Rice Economics* by Robin Andrews provides insight as to how this can be done.

Conclusions

The process of breeding and evaluating a new hybrid is data driven. The added value of a new hybrid involves the quantification of four areas of costs and benefits: the grain yield advantage, grain quality as reflected in the grain price, hybrid seed production costs, and hybrid grain production input costs. The first two areas combine to define the revenue advantage to the farmer and the second areas define the grain cost disadvantage or advantage. As a separate step, the added value has to be divided between the seed producer and the farmer by setting a seed price that is beneficial to all involved. The structure and ownership of the hybrid seed system vary from country to country but the basic principles involved in creating and sharing value are universal.

Notes

Authors' addresses: Robin D. Andrews, retired president and CEO, RiceTec, Inc.; Fangming Xie, senior scientist, International Rice Research Institute.

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Economics of hybrid rice seed production in India

B. Nirmala, P. Muthuraman, Shaik N. Meera, Mangal Sain, and B.C. Viraktamath

Rice is the staple food crop in India. It is grown on about 43 million hectares annually, with a production of around 90 million tons. It is projected that India needs to produce 115 million tons of rice by 2020 to maintain current self-sufficiency. There are two options for achieving this target: one is the horizontal expansion of area under rice cultivation, for which there is very little scope; the second is the vertical expansion—increasing rice yield through the adoption of hybrid rice cultivation and other yield-enhancing technologies. The large-scale adoption of hybrid rice depends on efficient and economical hybrid rice seed production.

In the new millennium, many eastern Indian states have taken up hybrid rice cultivation on a massive scale. This has created demand for good-quality hybrid rice seeds. Although sporadic attempts were made earlier to study the economics of hybrid rice seed production in the 1990s, under the recent changed scenario of supply and demand, no study was conducted. Hence, the economics of hybrid rice seed production will have more relevance than in the 1990s. With this backdrop, our study was conducted in Karimnagar and Warangal districts of Andhra Pradesh, where about 85% of the hybrid rice seed is produced in India.

Farm-level data from hybrid rice seed producers were collected through surveys in the selected study area. The data pertain to 2006-07. The total sample size was 96 seed growers. Data on input-output details were collected from the sample farmers through personal interviews with the help of a structured questionnaire.

The results have shown that the benefit-cost ratio for hybrid rice seed production is 1.82, indicating that this technology is economically viable. In addition, hybrid rice seed production requires about 35% more labor than the cultivation of high-yielding varieties. Thus, the labor intensiveness of hybrid rice seed production has created rural employment opportunities besides increasing farmers' income.

Rice is the staple food crop of India, providing 43% of the calorie requirement for more than 70% of the Indian population. Rice covered 43.66 million hectares, with a production of 91.79 million tons in 2006 (Agricultural Statistics at a Glance 2007). It is projected that India needs to produce 120 million tons of rice by 2020 to maintain current self-sufficiency. There are two options for achieving this target: one is horizontal expansion of the area under rice cultivation, for which there is a very little scope, the second is the vertical expansion that is increasing rice yield through the adoption of yield-enhancing technologies, including hybrid rice.

Hybrid rice is a feasible and readily adoptable genetic option to increase rice production, as has been amply demonstrated in China. Thus, having been convinced of the potential of hybrid rice technology to enhance rice production and productivity, the Indian Council of Agricultural Research (ICAR), New Delhi, launched a national project on hybrid rice in 1989. This project was implemented through a well-organized national network comprising 12 centers located across the country and was coordinated by the Directorate of Rice Research, Hyderabad, and it has another 20 voluntary centers represented by public, private, and NGO sectors (Viraktamath et al 2006).

The area under hybrid rice increased from 0.1 million ha in 1995 to 1.2 million hectares in 2007. It is projected that the area under hybrids will reach 3 million ha in 2010 and 6 million hectares by 2020. This increasing trend in area under hybrid rice reflects the commendable performance and accelerated adoption of hybrid rice in India. Because of its yield advantage, hybrid rice technology is very important for the food security of India, where arable land is diminishing and the population is steadily increasing.

The availability of good-quality seed at an affordable price is a crucial factor for the large-scale adoption of hybrid technology of any crop. This has been demonstrated by the success obtained through hybrids of maize, pearl millet, sorghum, sunflower, and cotton, all of which are supported by cost-effective seed production and distribution systems in the countries where hybrids are commercialized. The large-scale adoption of hybrid rice also depends on efficient and economical hybrid rice seed production.

Hybrid rice seed production in the country, starting with less than 200 tons of total production in 1995, surpassed 19,000 tons in 2007 (Table 1). India now has more than 95% of the hybrid seed being produced by the private sector. In total, around 30 private seed companies (10 large and 20 medium to small) are now engaged in large-scale hybrid rice seed production. The leading private hybrid rice seed production companies are Pro-Agro, Bayer Crop Science, Pioneer, Mahyco, Bioseed, J.K. Agrigenetics, Syngenta, Advanta, and Metahelix.

The hybrid rice seed requirement during 2010 and 2020 is expected to be around 50,000 tons and 100,000 tons, respectively.

Hybrid rice seed production is generally carried out mostly in Andhra Pradesh, a southern state of India, during the dry season (November to April). Though hybrid rice cultivation is confined to northern India, seed production is concentrated in southern India, where the conditions are congenial for obtaining higher seed yields. In recent times, it has been observed that farmers in the Northern Telangana Region of Andhra

Table 1. Area under hybrid rice seed production and quantity of seed produced (1995-2007).

Year	Area (ha)	Seed produced (t)
1995	195	200
1996	1,075	1,200
1997	1,485	1,800
1998	1,630	2,200
1999	1,660	2,500
2000	1,630	2,700
2001	1,625	2,900
2002	1,635	3,100
2003	2,865	4,000
2004	4,350	8,600
2005	6,800	12,500
2006	15,000	19,000
2007	15,000	19,500

Source: Manual on hybrid rice seed production technology. 2007. DRR, Hyderabad.

Pradesh have taken up hybrid rice seed production. The major players in large-scale hybrid rice seed production are private seed companies. Hybrid rice seed is produced through contract seed growers. In a majority of the cases, the seed companies are providing back-end and front-end support to seed growers by supplying seed of parental lines and other critical inputs such as GA_3 required for hybrid rice seed production. They are also offering technical expertise and guidance to seed producers by making visits to fields at regular intervals during the crop season. The hybrid seed thus produced is procured by seed companies at mutually agreed-upon rates. Seed producers in turn are reaping benefits from this technology.

In the new millennium, many eastern Indian states have started growing hybrid rice on a massive scale. This has created demand for good-quality hybrid rice seeds. Although sporadic attempts were made earlier to study the economics of hybrid rice seed production in the 1990s, in the recent past in the changed scenario of demand and supply, no study was conducted. The economics of hybrid rice seed production will now have more relevance than in the studies in the 1990s, as these studies were conducted in the initial stages when the farmers had no experience in seed production and hence seed yields were rather low. With this background, our study was conducted.

Methodology

A study was conducted in Karimnagar and Warangal districts of Andhra Pradesh, where about 85% of the hybrid rice seed is produced in India. Farm-level data from hybrid rice seed producers who also cultivated high-yielding varieties of rice were collected through surveys in the selected study area. The data pertain to 2006-07. From each of the two districts, four villages with an extensive area under hybrid rice seed production were selected. From each village, 12 hybrid rice seed producers were selected randomly. Thus, the total sample size was 96 seed growers. Data on input-output details were collected from the sample farmers through personal interviews with the help of a structured questionnaire.

Salient findings of the study

Cost of cultivation

A comparison of the cost of producing hybrid rice seed versus the cost of cultivating high-yielding varieties revealed that the cost for seed was almost the same (Table 2).

The expenditure on organic manure and irrigation was the same in both cases. The expenditure on fertilizers and plant protection chemicals was high for hybrid rice seed production in comparison with cultivating high-yielding varieties. The total number of person-days for hybrid rice seed production was 45% more than that for high-yielding varieties. An important point is that hybrid rice seed production is labor-intensive. It involves additional costs on operations such as GA_3 application, supplementary pollination, and roguing. GA_3 cost US$17.77 per hectare. Additional costs in hybrid rice seed production amounted to $182.24 per hectare (Table 3). The total cost of hybrid rice seed production was higher than that of high-yielding varieties by $344.44 per hectare.

The main sources of higher costs in hybrid rice seed production are the cost of plant protection, use of higher quantities of chemical fertilizers, and additional operations such as supplementary pollination, GA_3 application, and roguing, which require more labor. Extra care has to be taken in harvesting and threshing hybrid rice seed, which resulted in more labor involved and also more bullock labor and machinery cost, which accounted for higher cost of production than for high-yielding varieties.

Employment potential

Hybrid rice seed production requires 61 person-days ha^{-1} more than that of high-yielding rice varieties. Thus, it requires about 45% more labor than when growing improved varieties. The labor intensiveness of hybrid rice seed production has created employment opportunities in rural areas where hybrid rice is taken up.

The annual employment potential of hybrid rice seed production is around 45% more person-days ha^{-1} than the cultivation of high-yielding varieties, particularly for landless rural women. During 2007, the additional employment being generated was estimated to be around 1,080,000 person-days. Expected additional employment

Table 2. Cost comparison of hybrid rice seed production vis-à-vis cultivation of high-yielding varieties (HYV) (per hectare).

	Particulars/item	Hybrid rice seed production	HYVs
1	Seed		
	Quantity (kg)	20	75
	Value (US$)	17.77	18.96
2	Organic manure		
	Quantity (tractor loads)	3	3
	Value ($)	34.41	34.41
3	Chemical fertilizers		
	Quantity (kg)	632.5	562.5
	Value ($)	144.57	118.50
4	Plant protection chemicals ($)	32.91	21.68
5	Irrigation costs ($)	2.96	2.96
6	Human labor (person-days)	199	138
7	Total human labor charges ($)	331.68	226.92
8	Bullock labor cost ($)	28.44	21.33
9	Machinery cost ($)	142.20	127.98
10	Total cost ($) – (A)	734.94	572.74

Table 3. Additional costs for hybrid rice seed production (per hectare).

Item	Amount (US$)
GA_3	17.77
Human labor for GA_3 application	6.63
Supplementary pollination	136.51
Roguing	21.33
Total additional costs—(B)	182.24
Total costs (A (of Table 2) + B)	**917.18**

Table 4. Returns from HYV cultivation.

Item	Amount
Grain yield (kg ha^{-1})	5,730
Market price (US$ t^{-1})	159.71
Straw value ($ ha^{-1})	49.77
Returns from grain ($ ha^{-1})	915.14
Total returns ($ ha^{-1})	964.91

Table 5. Returns from hybrid rice seed production.

Item	Amount
Hybrid seed yield (kg ha^{-1})	1,935
Seed price (US$ kg^{-1})	0.70
Hybrid seed value ($ ha^{-1})	1,354.50
Restorer yield (kg ha^{-1})	1,824
Price ($ kg^{-1})	0.16
Restorer value ($ ha^{-1})	291.84
Straw value ($ ha^{-1})	44.17
Total returns ($$^{-1}$)	1,690.51

during 2010 and 2020 from hybrid rice seed production will be around 1,800,000 and 3,300,000 person-days, respectively, thus providing ample employment opportunities in rural areas.

Returns

The grain yield obtained from high-yielding varieties was 5,730 kg ha^{-1}. The average market price was $159.71 per ton. Thus, returns from grain amounted to $915 ha^{-1}. The straw value was $49.77 per hectare. The gross returns obtained from high-yielding varieties were $965 ha^{-1} (Table 4).

The average yield in hybrid rice seed production was 1,935 kg ha^{-1} (Table 5). The average procurement price was $0.70 kg^{-1}; hence, the return from hybrid seed was $1,354.50 ha^{-1}. Restorer yield obtained was 1,824 kg ha^{-1}, which was obtained at an average price of $0.16 kg ha^{-1}, and the returns from restorers were $291.84 ha^{-1}. The value of straw obtained per hectare was $44.17 ha^{-1}. The gross returns from hybrid rice seed production technology were $1,691 ha^{-1}.

Even though the total costs incurred for hybrid rice seed production were more than those of high-yielding varieties, both the gross and net returns were higher for

Table 6. Economic indicators.

Items	Hybrid rice seed production	HYV cultivation
Gross returns (US$ ha^{-1})	1690.51	964.91
Net returns ($ ha^{-1})	773.33	392.17
Benefit-cost (BC) ratio	1.84	1.68
Cost of seed production ($ kg^{-1})	0.47	–
Real cost of seed production ($ ha^{-1})	581.17	–
Real BC ratio	2.33	–

hybrid rice seed production than for high-yielding varieties (Table 6). Hence, the benefit-cost ratio was 1.84 for hybrid rice seed production and 1.68 for high-yielding varieties. The cost of hybrid rice seed production was \$0.47 kg^{-1}. The real cost of seed production, which is the gross costs excluding the returns from restorer seed yield and straw, was \$581.17 ha^{-1} and the real benefit-cost ratio was 2.33 for hybrid rice seed production. Since the benefit-cost ratio is high, hybrid rice seed production can be considered an economically viable technology.

One of the most important prerequisites for producing genetically pure hybrid rice seed is isolation distance. Normally, an isolation distance of 100 meters is recommended for hybrid rice seed production. This means that, when different hybrid combinations with the same flowering are grown in adjacent plots, there is a possibility of pollen contamination; hence, a 100-meter isolation distance should be maintained in between two combinations. It is very difficult to maintain isolation distance in intensive rice fallow areas. In most villages, this problem was solved by the farmers themselves through cooperative farming involving many like-minded farmers. Through mutual cooperation and understanding, seed production of the same hybrid combination is being taken up in a large area and sometimes the same hybrid occupies a whole village. In many seed villages, one particular private seed company carries out hybrid rice seed production of one or two hybrids in the whole village, thereby avoiding the problem of (isolation distance) pollen contamination. This type of farming facilitates easy supervision by company agents, timely farm operations, and effective use of resources such as water, nutrients, labor, and plant protection chemicals, and thereby helps to increase seed yield as well as the profitability of the technology.

Conclusions

Hybrid rice technology is a key strategy for increasing rice production and maintaining self-sufficiency and food security. However, the concerted efforts of both the public and private sector are vital to ensuring more rapid growth in the development and adoption of hybrid rice technology in India. The availability of good-quality seed at a reasonable price is a crucial factor for the large-scale adoption of hybrid rice technology. Since the benefit-cost ratio of hybrid rice seed production is more, it can be considered an economically viable technology. The high profitability of seed production that has also resulted in employment generation is now being realized on-farm. Hybrid rice seed production is a labor-intensive technology that requires about 45% more labor than the cultivation of high-yielding varieties. The advancing technology refinement by scientists, technology dissemination by the seed industry and extension workers, and policy and financial support by national and local governments contribute greatly to the success in the development and use of hybrid rice technology. For expansion of the area under hybrid rice, certain administrative and research policies have to be implemented to improve seed production, standardize the technologies, and encourage the participation of the public sector. For public-private partnerships, it is necessary to regulate the seed price, assure procurement, have a minimum support price, and carry out promotional activities and hybrid rice development programs.

Large-scale hybrid rice seed production is now concentrated in only two districts of Andhra Pradesh, Karimnagar and Warangal, where more than 90% of the hybrid seed is being produced. With the increasing demand for hybrid seed, additional areas are required for seed production. The area suitable for seed production in Karimnagar and Warangal has already reached saturation, with almost 15,000 ha under seed production during 2006. Therefore, there is an urgent need to identify new areas suitable for large-scale seed production in other states.

The performance of the public seed sector in hybrid rice seed production has not been encouraging so far, though many good public hybrids have been released. Seed production personnel in the public sector have to be motivated to undertake large-scale hybrid rice seed production. Hence, the National Seed Corporation, State Farms Corporation of India, and the State Seed Development Corporation have to be encouraged and provided with all the needed facilities and infrastructure to carry out large-scale hybrid rice seed production.

Though there were a few problems in the initial stages of large-scale hybrid rice seed production in India, the average seed yields obtained are now satisfactory and are increasing gradually over time with experience. It is possible to overcome the minor problems encountered. Hence, with regular refinements in seed production technology, the prospects for large-scale hybrid rice seed production in India appear to be bright and this activity will be very helpful for bringing prosperity to the farming community.

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Authors' address: Directorate of Rice Research, Rajendranagar, Hyderabad 500030, India.

Strategies to develop the hybrid rice industrial economy in China

Qing Xian-guo

The development of a hybrid rice industrial economy is an effective way to expand hybrid rice planting area, increase rice production benefits, and ensure both high yield and high efficiency of hybrid rice production. In this paper, the basic meaning of a hybrid rice industrial economy is discussed and its important social and economic functions are explained. The advantages and the factors that limited the development of a hybrid rice industrial economy in China are thoroughly analyzed. On the basis of these, strategies and policy suggestions are put forward.

Keywords: hybrid rice, industrial economy, advantage, limiting factor, development strategy in China

Since the introduction of hybrid rice, the area planted to it has reached 330 million ha and production has amounted to 450 million tons. In recent years, annual planting area of hybrid rice stabilized at 17.3 million ha in China, which is about 60% of the total rice area (Yuan 2004). To ultimately develop hybrid rice, one key issue is how to enlarge planting area to maximize the yield-increasing potential of hybrid rice to the fullest extent. Food is a special commodity with both social and economic properties. The social properties mainly emerge during the early years in the transition from a closed economy to an open economy. Economic properties dominate at the relatively open economy stage. In today's China, the high yield of food crops must be ensured. At the same time, the benefits of food production must be spread steadily. So, another key problem related to the development of hybrid rice is how to increase production benefits of hybrid rice ultimately. In my opinion, research and development of the hybrid rice industrial economy is an effective way to promote the development of hybrid rice, boost national and international influences of hybrid rice, enlarge the planting area, and increase production benefits derived from hybrid rice. In this paper, the basic meaning of a hybrid rice industrial economy, the advantages and limiting factors, and development strategies are discussed.

Basic meaning of a hybrid rice industrial economy

The hybrid rice industrial economy can be defined on the basis of food security; change in market demand; social and economic features of hybrid rice development and its industry; innovations in breeding, cultivation, processing, brand establishment, and marketing; and change in agriculture from traditional to modern. The significant characteristics of a hybrid rice industrial economy mainly lie on its base, the biological product of hybrid rice and its measures, processing, transformation, and marketing aspects. It is an economic activity that involves different trades, different industries, and different regions. It is different from traditional rice production. Hybrid rice belongs not only to a technology-intensive and labor-intensive industry but also to a soil resource-intensive industry. The formation of a new type of hybrid rice industrial economy must be based on planned development by using modern industrial concepts; improving production conditions; intensifying breeding of special-purpose varieties with super high yield; stabilizing outputs, processing, and transformation; lengthening the industry chain; improving integrative benefits; changing yield superiority into economic superiority; and promoting an increase in farmers' income and benefits from agriculture (Qing Xianguo 2003).

Social and economic functions

Ensuring food security

Food is an important component in national development, an integral part of people's lives. Almost 65% of the Chinese population eats rice as their main food. It is estimated that the population of China could reach 15–16 billion in 2030. If food consumption per capita is 400 kg and the percentage of rice production in total food production is 40%, 2.4–2.5 × 10^8 t of rice grains must be produced annually, which means that rice production must increase by 21.4–36.9% at the 2006 level. Because of political reasons and circumstances surrounding trade, transportation, and storage, China must depend mainly on its own resources to meet rice demand. At the same time, arable land is limited and there is a slim chance of increasing planting area. Thus, the increase in rice production must come from improvement in per unit area yield. We must increase per unit area yield of rice from 6.23 t ha^{-1} in 2006 to 8.19–8.55 t ha^{-1}, an increase of 30–37%. The yield of conventional rice is about 1.5 t ha^{-1} lower than that of hybrid rice; the probability of conventional rice yield exceeding that of hybrid rice is very small. As Yuan Longping has pointed out, "Use of heterosis in rice is at its primary stage now, and the huge yield potential of hybrid rice has not been fully exploited." To improve per unit area yield of rice, hybrid rice must be popularized. The development of the hybrid rice industrial economy is therefore an inevitable choice to ensure national food security.

Accelerating the course of modern agriculture in rice-growing regions

The hybrid rice industrial economy has five constituents—regional distribution, specialized production, standardized management, integrative exploitation, and multiple foci. Regional distribution means assigning to specific regions different varieties according to their biological properties, soil and climatic conditions, demands for processing and transformation, and consumption trends. Specialized production involves breeding appropriate varieties according to the needs of the market and targeting production to meet demand. Standardized management necessitates the setting up of a quality standard system and a quality monitoring system, organizing production according to national industry standards and marketing demands, and carrying out the whole course of standardized management with standardization of variety, production environment, production process, and product quality. Integrative exploitation requires a steady supply situation and a stable marketing relationship between processing groups and farmers by boosting the industrialization developmental modes—e.g., corporation linking to production base through a cooperative economic enterprise, corporation linking to the scientific research center, corporation linking to farmers, and so on. The focus is on forming a community with the same benefit, risk, and service. The hybrid rice industrial economy is a systematic engineering entity containing many domains and links. Its development demands resources from different channels and building management patterns and participatory modes that involve multiple systems, which adapt to the demands of the market economy and follow the regulations of the WTO. Organizing production according to economic factors will effectively solve existing problems in the current rice production scheme in China—e.g., incomplete structure, small production scale, adulterated rice, low-standard management, short industry chain, low production benefit, and so on. In promoting the development of modern agriculture and a new countryside, we accelerate the developmental course of modern agriculture in rice-growing regions in China.

Enhancing the rice industry in China

Along with urbanization, population increase, decrease in rice land, and increasing use of food crops as materials for industry and as forage, the demand for rice will increase, especially in developing countries. The amount of rice traded in the international market will increase significantly and, along with emerging trade freedom, will reach 3,000 × 10^4 t per year, affording a wide international market for China's rice industry. In recent years, FAO statistics show Chinese rice being superior in yield and price in the international market. The yield of rice in China was 6.3 t ha^{-1}, which was 4.1 t ha^{-1} and 2.2 t ha^{-1} higher than that of Thailand (the largest rice-exporting country in the world) and Vietnam (the second largest rice exporter). It was 3.4 t ha^{-1} higher than that of India, the biggest country in terms of area planted to rice. The yield superiority is also evident compared with other rice-growing countries. The high yield and wide adaptability of hybrid rice provide an important base for achieving lower production cost. The superiority in yield and price of Chinese rice will provide impetus for the development and export of hybrid rice. As to being a rice exporter, China is ranked as the third, rising from fifth to seventh place in the last 20 to 30 years because of its inconspicuous rice quality superiority. We predict that the competitive ability of hybrid rice in terms of low price and good quality will be fully revealed in the international market, if the quality of Chinese hybrid rice varieties can be improved, along with yield and production efficiency. By then, the green-box policy of the WTO could be fully applied.

Developing a hybrid rice industrial economy in China

Scientific and technological superiority

Hybrid rice is the great invention of China. China is the leader in the breeding, cultivation, and popularization of hybrid rice. The discovery of cytoplasmic male sterile materials established the basis of the three-line breeding system, which resulted in the production of hybrid rice. The successful application of photoperiod- and thermosensitive genic male sterile genes and wide-compatibility genes promoted the research and development of two-line hybrid rice, which showed a greater yield-increasing potential than three-line hybrid rice. Research on super rice in China started later than that in Japan and at IRRI, but significant research achievements have been made using a combination of conventional and molecular breeding. The result is a super high-yielding hybrid rice plant type with higher leaf canopy, lower panicle position, and medium to large panicles, which was proposed earlier by Academician Yuan Longping. China has reached the breeding targets set up by the Ministry of Agriculture for the first and second phases of the super rice project—10.5 t ha^{-1} and 12.0 t ha^{-1}, respectively. Furthermore, the Phase III yield target (13.5 t ha^{-1}) is expected to be realized by 2010. China has now made great progress in creating new germplasm and breeding super hybrid rice parents, including parental lines with huge panicles (900 spikelets per panicle). This was achieved through molecular breeding techniques, such as the introduction of exogenous DNA, transfer of high-yield genes from wild rice, transfer of genes for high photosynthetic efficiency from C_4 plants, etc. The world's leading hybrid rice technology of China ensured the country's food security, effectively solving the food insufficiency problem. The successful Chinese experience gives a good example and shows the way for some Asian and African countries to address their food security concerns. China could provide them with technical assistance in developing hybrid rice. Chinese hybrid rice technology has been described by FAO as the primary technical means to help countries overcome food shortages.

Natural resource superiority

China is located in a transitional area from subtropical to warm temperate climate. Total solar radiation is 284.7–334.9 kJ cm^{-2}, mean sunshine hours range from 12.8 to 13.8 h, mean air temperature is 21.5–23.5 °C, accumulation temperature (>10 °C) is 3,770–4,780 °C, and there is adequate precipitation during the duration of rice growth. The natural resources, including light, temperature, water, and soil, support the development of indica hybrid rice and japonica hybrid rice under double cropping and provide an advantage of superior yield and quality of the planted varieties.

Cost and benefit superiority

The unit production cost of major food crops in China falls within the range of 900–1,200 RMB t^{-1} (US\$1 = 7 RMB). The unit production cost of hybrid rice is 120

RMB t^{-1} lower than that of wheat but 140 RMB t^{-1} higher than that of maize. Cash cost is the main cost item in food-crop production cost, about 440–460 RMB t^{-1}, with the percentage of cash cost to total cost being 50–60%. The cash cost of hybrid rice is 100 RMB t^{-1} lower than that of wheat and 120 RMB t^{-1} higher than that of maize. The percentage of labor cost to total cost is about 30–40%. The labor cost of wheat is relatively lower (30%), whereas material and service costs are the highest; the labor cost of hybrid rice is superior to some extent. For rice production cost in China, the costs of labor, machinery, and cash accounted for 33%, 12%, and 50%, respectively, with 90% of the labor cost coming from own labor. The corresponding figures in the U.S. are 12%, 27%, and 75%, respectively, with 62% of the labor cost coming from own labor. According to 2002-04 data, the total cost and cash cost of producing 50 kg of rice grains in China are 28 and 32 RMB lower than those in the U.S., respectively. These results show that Chinese hybrid rice production is clearly superior in terms of cost and benefit not only to other food crops in China but to U.S. rice as well.

Efficient agroecological superiority

In recent years, as measures to increase yield, save on cost, and increase the benefit and income of rice farmers, innovative farming systems were established. Many types of efficient planting models and cropping patterns were tried: food-food combinations (potato under no-tillage and straw mulching–double crop of rice or medium rice, wheat or barley–double crop of rice or medium rice); food-feed combinations (ryegrass or rye under no tillage–double crop of rice or medium rice); food-economic crop combinations (winter vegetable–spring maize/watermelon–super hybrid rice, winter vegetable–cured tobacco–late hybrid rice); ecological planting and feed type (ryegrass-goose, rice-duck); shrimp-crab-pearl feeding model in lake regions; and so on. This was achieved by first doing research on efficient multiple cropping systems, conservation tillage, and returning of straw to paddy fields. The next step involved assembling the existing new varieties, planting techniques, and tillage models. The third method added economic crops, forage crops, and vegetables under a rotation system in the field. These efficient planting models optimized the allocation of resources and improved the ecological benefits derived from the rice planting area.

Multiple bases for industrial exploitation of hybrid rice

Chinese scientists have, for many years, studied rice specialization. They classified rice into three types, according to use: high-quality edible rice, high-protein forage rice, and special industrial rice. Studies on variety breeding, cultivation techniques, and processing techniques were done, and many high-quality edible rice and high-protein forage rice varieties were bred. Varietal standards and cultivation technique systems were established. At the same time, regional distribution of new varieties was planned. Furthermore, following the “corporation + base + farmer” idea, the Chinese government encouraged many leading corporations to engage in national and international efforts to exploit hybrid rice. The Longping High-tech Company Ltd., for example, is marketing hybrid rice seed; the Jinjian Rice Industry Company Ltd. and the Longping Rice Industry Company Ltd. are mainly engaged in refining and deep processing of rice. Today, leading corporations that engage in the exploitation of hybrid rice are distributed in almost all major rice regions and provinces. We thus have a good base to industrially exploit hybrid rice and develop the hybrid rice industrial economy.

Factors that limit the development of a hybrid rice industrial economy

Frequent floods and droughts, poor irrigation infrastructure, and severe insect and disease incidence

Many problems in rice production exist in the regions—low temperature and rainy weather in early spring, floodwater in early summer, drought in summer and autumn, waterlogging in paddy fields, and so on. Low temperature and rainy weather usually result in a decay in rice seedlings. Rainstorms in late spring and early summer could induce flooding in mountainous areas and then cause waterlogging in the plains, lake regions, and along river regions. Summer drought, autumn drought, and summer-autumn drought that occur from July to September (when water requirement is maximum) greatly affect the growth of hybrid rice. At the same time, heading, flowering, and grain filling of late rice are greatly affected by low temperature in the middle and latter part of September. Although there are water-conserving facilities on farmland, these are mostly obsolete, thereby lessening their effectiveness during natural disasters. In the regions, especially in the southern areas where indica rice is grown, serious diseases and insect pests threaten the crop. These were induced by high temperature and high moisture (which resulted in serious disease incidence), abundant cropping systems (which gave a favorable growth environment to insects), and inappropriate use of pesticides (which resulted in a decrease in number of natural enemies, the death of the agroecosystem, and the buildup of insect resistance to pesticides). All of these proved disadvantageous to a sustained development of hybrid rice production (Yang et al 2004).

Commercially inferior rice product, low industrial level, and lack of techniques for rice refining and deep processing

Foremost among the constraints to the development of the hybrid rice industry is rice quality. Although the degree of quality of Chinese hybrid rice has been greatly improved, its quality is still poor compared with that of foreign high-quality rice, especially in appearance. The second limiting factor is the discord between the structural status of hybrid rice production and market requirement, which greatly affected the motivation of farmers to plant. The third one is the lack of high-quality rice varieties to meet requirements of consumers, manufacturers, and farmers at the same time. Consumers want high-quality rice with good appearance, good taste, and low price; rice processors demand rice varieties with high head-rice recovery and long storage time; and farmers like high-yielding and stress-resistant varieties. The current rice varieties cannot meet all of these competing demands. The fourth restriction is the limited type of paddy grown at present and the unitary use of rice. This is mainly indicated by much less japonica rice in production compared with indica rice and the small scale of R&D on high-quality edible rice, forage rice, and processed rice, thus limiting the exploitation of the market and use of rice (Li and Li 2004). The fifth factor is the sanitation quality of rice. Heavy metal and pesticide residues were detected in rice in some regions. The sixth limiting factor pertains to significant differences, compared with developed countries, in terms of enterprise scale, processing equipment, technical processing flow, and processing techniques of leading rice corporations. At the same time, refining and deep processing techniques of rice are lacking. The seventh limiting factor has something to do with price policies that govern the purchase, storage, and processing of rice. The lack of guidelines affected exploitation and processing aspects, thus stifling the growth of the rice industry (Zhang et al 2005).

It behooves China to strengthen the leading rice corporations and promote rice product brands, prolong the industrial chain, and intensify research on refining and deep processing techniques to further develop the Chinese rice product industry.

Development strategies

Establishing national development strategies and drawing up a national plan

Establishing national development strategies for the hybrid rice industrial economy, regarding it as a major component of national development strategies for the whole economy, augurs well for the development of agriculture and high-tech industry. This could make possible the transfer of scientific and technical outputs related to hybrid rice, thereby increasing productivity and promoting general competitiveness. As to product strategy, we should focus on developing middle- and high-grade indica rice and high-grade japonica rice. We should concentrate on high-grade edible hybrid rice with long grains and, as much as possible, avoid producing the same quality level and type as Thai and U.S. rice. At the same time, we should pay attention to developing a special type of hybrid rice for industry and forage use. For market development, the strategy should be to engage in market segmentation and localization. High-grade products should be sold mainly to developed countries, whereas middle-grade and middle- to high-grade products should go to developing countries.

Carrying out a national plan is crucial in organizing and synchronizing activities to develop a hybrid rice industrial economy. We should use the same approach in developing the information, robotization, energy, space flight, new materials, and other high-tech industries. Policies must be established to accelerate the development of the hybrid rice industrial economy by providing adequate support and protection in various areas such as trade, financing, taxation, exchange rate, personnel, intellectual property, and system of organization.

The state should consider hybrid rice as a superior agricultural product and bring it into national focus. Efforts must be made in developing the southern China indica hybrid rice industrial belt and northern China japonica hybrid rice industrial belt, setting aside a special rice production area for different purposes in the industrial belt. In double-cropped and single-cropped rice regions of central China, an indica hybrid rice industrial belt in the valleys near the Yangtze River should be targeted.

Likewise, a community of production areas dealing with indica hybrid rice should be established in the Tai Lake region, Dongting Lake region, Poyang Lake region, hilly area in central Jiangxi, Jianghan Plain, Jianghuai region, Sichuan Basin, and Zhujiang River Delta. Japonica hybrid rice, on the other hand, should be grown in the Liao River Plain and the Songhua River Plain. These production bases, forming a community of bases, should support the industrial belt in terms of breeding, seed production, seed processing, rice product processing, comprehensive use of by-products, farmland improvement, machine manufacture, restaurant operation, landscape tourism, and rice culture. Demonstration projects should be carried out using these production bases, especially with respect to investment policy. Problems such as separate targets, less prominent emphasis, and small investment scale should be overcome. Financial support should be available to foster and support leading enterprises and superior regions. The nation should adopt rigorous measures to protect variety rights and patents under the hybrid rice economy.

Maximizing the use of science and technology to ensure a prosperous hybrid rice industry

Every province in the hybrid rice production areas has more than 10 scientific research organizations, and the number of persons engaged in scientific research and education has reached more than 30,000. Of these, nearly 10,000 have advanced degrees. We should make good use of these human resources to actualize the "Strategy of Achieving Rice Prosperity with Science and Technology." First, we should strengthen breeding programs to develop super hybrid rice varieties with high yield, high quality, multiple pest resistance, and wide adaptability. We should aim to make breakthroughs in molecular breeding technology, strengthen the application of biotechnology, and develop a systematic study on C_4-type super hybrid rice. Second, we should strengthen research on high-quality, low-investment, and high-yielding applied techniques; decrease production cost; and ensure food safety by minimizing the accumulation of heavy metals and pesticide residues. At the same time, we should explore the use of cultivation techniques that can adapt to new types of farming systems. Third, we should strengthen research on storage and processing, especially with respect to deep processing. Fourth, we should perfect the scheme to disseminate agricultural techniques through networking; and training of farmers to bring new farmers up to speed on production techniques, market management, and modern administration. Fifth, we should increase investment on science and technology. To realize the goal of achieving rice prosperity with science and technology, governments at all levels should increase scientific research funds to sustain research on the hybrid rice industry.

Establishing a brand name and implementing a brand-leading strategy

A brand name is the symbol of the product market. It gets the benefit, wins the market, and becomes the victor in market competition. At the same time, the brand name adjusts the industrial structure and eliminates inferior products. A brand name needs creation and protection, through which profitability and stability can be reached. Economists say that branding is at the core of commodity market competition. We should establish a rice brand development strategy, cultivating and developing famous brands of rice. We should strengthen the hybrid brand “Longping Rice” and make name brands (such as “JinJian” in Hunan, “AnHe” in Jiangxi, and “ZhengTai” in Hubei) easily recognizable by improving their quality, enlarging their market share, and retaining their good reputation. Leading enterprises should choose readily marketable hybrid rice varieties according to market demand, thus soldifying the production base of high-quality hybrid rice in some countries and producing it as ordered. At the same time, we should develop a regional production hub, encouraging leading enterprises to participate in industrial exploitation of hybrid rice (Qing et al 2006).

Implementing hybrid rice diplomacy to benefit people all over the world

In the past decades, China has been sharing hybrid rice technology with the world through international hybrid rice training and experts’ direct R&D activities outside China. In recent years, great progress has been made in Vietnam, India, the Philippines, Bangladesh, and the U.S. However, this is still far from “developing hybrid rice to benefit people all over the world,” a slogan coined by Yuan Longping. There are persisting problems such as slow international efforts and many barriers. Some developing countries, especially those beset by food shortages, have been showing interest in trying this technology to increase rice production potential. At the same time, more and more foreign technical assistance is being sought every year. Hybrid rice technology could thus be used as a main component of foreign agricultural assistance of the Chinese government. This not only fosters friendship between China and other countries, it could also be a good source of international business. So, it would have important political and economic implications for the state to encourage the development of hybrid rice internationally. Implementing hybrid rice diplomacy results in a win-win situation, provided the following measures are taken. First, we should strengthen the protection of intellectual property and international patent declarations to ensure benefits to China. Second, we should encourage hybrid rice research centers and enterprises to engage in research and exploitation of hybrid rice abroad, and the government should give them financial and policy support. Third, we should remove the policy barrier on exporting hybrid rice techniques. We should fully encourage germplasm and information exchange under international and country IP laws. To satisfy research requirements, the export of a small amount of hybrid rice seeds and breeding materials should be allowed. Fourth, we should share established hybrid rice techniques to ensure the sustainability of technology development.

Strengthening the support system and optimizing and innovating control and management modes

Operation buildup under guidelines of industrialization. Considering the actual conditions of agriculture and the rural areas, two operation modes are proposed. One is an organizational system that comprises “leading enterprises + rice-producing cooperative consortium + major rice households.” Without change in the household-contracting management system, the farmer cooperative consortium, which is built under the principles of equality, free will, and mutual benefit, is useful for managing united production, extending advanced techniques, and controlling production elements, such as land, funds, and methods. The cooperative consortium is also useful for specialized production, regional distribution, and branding. At the same time, it can enforce production standards, unify the brand, control product quality, and improve market competitiveness. The other model is a farmer company—the farmer becomes a shareholder of the corporation by investing resources or other assets. This makes the union structure more stable and the activities more standardized. It is useful for scale operations, focusing resources more on the predominant industry and product.

Developing "green" and pollution-free production systems to realize sustainable development. In the 21st century, what is dominant is ecological agriculture, and the dominant food is green food. We should pay more attention to checking and supervising the food and break through the international trade barrier. At the same time, we should protect the ecology and allow neither the destruction of arable land nor its conversion for nonagricultural use. The agricultural structure should be adjusted and planting fruit trees on arable land must be forbidden as that would destroy productivity. We should make good use of policies that support rice development, strengthen the infrastructure, and improve the hybrid rice production environment in order to sustain its development. We should protect the agricultural environment, reduce pollution, and improve the sanitation quality of rice to ensure food safety.

Strengthening policy support to participate in the international trade system. First, China should join in efforts to constitute new rules of rice trade, try to introduce initiatives, and participate in multilateral trade negotiations about market access and tariff quotas. Second, we should be familiar with WTO and international trade rules; our production and operations should coincide with international trade norms, technology standards, and financial standards. The laws and policies of our country should be adjusted to strengthen the competitiveness of hybrid rice. Finally, we should know the current technology advances, adjust the structure of export commodities, eliminate out-of-date methods of production and products, overcome all kinds of trade barriers, and try to avail of more market space and trade opportunities.

Making reforms in the controlling mechanism of the hybrid rice industry. First, we should cultivate a market entity, go on with the reform on enterprise property, absorb all kinds of social capital, diversify property entities, and perfect the governance of corporate business. At the same time, we should encourage more nongovernment food enterprises to join in the development of the rice industry and to take part in market competition. Second, we should revise the circulation controlling system of hybrid rice. To make the management of the hybrid rice industry more effective, we can incorporate the governmental hybrid rice circulation administration into the agricultural administration and create a special department to manage all links with hybrid rice production, processing, and marketing. Finally, we should create a normal, orderly, and healthy market environment. China should institute a good market information system on hybrid rice, advance standardization procedures, standardize market orders, optimize the market environment, and improve the competitiveness of hybrid rice.

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Author's address: China National Hybrid Rice Research and Development Center, Changsha, Hunan 410125, China.

A short review of the hybrid rice seed industry in China

Yan Weibin, Chen Zhixin, and Hong Haoru[1]

After 30 years of development, the business model of the Chinese hybrid rice industry has changed markedly, from a 100% government business to a nearly 100% market-oriented one. Industry concentration has also increased. But the disorderly competition is still there and will last for a while, such as with the homogenization of products. The limited entry for businesses allows small companies to operate in a very flexible and irregular way, which could give them more advantages in promotion and sales policies. But this is rather harmful to the benefit of growers and other business players who strictly follow government regulations and fully consider the benefit of farmers. Meanwhile, China is turning toward a free-market economy and seed companies, even if not big right now, must become major players in the market. Their weaknesses are obvious—R&D with their own intellectual property and control, human resources with the idea of market competition, investment capabilities, etc. Two things make their survival more difficult—technology upgrading and international competition. This paper gives a brief review of the hybrid rice industry in China, its history, current situation, and development trends.

Keywords: Hybrid rice seed, history, trends

China has been the driving force in the world in the search for and use of hybrid rice heterosis in the past 30 years. Nowadays, the annual planting area of hybrid rice occupies 59% of the total rice planting area and 65% of total rice yield. The large-scale extension of hybrid rice plays an important role in assuring national food security and also accelerates the hybrid rice seed industry.

Since the success of hybrid rice research in 1974 and large-scale popularization starting in 1976, there have been three stages of seed industry development in China.

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[1] Disclaimer: the views in this paper are the personal views of the writers and do not necessarily represent the official views of the Yuan Longping High-Tech Agriculture Co., Ltd.

Stage 1: 1976-95 was the government-planned economy period, with the lead and support of the government. County-level state-owned seed companies were responsible for producing and distributing hybrid rice seeds according to the plans and requests of each regional rice production unit. Four segments played different and separate roles regarding R&D, seed multiplication, promotion, and marketing, including the People's Cooperative (which was a town-level administrative unit), production team (village-level administrative unit), agri-tech station, and seed supply station. In this stage, the advantage was the speed of the extension of new varieties. The main problems were the uniqueness of varieties, R&D deficiency, and lack of incentive to provide elite seeds. During this period, the agricultural system in China changed greatly to a family basis, and the conflict of decentralization of requirements and centralization of supply became an important problem, which was actually a conflict in government planning, operations, and the market economy. Nobody was favored by such an arrangement and nobody was happy.

Stage 2: 1995-99 was the reform period. From that time, the government became aware of the lack of development of the seed industry, such as the conflict between suppliers and customers, and the lack of investment in R&D and seed-processing facilities. To improve the situation, one government program (called the "National Seed Project") was launched to help regional- and state-owned seed companies to improve their facilities and transform themselves from state-owned businesses to market-oriented operations. Regional seed companies were formally split off from seed stations and market-oriented seed businesses started to grow. Meanwhile, the county-level state-owned seed companies went into a decline. Each segment had been mixed. Some public research institutes went directly into a seed business, and a richness of varieties became available. Businesses became competitive and sales terminals were moved to villages and towns. New types of seed companies arose, and some of them became multifunctional with an industry chain involving seed production, seed processing, and seed marketing. The main problem at this stage was the protection of intellectual property because laws were absent, good and bad companies were intermingled, irresponsible sellers swindled farmers by giving them fake seeds, and exaggerated advertisements were popular. There was no clear line between the functions of the government and enterprises. Most companies were small and all-inclusive. This situation became a constraint to the development of the industry.

Stage 3: 1999-now is the development period. With the launch of the "Seed Law" and "Regulations on the Protection of New Varieties of Plants," the hybrid rice seed industry entered a real market-economy stage. Different types of capital entered the hybrid rice seed industry, the same as for the whole seed industry. The seed industry was centralized quickly. Several companies were listed on the stock exchange in China, two of them mainly for hybrid rice. AVA Seed (000918 SZ) made its public offering on the Shenzhen stock exchange in 1999 and LPHT (000998 SZ) made its public offering in 2000. The industry players and the total market volume increased at the same time. The price of varieties with intellectual property grew rapidly, and the seed commercial rate increased. Various types of competition with a sales network, sales promotion, commercial campaign, and service upgrading, etc., among largesized companies emerged. Homogenization of product, infringement and confusion of varieties, seed quality irregularities, and extensive management were the main problems of this period, and the supply was exceeding demand.

Analysis of the current situation of the hybrid rice seed industry

The market environment of seed businesses in China has matured after 30 years of development. After the (2006) #40 Document issued by the State Council, agricultural administrative authorities were no longer permitted to have their own seed business or investment in seed companies, and marketing enterprises gradually became mainstream in the market. Industry concentration also increased. There is no doubt that those companies that had comprehensive advantages in R&D, production, and sales would be the market leaders. However, as the industry is still young, low standards of access to the industry will still allow the existence and founding of small companies. The hybrid rice seed market is still disordered. Under such circumstances, disorderly competition will last for a certain time.

Industry concentration

Industry concentration is still low, although it has risen in recent years, and administrative forces still play an important role in the market.

Because of the implementation of a long-time government-planned economy, the regulations and rules for seed industry administration, especially for its implementation, are still not completed. The industry also still needs some time to change its way of thinking as well as the behavior of agricultural administration agencies. The quantity of players in the market is too many and the seed supply is overly abundant, but the product (seed) has a short life, and dumping of seeds onto the market becomes a normal practice, which makes the whole industry unprofitable and decentralized. The limited business scale and low profitability of operations put strong pressure on seed companies to invest in their R&D, human resources, and facility improvements. Of course, there is some good news for the industry. With the promulgation of the Seed Law and Protection Regulations for New Plant Varieties, seed production bases are linking to large companies rapidly. The top five seed companies in Hunan, Sichuang, and Jiangxi got more than half of the market share based on market research in each of these three provinces. All kinds of private seed operators are going to the market while old state-owned seed companies become bankrupt; hence, industry concentration is increasing.

Another important issue is the “Elite Seed Subsidy Program” and other favored policies that were originally launched to stimulate growers to choose elite seed, encourage them to make plantings, and improve returns. Such policies did not make as good an impact as predicted, as the labor costs and price of fertilizer increased drastically for the major seed companies. The times became even more difficult as the list of “Elite Seeds” should be decided by the local agricultural bureau, which did tremendous work for communication with each local bureau. This program also became a barrier for national seed players rather than local ones, which in some places became a new barrier for the protection of local small seed companies.

Research and development

Public research institutes and universities are the main forces in hybrid rice research, and resources are still concentrated in these organizations, such as genetic materials, human resources, and facilities, and they receive around 95% of the public financing in this field. Companies started their own R&D mostly after 2000 and all major companies have realized their lacks and are making an effort to build up their own research. Public research organizations are still very active in hybrid rice research and, for the top five hybrid rice ones, their investment in conventional programs surpasses that of the top five companies, and they are additionally financed by the government for upstream bio-tech programs, which is very good for the industry and the whole country. The threat to the industry is that such public organizations will go directly to seed businesses and compete with the seed companies in the market while they are financed by the taxpayers. So, the government and the whole industry have a lot to do to properly link the results of public research to private business, and to promote and help the private sector to transform its strengths from conventional methods to modern technology.

Seed production

Seed production is low, and supply exceeding demand is the normal condition. Hybrid rice seed production in China is still continuing in the form of sowing and harvesting by individuals. Mechanized production is restricted by the land form, which leads to seed production scale not being economical. In addition, standardized seed production can't be achieved and seed quality can't be improved. At the same time, because of over-anticipation of the market, the supply of hybrid rice seeds exceeded demand in recent years.

Planting area of hybrid japonica rice

The planting area of hybrid japonica rice is not growing fast. When compared with hybrid indica rice, the development of hybrid japonica rice was slow before 2005. According to statistics, in the mid-1980s, the planting area of hybrid japonica rice occupied 2% of the whole japonica rice area. Even in the northern rice area, it occupied about 6%. In the early 1990s, because research on hybrid japonica rice was still limited, the planting area occupied 1% of the entire japonica rice area. The reasons for this phenomenon were as follows: the growth duration of hybrid japonica rice is long, and the effective accumulated temperature can't meet the requirement, so the yield advantage can't be achieved. Hybrid japonica rice has shortcomings in resistance, quality, and reaction to temperature and photoperiod, leading to a high empty-grain rate, low seed production yield, and low purity. In recent years, the planting area of hybrid japonica rice has been increasing. In some provinces, the planting of hybrid indica rice has been changed into hybrid japonica rice. For example, rice production in Jiangsu Province, which used to be indica rice, now has nearly all the area in japonica rice. Japonica rice is welcomed by customers in domestic and overseas markets because of its good grain and eating quality. So, if the key technical problems such as yield advantage, quality, and seed production could be solved, the sowing area could increase quickly.

Main constraints to the development of the hybrid rice seed industry

As stated above, the scale of seed enterprises in China is generally small. Until now, no hybrid rice seed enterprise has net capital or annual hybrid rice seed sales above 1 billion RMB. Their history in research is short, and capabilities of further investment are very limited. The average profit of this industry does not allow quick internal growth. The industry also suffers from a serious lack of human resources, especially talent with an open mind, market-oriented way of thinking, and learning spirit.

The trend of hybrid rice seed industry development

Every coin has two sides. Although the industry is suffering from hard times, some companies are changing their business model, from sales volume-oriented to profit-oriented, from business-scale advantages to variety advantages, and from technology pioneering to comprehensive competitive ability. The government has also become aware of some of the main problems in the industry and is making step-by-step efforts. Generally speaking, the hybrid rice seed industry is moving into an upgrading period, and, with the increase in food prices, seed businesses, which were the very upstream bottleneck of agriculture, not only caused concern about the industry itself but also attracted more attention from outside of the industry. More players are entering the seed business, and capital investment in the industry is increasing. Market concentration increases fast as foreign seed companies gain access to this market. Major seed companies are paying more attention to brand awareness, talent, R&D, corporate governance, management, and service improvement.

Competition goes from product and price to the value of supply and services. Companies focus on farmers' awareness of their brands rather than on retailers. With an increase in their strength and competition, companies started research programs with their own intellectual property rights. As their advantages are obvious, such as the experience of breeders, vast resources of genetic materials, and rich experience in seed production, once the awareness is there, action will be very fast and results will emerge quickly. Together with an increase in resource inputs to the hybrid rice seed business, Chinese hybrid seed businesses will steadily enter the international market, which requires a higher standard for companies, especially in their human resources. In the meantime, this enables companies to have a larger market size and business scale. After 5 to 8 years' effort, it appears that some Chinese hybrid rice companies, such as LPHT, will surely become an internally operated seed organization, and that small companies will turn their position from a full-range seed company to a specialized actor, and business concentration in the Chinese market will increase, so that the industry could become re-organized in the coming years.

Notes

Authors' address: Yan Weibin, Chen Zhixin, and Hong Haoru, Yuan Longping High-Tech Agriculture Co., Ltd., 9f Securities Building, No. 459 Chezha (N) Road, Changsha, Hunan 410001 China.

The International Treaty on Plant Genetic Resources for Food and Agriculture: implications for management of intellectual property[1]

Ruaraidh Sackville Hamilton

The concept of intellectual property (IP) was formulated to encourage creativity and innovation. In the area of crop genetic resources, the goal is to encourage the development of new varieties and associated discoveries. The principal devices for IP protection in this area are trade secrets, patents, plant variety protection, and material transfer agreements. However, these new varieties and associated knowledge are derived from traditional varieties and wild crop relatives, which are under the 1993 Convention on Biological Diversity (CBD), and therefore governed by a totally different set of concepts relating to the protection of biodiversity and sovereign rights.

The International Treaty on Plant Genetic Resources for Food and Agriculture ("the Treaty") fills the gap between the CBD and IP protection, establishing a pathway that links the exchange of raw genetic materials under the CBD to the creation of new products such as improved varieties. It defines new rules for germplasm exchange, linked to commercialization and IPR protection. This paper addresses the implications for the documentation and protection of intellectual property. Although there are no fundamental changes in operating practices for research, breeding, and commercialization of varieties, breeders need to know the new rules and how to apply them to their systems for managing IP, tracking pedigrees, monitoring sales, and protecting their intellectual property. For breeders that already have such systems in place, as already required for other purposes, adapting procedures to comply with the Treaty is straightforward.

This paper assesses the implications of The International Treaty on Plant Genetic Resources for Food and Agriculture (the "Treaty") for the management of intellectual property on genes and improved varieties of crops. To do so, it is necessary first to discuss the concept of intellectual property as it applies to genes and varieties of crops, the range of international agreements relevant to rights to use crop genetic resources, and the rights and obligations set out by the Treaty on the conservation and use of crop genetic resources.

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[1] Disclaimer: although the information here is presented in good faith, it is not legally authoritative. Readers should consult the legal texts of the international agreements discussed in this paper or their countries' authorized representatives.

Intellectual property

The concept of intellectual property (IP: "creations of the mind"; www.wipo.int) was formulated to encourage creativity and innovation. The invention of a new product and its delivery to the market place require intellectual investment, not just in having an idea, but also in research and development to design, test, and enhance the product until it is ready for commercial sale. If the knowledge acquired by the inventor during the process is freely available to competitors, those competitors would have a commercial advantage: they would not need to use a proportion of their sales to pay for the initial investment in R&D, and therefore they would be able to market the product more economically than the inventor. This scenario places inventors at a disadvantage, thus discouraging innovation.

The concept of claiming and protecting intellectual property rights (IPR) was introduced to avoid this problem by giving the inventor preferential marketing rights that provide an opportunity to recoup the initial investment. The inventor's rights are protected for a fixed limited duration, after which the market is opened to free and fair competition.

The World Intellectual Property Organization (WIPO) establishes that all in its 184 member states (listed at www.wipo.int/members/en/) have the right to protect their own IP. How these rights should be recognized was negotiated through the World Trade Organization (WTO www.wto.org) in the "TRIPS agreement" (Trade-Related aspects of Intellectual Property rights). Originally agreed upon in 1994 (text at www.wto.org/english/tratop_e/trips_e/t_agm0_e.htm), amendments were approved in 2005 but have not yet taken effect. Some details of the TRIPS agreement remain disputed, including the sections on protecting genes and varieties of plants. However, the basic principle remains intact: each of the 153 member countries of the WTO must establish a legal system enabling the protection of IPR.

Protecting IPR on crops

Four forms of IPR protection are particularly relevant to crops. Two of them are addressed in article 27.3(b) of the TRIPS agreement, by which members may exclude plants and/or their genes from patentability, but must "provide for the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof."

Patents. As indicated above, under TRIPS article 27.3(b), a country may choose to provide for the protection of genes and/or varieties by patents. This form of protection provides full protection over use of the genes or varieties within the country where the patent claim is filed. Patents do not provide international protection except by patent agreements between countries.

Plant variety protection (PVP). *Sui generis* (literally "of its own kind"—meaning each member country can work out its own system for protection) PVP is an alternative or complement to patents for plant varieties. Like patents, it provides only national protection, except through specific agreements between countries. The best known internationally-agreed-upon *sui generis* PVP system is UPOV (Union internationale pour la Protection des Obtentions Végétales, or the International Union for the protection of new varieties of plants: www.upov.org), currently with a membership of 65 countries. Two important features of UPOV are that

- It distinguishes between use for commercial sales and use for further breeding and research. It recognizes and protects a breeder's right to market a variety, but it denies the breeder any right to prevent others from using the variety for further breeding: seeds must be made freely available to others for further breeding and research.
- It provides international protection, in the sense that no one else can claim PVP over the same variety in other UPOV countries. However, like other forms of protection, the extent of protection of the breeder's right to market is only national.

Trade secrets. An inventor is entitled to protect IPR by denying access to information or materials. This is common practice in all sectors. Even in organizations working for the global public good, scientists sometimes choose to restrict access to their data at least until the results have been published (thus proving "prior art" and preventing others from claiming the discovery as their own). In the private sector, plant breeders typically seek to gain competitive advantage by restricting access to key information such as the pedigree data.

Material Transfer Agreements (MTAs). An MTA is a legally binding contract, usually between two parties, specifying the rights and obligations of each party when material is transferred from one party (the provider) to the other (the recipient). It is typically used when there is a need to protect IPR by specifying in detail what rights the provider and recipient have over the material, that is, what they are authorized to do if they wish, what they are obliged to do, and what they are forbidden to do. Unlike patents and PVP, the legal scope of an MTA can be global, enforceable under any specified national or international legal system.

International frameworks governing genetic resources

Time-bound legal protection of IPR on improved varieties and genes under TRIPS deals only with one element of rights to use germplasm. Other elements include rights over wild relatives and traditional varieties, and the rest of the precommercial chain from genetic resources in the field to using research for development.

A key international agreement addressing precommercial elements of the chain is the 1993 Convention on Biological Diversity (CBD; www.cbd.int). The CBD establishes the sovereignty of each of its 191 member nations over all biodiversity present in and originating in its territory, including not only wild species but also traditional varieties, breeding lines, and other crop genetic resources. If one nation wishes to gain access to genetic resources under the sovereignty of another nation, sovereign rights include the right to grant or deny access, the right to know and negotiate the intended use of the genetic resources by the requesting nation, and the right to share in any benefits that may arise from such use. In marked contrast to the time-bound nature of IPR protection under TRIPS, protection of sovereign rights under the CBD is indefinite.

Since germplasm governed by the CBD provides the basic raw material for all improved varieties protected under TRIPS, it is necessary to link these two international frameworks. The Treaty (www.planttreaty.org) provides an agreed-upon standard link between the two for a defined set of plant genetic resources for food and agriculture. Like all international agreements, the Treaty applies only to its member countries: for countries that are members of the CBD but not the Treaty, the gap between the CBD and commercial use of crop varieties remains.

The International Treaty on Plant Genetic Resources for Food and Agriculture

The Treaty governs crops and their wild relatives that are considered essential for food security and for which countries are mutually interdependent. This includes all species of the genus *Oryza*, that is, cultivated rice and its wild relatives (but not of the genus *Zizania*, even though it is commonly sold under the name “wild rice”).

The 120 members of the Treaty have agreed upon and adopted a single standard system for the transfer of plant genetic resources for food and agriculture (PGRFA) in a “Multilateral system of access and benefit-sharing” (MLS). Under the MLS, members agree to facilitate access to genetic resources under their control and to ensure the equitable sharing of benefits that arise from their use. When PGRFA are transferred to a recipient, the recipient is authorized to use the material for breeding, research, and training for food and agriculture, and to protect and commercialize derived products; and is required to share benefits of use. The nature of benefit sharing is linked to the nature of commercialization and IPR protection.

The research centers of the Consultative Group on International Agricultural Research (CGIAR) have signed agreements with the Governing Body, committing them to share genetic resources under the same conditions as member countries.

The Standard Material Transfer Agreement (SMTA)

Each transfer of genetic resources under the Treaty from a provider to a recipient must be governed by a Standard Material Transfer Agreement (SMTA). This is a legal contract under international law between the provider and the recipient, specifying the rights and obligations of the provider and recipient over the germplasm being transferred. The text of the SMTA, available in six languages at www.planttreaty.org/smta_en.htm, was negotiated by the Governing Body of the Treaty and must not be changed. This section summarizes the key rights and obligations of the provider and recipient that have implications for the management of IP.

Rights of the provider. If the provider is providing access to “PGRFA under development” (essentially breeding lines developed from germplasm previously received with an SMTA), ancillary conditions may be attached to the SMTA, provided these do not change or conflict with the other terms and conditions of the SMTA.

Obligations of the provider. The provider must

- Provide access expeditiously, without the need to track individual accessions, and either free of charge or for a fee not exceeding the minimal cost involved.
- Grant access to all available passport data and any other associated available nonconfidential descriptive information.
- If transferring germplasm protected by intellectual or other property rights, ensure that the transfer is consistent with relevant international agreements and national laws.
- Inform the Governing Body about the transfer, according to a schedule to be established by the Governing Body.

Rights of the recipient. Subject to certain conditions, the recipient is authorized to undertake any activity that is a normal part of the fair use of genetic resources for improving agriculture. The recipient may

- Conserve the germplasm.
- Use it for breeding, research, and training for food and agriculture.
- Develop and commercialize products derived from it.
- Claim IPR over the product(s) that the recipient develops from the material received.
- Distribute samples of the original received germplasm to others.
- Distribute derived breeding and research materials to others.
- Add further conditions to distribution of breeding and research materials.

Obligations of the recipient. The following actions are prohibited for the recipient:

- The recipient must not use the germplasm for “chemical, pharmaceutical, and/or other nonfood/nonfeed industrial uses,” or any other purpose except research, breeding, and training for food and agriculture. The Treaty and the terms and conditions of the SMTA were negotiated on the assumption of conservation and use for food and agriculture; other uses fall outside the scope of the Treaty, and the conditions of the SMTA are not necessarily appropriate for such uses.
- The recipient must not claim “intellectual property or other rights that limit the facilitated access to the material…, or its genetic parts or components, in the form received….” This careful choice of words does not merely prevent the recipient from claiming IPR over the germplasm received; it also prevents any form of claim that restricts access to the original germplasm or its genetic parts or components. For example, if the recipient discovers a gene in the germplasm received and seeks IPR on the discovery, the form of IPR claim over the gene cannot restrict access to that gene in the original germplasm.

The following actions are obligatory for the recipient:

- The recipient must make available all nonconfidential information resulting from the recipient’s own R&D on the germplasm.

- If the recipient chooses to conserve a sample or copy of the germplasm, (s)he must make it available to others.
- If the recipient transfers samples of the original germplasm or derived breeding lines or other materials to a third party, the transfer must be subject to a new SMTA in which the recipient is now the provider, and as such must comply with the provider's obligations.
- If the recipient commercializes a product (e.g., improved variety) developed using germplasm received with an SMTA, then one of two conditions applies depending on the availability of the product:
    - If the product *is not* available without restriction to others for further breeding and research, the recipient *must* pay to the Governing Body annual payments amounting to 0.77% of the gross sales of the product. The percentage does not depend on how much of the genome of the product is derived from germplasm received with an SMTA: 100% or just one gene both incur the 0.77% annual payment. The recipient must also prepare an annual report on his/her liability to payment and submit it to the Governing Body together with the payment.
    - If the product *is* available without restriction to others for further breeding and research, the recipient *is encouraged to* make the same annual payments as described above.
- As an alternative to the financial benefit-sharing mechanism described in the previous bullet point, the recipient may opt for an alternative form of payment. The alternative involves a lower rate (0.5%), payable on the sales of all products of the same crop for 10 years renewable starting on the date of the first SMTA, regardless of whether or not the products are derived from the received germplasm, and regardless of whether or not the products are available without restriction on use for further breeding and research. After 10 years, the recipient may choose to continue this alternative scheme, or to change to a scheme that is the same as the standard except that the lower rate of 0.5% applies. If the recipient chooses this option, (s)he must inform the Governing Body.

Implications of the Treaty for the management of intellectual property

Based on the factors set out above, this section addresses implications for two aspects of IP management: the need for documentation and the need for protection.

Documenting the IP status of each line

The rights and obligations of a holder of germplasm differ between germplasm samples. It is therefore necessary to document the status of each germplasm sample. The following categories of germplasm must be distinguished:

1. Germplasm to which the holder is bound to grant access on demand using the SMTA, provided the requestor is in a country that is party to the Treaty or an international organization such as IRRI that has signed an agreement

with the Governing Body, and provided the intended use of the material is for research, breeding, or training for food and agriculture. This includes

- Germplasm of crops listed in Annex 1 of the Treaty, in the public domain and under the management and control of a contracting party to the Treaty.
- Germplasm held by an international organization that has signed an agreement with the Governing Body placing the germplasm under the multilateral system.
- Germplasm received with an SMTA, if the recipient still conserves it. Note that this obligation applies to any recipient anywhere, regardless of the status of the recipient or of the recipient's country.

2. Germplasm to which the holder is not required to grant access, but in the event that access is granted it must be with an SMTA, optionally with ancillary conditions agreed upon bilaterally between the holder and recipient. This applies to "PGRFA under development," typically breeding lines developed by the holder with at least one sample received with an SMTA in its ancestry.
3. Germplasm that is not subject to the SMTA but which the holder may voluntarily make available with an SMTA without ancillary conditions. This applies to germplasm that is not legally protected by IPR with contrary conditions and which is held by any individual or organization:
   - in a country that is a party to the Treaty (other than germplasm in the public domain and under the management and control of the government, which is addressed in the first bullet point above)—all such individuals and organizations are formally encouraged by their government to grant access to the germplasm they hold, using the SMTA;
   - in a country that is not a party to the Treaty but is a party to the CBD, and the individual or organization has the required authorization from the country's CBD authorities; and
   - in a country that is not a party to the Treaty or to the CBD.
4. Commercialized varieties and other products that contain in their ancestry at least one sample received with an SMTA and that either are not available to be used by others for further research and breeding or are available but subject to legal or contractual obligations or technological restrictions that preclude using them in the manner specified in the Treaty. This includes not only varieties with IPR formally protected under law using a form of IPR protection that precludes their being distributed with an SMTA, but also varieties that for any other reason cannot be so distributed.
5. Commercialized varieties and other products that contain in their ancestry at least one sample received with an SMTA and that are available to be used by others for research and breeding without any legal or contractual obligations, or technological restrictions, that would preclude using them in the manner specified in the Treaty. These must be distributed with an SMTA on demand—if they are not, they would be implicitly grouped with varieties

in the previous bullet point that are not available without restriction. This includes

   - Varieties commercialized without legal protection of IPR.
   - Varieties with IPR protection that does not preclude distribution with an SMTA. This should normally include IPR protection with UPOV-compliant PVP.

6. Germplasm that is not subject to an SMTA and must not be distributed with an SMTA. This includes

   - Germplasm under the sovereignty of a country that is not a party to the Treaty but is a party to the CBD, held without permission from the country's CBD authorities to distribute with an SMTA.

The need to document the IP status of germplasm already existed years before the SMTA was introduced to ensure compliance with the CBD and with IPR laws and to prove the germplasm holder's "freedom to operate" (a legal term describing someone's right to undertake an action, in this case to use germplasm). Thus, the Treaty has merely modified the categories of germplasm, without changing the concept of documenting IP status.

Documenting pedigrees

A breeder must use an SMTA for distributing breeding lines that have breeding lines with pedigrees that include one or more samples received with an SMTA. In addition, in this case, the provider must identify in annex 1 of the SMTA all such ancestral lines received with an SMTA. This obligation persists indefinitely, regardless of the number of crosses or generations of selection between the breeding line and the ancestors received with an SMTA. Similarly, commercializing a variety bred from material received with an SMTA triggers relevant benefit-sharing clauses of the SMTA—again indefinitely, regardless of the number of crosses or generations of selection between the breeding line and the ancestors.

All of these factors require an effective pedigree management system enabling breeders to document pedigrees of their breeding lines and varieties indefinitely, together with their associated IP status.

The need to document pedigrees is not new to the Treaty. IPR protection by patents and PVP in many countries requires disclosure of the origins of improved varieties. Accurate knowledge of pedigrees is also essential scientifically for many genetic analyses and hence for more efficient breeding. Thus, again, the introduction of the SMTA has not fundamentally changed the requirements for documenting the breeding process, but has only introduced a new reason for doing so.

Recording gross sales by variety

Annual payment of a fixed percentage of gross sales to the Governing Body of the Treaty is normally mandatory only on sales of commercial varieties that include in their pedigree at least one sample received with an SMTA and that have IPR protection restricting their use for further breeding and research. Compliance with this requirement therefore requires a breeder to record gross sales for each variety except in the following situations:

- The breeder chooses to make voluntary payments on sales of all varieties, not just the obligatory payments.
- The breeder commercializes varieties without restricting access for further breeding and research, so that no payment is required.

Again, this is not a new requirement. Breeders should normally record their sales by variety as part of planning their breeding and seed production activities. Thus, the SMTA merely introduces an additional reason for recording this information.

Protecting IPR on breeding lines

The SMTA requires that it be used for all distribution of breeding lines derived from material received with an SMTA (described in the SMTA as "Plant Genetic Resources for Food and Agriculture under Development"). However, breeding lines do not have to be shared at all, and, if they are shared, the SMTA allows ancillary conditions to be attached to them. This allows considerable flexibility for protecting IPR on breeding lines using a Material Transfer Agreement as the legal instrument. It could, for example, be used to protect trade secrets by denying any access to breeding lines, or by providing them to collaborators while preventing them from publishing confidential information or sharing the germplasm with competitors, or seeking royalty payments from the recipient, or requiring that the recipient reports to the provider all use of the material.

The definitions of phrases given in the SMTA must be read carefully to understand the scope of this option. In particular, "Plant Genetic Resources for Food and Agriculture under Development" means material derived from the **Material**,[2] [where **Material** refers to the PGRFA transferred under the SMTA] … The period of development for the **Plant Genetic Resources for Food and Agriculture under Development** shall be deemed to have ceased when those resources are **commercialized** [where to **commercialize** means to sell a **Product** or **Products** for monetary consideration on the open market] as a **Product**.

Thus, attaching ancillary conditions to an SMTA is permitted only for breeding lines derived from germplasm received with an SMTA, and the option remains possible right up to the moment the line is sold on the open market. In many cases, there may be a protracted period of testing and registration after a new variety has been created before it can be protected and commercialized. Thus, the period of "development" includes not only genetic development through breeding but also the subsequent period of testing and registration when the period of genetic development has finished.

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[2] Bold type face in the SMTA is used for terms that are defined within the SMTA. Thus, bold capitalized "**Material**" refers to the PGRFA transferred under the SMTA, whereas "material" refers to other material; and bold capitalized **"Product"** means **Plant Genetic Resources for Food and Agriculture** that incorporate the **Material** or any of its genetic parts or components … whereas "product" refers to other products.

Protecting IPR on commercial varieties

The option chosen by breeders for protecting IPR on their varieties affects the benefit-sharing obligations. In particular, financial benefit sharing, to the extent of 0.77% of gross sales, is obligatory for a variety developed from germplasm received with an SMTA if it is "not **available without restriction** [i.e., "when it is available for research and breeding without any legal or contractual obligations, or technological restrictions, that would preclude using it in the manner specified in the Treaty"] to others for further research and breeding," but that payment is voluntary if the variety is available without restriction to others for further research and breeding. Breeders may wish to take this into account when deciding how to protect IPR on commercial varieties.

Typically, it is assumed that protection by patents will normally restrict use by others for breeding and research, whereas PVP under UPOV does not. In this case, IPR protection by patents would trigger obligatory benefit sharing whereas PVP under UPOV does not. However, neither case is necessarily true. Patented varieties could be freely available for further breeding and research. Under UPOV, the "breeder's exception," by which a protected variety must be freely available for use by other breeders, may be implemented only nationally, thus restricting use for breeding and research in other countries and thus triggering obligatory benefit sharing. Indeed, even a variety commercialized without any form of IPR protection would trigger obligatory benefit sharing if the breeder does not make the variety available without restriction to others for further research and breeding.

Thus, the critical question to be addressed by a breeder is not just the general form of IPR protection to be sought on the breeder's varieties. Rather, the breeder has to decide among

(a) restricting access for further breeding and research, paying the obligatory 0.77% annual royalty fee incurred by this choice;
(b) opting for the alternative form of financial benefit sharing, paying 0.5% of gross sales on all products of the crop;
(c) providing free access to anyone for further breeding and research and not making any payments; and
(d) providing free access to everyone for further breeding and research and making voluntary 0.77% payments.

Conclusions

In conclusion, the Treaty introduces new rules for germplasm exchange, linked to commercialization and IPR protection. The new rules have several implications for the management of intellectual property. However, these implications involve no fundamental changes in operating practices for research, breeding, and commercialization of varieties. Breeders need to know the new rules and how to apply them to their systems for managing IP, tracking pedigrees, monitoring sales, and protecting their intellectual property. For breeders that already have such systems in place, as already required for other purposes, adapting procedures to comply with the Treaty should provide little problem.

Notes

Author's address: International Rice Research Institute, Los Baños, Philippines.

Economics of hybrid rice in tropical Asia: major issues and opportunities

Sushil Pandey and Humnath Bhandari

Hybrid rice accounts for more than 50% of the total rice area in China and it is now spreading in tropical Asia also. However, its spread in tropical Asia has been somewhat slow and patchy despite its considerable potential yield advantage over inbred varieties. Adoption patterns across the major rice-growing countries of Asia are analyzed in this paper and economic and institutional factors that have limited the spread are analyzed. The likely future economic impact of hybrid rice is assessed and some guidelines on future targeting of hybrid rice are suggested. Finally, a two-pronged strategy consisting of increased investment in research and promotion of hybrid rice is suggested.

Of the world's 1.1 billion poor people, almost 700 million people with income of less than a dollar a day reside in the rice-growing countries of Asia. Rice is a staple food in Asia and it accounts for more than 40% of the calorie consumption. Poor people spend as much as 30–40% of their income on rice. The amount of rice produced and prices are thus important factors in determining progress toward poverty reduction. Keeping the price of rice low and affordable to the poor is crucial in this regard. The critical role of yield-increasing technologies for poverty reduction was clearly demonstrated by the Green Revolution that led to rapid growth in rice productivity and a long-term decline in rice prices from 1975 to 2000.

The food crisis of 2008 clearly indicates that there has been an imbalance between the long-term demand and supply growth of rice. Yield growth of rice in recent years has been slower than population growth. This has resulted in a supply shortfall, which can be overcome in the long run only by boosting the production growth rate. This production growth must ultimately come from an increase in yield growth as the possibilities for expansion of rice area are now limited.

In this context, hybrid rice provides an important opportunity for boosting the yield growth of rice. It has a substantial potential yield advantage over inbred varieties and new scientific approaches for incorporating desirable traits into hybrids offer considerable promise. Despite these scientific opportunities, there are some critical socioeconomic, institutional, and policy factors that determine the pace at which farmers will take up this important technology. The spread of hybrid rice technology has

Table 1. Research status and adoption rate of hybrid rice in Asia, 2008.

Country	Year research initiated[a]	Year of first hybrid rice released[b]	Hybrid varieties released until 2008 (no.)[c]	Hybrid rice adoption rate in 2007		
				Total rice area (000 ha)	Hybrid rice area (000 ha)	Adoption rate (% of total rice area)
Bangladesh	1996	1998	57	11,000	647	5.9
China	1964	1976	Over 1,000	28,919	15,786	54.6
India	1989	1994	33	43,770	1,200	2.7
Indonesia	1998	2001	35	11,900	135	1.1
Malaysia	2004	2007	1	660	1	0.2
Myanmar	1997	2003	1	7,085	45	0.6
Pakistan	2001	2003	7	2,550	61	2.4
Philippines	1993	1994	13	4,346	247	5.7
Sri Lanka	1994	2005	1	800	0.2	0.0
Thailand	1991	2007	2	10,600	[d]	0.0
Vietnam	1992	1992	58	7,412	600	8.1
IRRI	1979	–	–	–	–	–
Asia	Since 1964	1976	Over 1,200	137,547	18,722	13.6

[a]Year of mission mode R&D initiated. [b]Most countries used Chinese hybrid varieties for their research, development, and commercial cultivation. [c]Includes both nationally produced as well as imported and locally tested varieties. [d]Negligible area under hybrid rice.
Source: various publications (available upon request from authors).

been limited and somewhat patchy in tropical Asia. This paper focuses on analyzing these factors and offering strategies to move forward.

Hybrid rice adoption patterns in Asia

Hybrid rice has been developed in Asia for more than 30 years. Hybrid rice varieties for commercial cultivation have been released in 11 countries. Current hybrid rice area in Asia is approximately 19 million ha (14% of the total rice area), with nearly 85% being in China (Table 1). Area under hybrid rice in China reached 15% (5 million ha) within five years after the release of the first commercial varieties in 1976. The spread of hybrid rice continued to increase and, by 1990, more than 50% of the rice area in China was under hybrids. Its share in the total rice area peaked at 60% in 2003, but, since then, has decreased slightly and has stabilized at around 55% (Fig. 1).

Several factors contributed to the early success and fast development of hybrid rice in China (David 2006, Mao 2008). First, a centrally planned governing system directly influenced farmers' behavior and facilitated faster diffusion. Second, more

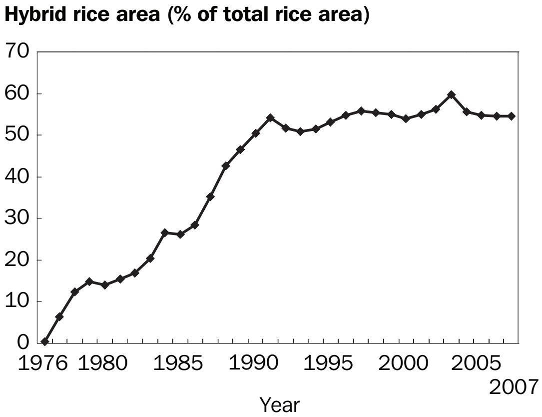

Fig. 1. Hybrid rice adoption pattern in China, 1976-2007.

than 90% of the rice area is irrigated and transplanted, which means that the production environment and crop establishment method are conducive to hybrid rice adoption. Third, until the late 1980s, rice quality was less of a concern under the quota system of rice marketing, in which farmers were obliged to sell a portion of their rice production at a predetermined price regardless of the grain quality. Finally, strong government support and heavy investment in research and development, seed production, and distribution of hybrid rice occurred up to the 1990s.

Outside China, hybrid rice area is only 2.9 million ha, which is less than 3% of the total rice area in Asia (Table 1). Only five countries have more than 100,000 ha under hybrid rice. Hybrid rice area is highest in India (1.2 million ha), followed by Bangladesh, Vietnam, and the Philippines. The adoption rate is highest in Vietnam (8.1%), followed by Bangladesh, the Philippines, India, and Pakistan (Fig. 2). In Vietnam, hybrid rice area steadily increased from less than 0.1 million ha in 1995 to 0.6 million ha in 2005 and has since leveled off somewhat. It is concentrated in the north and central regions where farmers' cooperatives and state farms are heavily subsidized and strongly influenced by government decisions. Similar agroecological, political, socioeconomic, and institutional settings, as in southern China, favored the spread of Chinese hybrids in these regions (David 2006). However, hybrid rice adoption has been minimal in southern Vietnam, which is a major rice bowl.

In Bangladesh, India, and Indonesia, hybrid rice adoption has been expanding rapidly in irrigated areas in recent years. Strong public investment in research and development, active promotional activities, provision of seeds at subsidized prices, tax breaks to seed companies, active participation of the private sector, international collaboration, and spillover effects of Chinese hybrid rice technology are considered key factors contributing to the fast adoption of hybrid rice in these countries. In the Philippines, a hybrid rice commercialization program began in 2001. Within five years, the adoption rate reached 10%, but it did not sustain itself, and declined to 6%

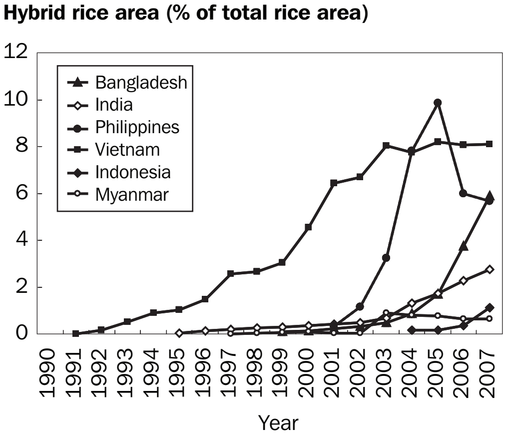

Fig. 2. Hybrid rice adoption pattern in tropical Asia, 1990-2007.

in 2007. The provision of a subsidy on seeds and other inputs has been a key for the fast initial diffusion of hybrid rice technology in the Philippines (Sebastian et al 2006). A gradual phase-out of the seed and input subsidies resulted in a decline in hybrid area in 2006 and 2007.

Farm-level studies in different countries reported a fast spread but also a fast dropout of many farmers from hybrid rice cultivation (Janaiah and Hossain 2003, Casiwan et al 2006, Mustafi et al 2006). The technology is still in the tryout phase among farmers, with most adopters planting only a small proportion of rice area under hybrids. Current coverage in the Asian tropics is small despite the active promotion of hybrid rice technology by both the public and private sector.

Economics of hybrid rice production

Farm-level studies in different countries in Asia demonstrated an average yield of 6.6 t ha^{-1} for hybrid rice and 5.7 t ha^{-1} for the best-yielding inbred rice—0.9 t ha^{-1} higher yield of hybrids (Table 2). The yield advantage of hybrids over inbreds averages 17%, with a range of 2–28% (0.1–1.6 t ha^{-1}) across countries. The incremental gross return across countries ranged from −3% to 24%. Despite the higher yield advantage, a lower market price reduced the incremental benefits of hybrids over inbreds. Most hybrid varieties fetch a 5–10% lower market price than inbred varieties because of poor grain quality. This lower price thus reduces the benefit from the yield increase. For example, in India during the 2001 crop season, hybrids produced 13% higher yield than inbreds, but a lower price in the market resulted in a 3% lower gross return than for inbreds. The lower price of hybrid varieties was due to the perceived low quality of the hybrid rice grain (Hossain et al 2006).

Table 2. Yield advantage and profitability of hybrid rice cultivation in Asia, 2008.

Country/ season	Yield			Gross return[g]			Total costs			Net returns		
	Hybrid (t ha^{-1})	Inbred (t ha^{-1})	Diff. (%)	Hybrid ($ ha^{-1})	Inbred ($ ha^{-1})	Diff. (%)	Hybrid ($ ha^{-1})	Inbred ($ ha^{-1})	Diff. (%)	Hybrid ($ ha^{-1})	Inbred ($ ha^{-1})	Diff. (%)
Bangladesh[a]												
2005 boro	8.2	6.6	24	1,146	937	22	547	524	4	599	412	45
2007 boro	7.3	6.0	21	1,294	1,043	24	808	820	–1	486	222	119
China[b]												
1984	7.4	6.4	16	976	827	18	512	488	5	464	339	37
2004	6.6	5.3	24	–	–	–	–	–	–	–	–	–
India[c]												
2001	6.8	6.0	13	845	869	–3	377	320	18	468	549	–15
2002	6.4	5.2	23	706	587	20	450	414	9	257	173	48
Indonesia[d]												
2003 WS	7.0	5.4	28	–	–	–	–	–	–	–	–	–
2008 DS	7.5	6.4	17	–	–	–	–	–	–	–	–	–
Philippines[e]												
2003 WS	5.5	5.1	8	861	793	9	530	493	7	331	300	10
2004 DS	5.3	4.8	11	962	835	15	564	539	5	398	297	34
Vietnam[f]												
2004 WS	5.4	5.3	2	737	741	–1	583	541	8	154	200	–23
2004 DS	6.3	5.8	10	844	787	7	565	564	0	279	223	25
All countries	6.6	5.7	17	930	824	13	548	523	5	382	302	27

Sources: [a]2005 boro: Samad Azad et al (2008); 2007 boro: Hossain (2008). [b]1984: He et al (1988); 2004: Zhu et al (2008). [c]2001: Janaiah and Hossain (2003); 2002: Singh (2006). [d]Satoto and Sembiring (2008). [e]Casiwan et al (2006). [f]Hossain et al (2006). [g]Exchange rate used—Bangladesh: US$1 = 62 taka; India: US$1 = 47 rupees; Philippines: US$1 = 50 pesos; Vietnam: US$1 =15,000 dong.

Past studies indicate that the additional cost of production for hybrids averages 5% (US\$30 ha^{-1}), with a range from −1% to 18% across countries. The higher cost of hybrids over inbreds was primarily attributed to the higher cost of hybrid seeds, pesticides, and fertilizers. In Bangladesh, the cost of seeds, pesticides, and fertilizers for hybrids was found to be about 60%, 55%, and 10% higher than for inbreds, respectively (Hossain 2008). The use of labor input was only marginally higher in hybrids as compared with inbreds (Mustafi et al 2006, Singh 2006).

The production of hybrid rice seeds requires not only special technical knowledge and skills but also a significantly higher amount of investment in labor and capital. As a result, the price of hybrid rice seed is much higher than that of inbred seed. The total seed cost is higher even if the seeding rate is reduced considerably to about 15 kg ha^{-1}. The high seed cost is one of the major constraints to the spread of hybrid rice cultivation in Asia (Hossain et al 2006).

The yield advantage of hybrids is partly offset by additional input costs and a lower grain price. The marginal net returns of hybrids over inbreds averages 27% (US\$80 ha^{-1}), ranging from −23% to 119% (–\$50 to \$260 ha^{-1}) across countries. The benefit-cost ratio of hybrids (1.7) is only marginally higher than that of inbreds (1.6). The relatively small additional returns to hybrids over inbreds indicate that the existing hybrid rice technology may not be sufficiently profitable to induce large-scale adoption.

When compared between seasons, the profitability of hybrid rice cultivation was relatively small and the marginal benefit of hybrids over inbreds was insignificant in the wet season. On the other hand, both the absolute amount of profit and marginal net benefits were significantly higher in the dry season. This implies that hybrid rice cultivation is more remunerative in the dry season than in the wet season.

Constraints to the spread of hybrid rice

Hybrid rice adoption has been slow in tropical Asia (Table 1). The reasons for this low adoption rate are varied. Broadly, the major constraints to a wider spread of hybrid rice can be grouped into two types: technological and socioeconomic.

Technological constraints

Technological constraints include limited heterosis, poor grain quality, and poor resistance to major pests and diseases of hybrids under tropical conditions. Scientific opportunities exist to overcome these constraints through additional investments in research and technology development as is shown by the scientific breakthrough in developing superior hybrids in China. Other papers contained in this volume describe well some of the scientific opportunities to overcome these constraints. Grain quality can be improved through the use of suitable parental lines and resistance to pests and diseases can be built in similarly. To promote future adoption, grain quality improvement can be a critical factor as consumers are likely to demand higher quality rice with urbanization and income growth.

Poor resistance to pests and diseases contributes to unstable heterosis expression and large crop damage. Substantial progress has been made in developing pest- and disease-resistant hybrids in China. However, most hybrids developed for tropical environments have inadequate resistance and are largely susceptible to major pests and diseases. Hybrid rice farmers spend about 2–3% of the total cost on pesticides (Singh 2006, Samad Azad et al 2008). The lack of pest- and disease-resistant hybrids, especially for the wet season, increases the risk to farmers.

The importance of good-quality seed at a reasonable price to speed up the large-scale adoption of any crop can hardly be overemphasized. High seed cost is an important constraint to wider adoption of hybrid rice. In China, hybrid rice seed costs about \$1 kg^{-1}, but outside China it costs about \$1.50–3 kg^{-1} or about 5–10 times higher than inbred seed. The high cost of hybrid rice seed is due to the seed production technology that requires skilled labor, the high labor and capital requirements, low mechanization, and low seed yield. The Chinese experience provides evidence that the increased seed yield and economies of scale would reduce the cost of production and hence the market price of hybrid rice seeds. The growing competition in the seed business is also likely to lower the price of hybrid seeds in the long run.

Socioeconomic constraints

Hybrid rice is an input-intensive technology. Hybrid rice production demands investment in seed, labor, fertilizers, pesticides, and irrigation. As a result, hybrid rice incurs a 10–15% higher cost (Vijayalakshmi and Hopper 2000). For cash-strapped poor farmers who are unable to afford agricultural credit, this higher cost can be a major barrier to adoption.

Existing hybrid rice varieties are suitable for irrigated environments only, though hybrids for rainfed and other unfavorable environments are now being developed. Only about 60% of the rice area is irrigated in Asia (IRRI 2008a). A large proportion of the unfavorable rainfed environment thus constrains the expansion of hybrid rice area.

Unlike inbred varieties, hybrid rice farmers cannot use their own harvest as seeds and thus they have to purchase seeds every year. More importantly, farmers have to rely on the market for the supply of this key ingredient of crop production. Although farmers depend on the market to purchase fertilizers and other cash inputs, production can take place even without these inputs. On the other hand, no production can take place without seeds and poor subsistence farmers may be reluctant to rely solely on markets for their seed supply.

Opportunities for hybrid rice

The recent global food crisis, irrigation development, and continued technological progress in hybrid rice are likely to expand its opportunity in the future.

The rice price increased very rapidly during the early part of 2008, reaching as high as \$1,000 t^{-1}. Although the rice price has come down since then, it is still much higher than the corresponding value in 2007. The high price of rice has improved the profitability associated with rice production, including for hybrid rice. The rise in price thus provides a major incentive for expanding the area under hybrid rice, and in fact such expansion is already taking place in Bangladesh.

Another factor improving the potential for hybrid rice is the expansion of irrigation facilities, especially the use of privately owned shallow tubewells. Groundwater is an important source of irrigation in South Asia. The development of groundwater irrigation is a relatively recent phenomenon in South Asia but its use has been increasing rapidly over time (Shah et al 2007). Of the total irrigated area, groundwater accounts for about 70% in Bangladesh (Mainuddin 2005), 60% in India (Shah et al 2007), and 20% in Nepal (Bhandari and Pandey 2006). Continuous expansion of groundwater irrigation and the subsequent increase in the area of dry-season rice (boro) provide new opportunities for expanding hybrid rice, particularly in South Asia.

Rice is becoming a popular food in Africa and demand for it is increasing steadily. Rice imports into Africa account for almost one-third of the total world trade (IRRI 2008b). Demand from Africa is expected to continue to grow in the coming years. This rising demand from Africa provides new opportunities for hybrid rice commercialization.

Although hybrid rice was originally targeted to irrigated areas, recent evidence indicates that these varieties may be suitable to rainfed areas also. Marginal and small farmers in rainfed areas grow rice mainly for home consumption. Their immediate priority is to ensure the availability of sufficient food for family consumption through the efficient use of available resources—limited land and capital. Farm-level empirical studies in Bangladesh, India, and Vietnam showed a higher adoption of hybrid rice among small subsistence-oriented farmers than among large farmers (Vijayalakshmi and Hopper 2000, Janaiah and Hossain 2003, Hossain 2008). Hybrid rice has also been established to have some degree of tolerance of drought, making it suitable to drought-prone rainfed areas (Atlin et al 2008).

Technological progress is also improving the future potential of hybrid rice. A majority of the existing hybrid rice varieties are three-line hybrids. To break the yield ceiling of three-line hybrids, rice scientists in China have developed two-line hybrids, which have a 5–10% yield advantage over the popular three-line hybrids. The two-line hybrid varieties thus have a yield advantage of 20–30% over high-yielding inbred varieties. Two-line hybrid varieties were released in 1995 for commercial cultivation in China. Another breakthrough in hybrid rice is the success of developing two-line super hybrid rice varieties through intersubspecific (indica/japonica) heterosis (Yuan 2008). These super hybrids have potential yield of 13.5 t ha^{-1} with farm-level yield of 10 t ha^{-1}. Similarly, significant progress has been made in China for increasing seed yield (3.0 t ha^{-1}) and reducing seed costs. Seed costs are expected to drop sharply as these Chinese seed technologies spill over to other countries. New advances in molecular biology, genomics, and biotechnology have tremendous potential for technological breakthroughs in grain and seed production as well as for value addition on hybrids through trait development, including high yield, premium grain quality, and resistance to major pests and diseases. All these developments point toward higher returns to the adoption of hybrid rice in the future.

A major institutional innovation that is likely to favor the production of hybrid rice is public-private partnerships in agriculture. It is now generally agreed that the production/distribution of hybrid rice can best be done by the private sector. In India, hybrid rice production and diffusion are dominated by the private sector (Redoña and Castro 2006) and the hybrid rice adoption rate is higher in states where private seed companies are more active (Wanjari et al 2006). The public sector can support this by providing suitable parental lines for hybrid rice and by formulating enabling policies. A strong public-private partnership can play an instrumental role in developing and disseminating hybrid rice technologies. The Hybrid Rice Development Consortium (HRDC) at IRRI and the Asia Pacific Seed Association (APSA) play an important role in fostering public-private partnerships in hybrid rice.

Technology targeting

The identification of target domains is essential not only for technology design but also for efficient dissemination. Various biophysical and socioeconomic factors can be considered in delineating the target domain. Following an earlier approach (Lin and Pingali 1994), a simplified typology based on population density and proportion of irrigated area is used here for a broader delineation of the target domain. Population density is an important factor driving land-use intensification (Boserup 1965). Yield-increasing technologies are increasingly demanded as population density increases. Hybrid rice (a yield-increasing technology) is thus likely to be demanded more in areas with high population density than in areas with low population density. Similarly, currently available hybrid rice technologies that are in the pipeline are more suited to irrigated areas than to rainfed areas. Given these features of hybrid rice technology, a simple typology depicted in Figure 3 can be used for targeting.[1]

The most suitable domain for hybrid rice is fully irrigated areas with high population density (Type IV). These are indeed the areas where hybrid rice is currently spreading. The coverage in these areas will increase with further technological improvements to overcome the major constraints discussed above. Excepting China, where the area under hybrid rice is substantial, good opportunities exist for its spread in Bangladesh, the Philippines, Sri Lanka, and Vietnam. Hybrid rice is least suitable in Type I systems that have low population density and limited area under irrigation. Thus, hybrid rice technology is likely to spread rapidly if it is targeted primarily to Type III and Type IV systems.

Ex ante impact assessment

The potential economic impact of hybrid rice can be estimated under alternative assumptions on yield gain and adoption rate. Table 3 presents such an analysis, assuming

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[1] This simplified construct facilitates a broader delineation. The countries (and states/provinces) do not necessarily neatly fall in one of the quadrants but may straddle several quadrants. The intention here is to capture the dominant features of the countries/states/provinces.

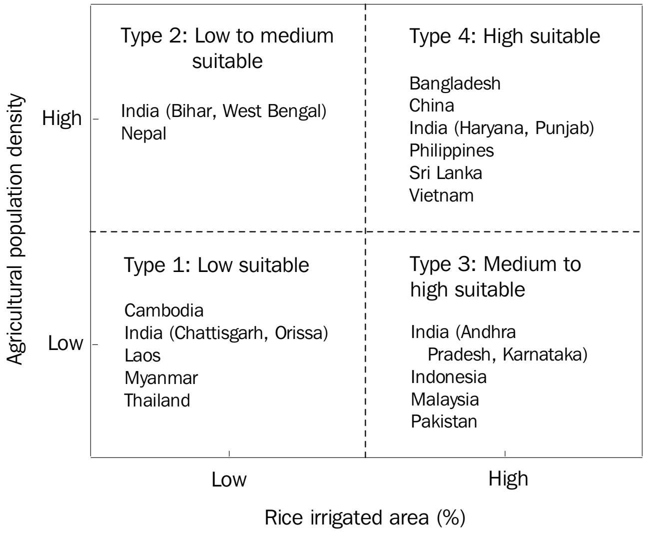

Fig. 3. Suitability of hybrid rice in Asia, by population density and irrigated area.
Note: Population density was calculated as agricultural population per unit arable land (persons/ha).

a 10-year period for adoption for rainfed and irrigated areas in Asia (excluding China). Yield gain (relative to inbred varieties) in irrigated areas is assumed to be twice that in rainfed areas. Similarly, the level of adoption in irrigated areas is assumed to be 25% compared with only 15% in rainfed areas. The total quantity of incremental production under these assumptions is estimated to be 15 million tons, which at a farm-gate price of \$250 t^{-1} is equivalent to \$3.7 billion. This is an estimate of the incremental gross benefit from research and extension. Of this total, 90% of the gross benefit arises from irrigated areas (Type III and IV systems). South Asia accounts for the major share of this gross benefit.

Policy and institutional issues

What policies and institutional developments are likely to promote the development and rapid diffusion of hybrid rice technologies? What are the pros and cons associated with the currently used policy instruments? It is beyond the scope of this paper to delve deep into these issues. However, it is important to explore answers to these questions for guiding policy reforms and institutional development in support of hybrid rice technologies.

The main policy instrument used in various countries in Asia (outside China) seems to be the promotion of hybrid rice technologies through subsidies on inputs (seeds, fertilizers, etc.). The usual “infant industry” argument is put forward to justify such policies. It is argued that, during the initial phase, the public sector has to

Table 3. Ex ante impact assessment of hybrid rice adoption in Asia (excluding China).

Region and ecosystem	Total rice area (million ha)	Adoption rate in year 2020 (%)	Yield gain over inbred varieties (%)	Total production increase (million tons)
South Asia				
Rainfed	29	15	10	0.9
Irrigated	31	25	20	7.8
Southeast Asia				
Rainfed	26	15	10	1.0
Irrigated	19	25	20	5.2
Total	105			14.9

Source: Authors' computation.
Note: Yield rate used—South Asia: rainfed (2.0 t ha^{-1}) and irrigated (5.0 t ha^{-1}); Southeast Asia: rainfed (2.5 t ha^{-1}) and irrigated (5.5 t ha^{-1}). Adoption rate and yield gains were assumed based on existing trends.

subsidize these costly inputs to draw the private sector into hybrid seed production and to make hybrid seeds affordable to farmers. These subsidies are supposedly to be removed when the economy of size reduces costs as the private sector becomes fully established.

The main concern is that the provision of such subsidies will result in substantial wastage of public resources, will give wrong signals to the private sector, and will not necessarily lead to the long-term development of the hybrid rice industry. Concerns are being expressed regarding the problems with subsidy programs in some countries (MARD 2003, David 2006).

The most effective means through which the public sector can support the development of a hybrid rice industry is through increased investments in research, training, and extension. All these activities have a "public goods" nature and rightfully belong to the public sector. The development of suitable parental lines through basic research by the public sector could form a good platform that can help the private sector to further develop specific types of hybrids for commercial production. A public-/private-sector partnership in which the public sector focuses on the development of basic breeding materials, which are then supplied to the private sector to produce and distribute specific hybrids, would use the comparative advantage of each sector. A clearly defined policy on intellectual property rights such as plant varietal protection (PVP), however, is essential to ensure adequate financial returns to the private sector for its investment in hybrid rice research and development.

The public sector could also provide institutional support by creating enabling conditions for the private sector to function efficiently. This includes establishing seed certification and quality control standards, protecting the trademark of seed companies, and developing legal mechanisms for enforcing compliance. The other main area for public-sector involvement may be to invest in strengthening the extension and information dissemination systems that complement private-sector efforts to commercially promote hybrid rice.

Concluding remarks

Hybrid rice technology, despite some initial technical problems, is now maturing rapidly. Scientific opportunities now exist for overcoming many of the initial problems such as poorer grain quality, lack of resistance to pests/diseases, and low seed productivity. The impressive success of hybrid rice in China clearly attests to this. A two-pronged strategy consisting of increasing investments in research and the development of hybrid rice and promoting institutional innovations in the form of public-/private-sector partnerships holds much promise for the future expansion of hybrid rice area in Asia.

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Notes

Authors' address: S. Pandey, senior agricultural economist and leader, Rice Policy and Impact Research Program; H. Bhandari, postdoctoral fellow, International Rice Research Institute.

Country reports

Hybrid rice in Bangladesh

A.W. Julfiquar, Md. Jamil Hasan, Umma Kulsum, and Md. Hazrat Ali

Rice alone constitutes about 92% of the total food grain produced annually in Bangladesh. It provides 75% of the calories and 55% of the proteins in the average daily diet of the people. However, Bangladesh's population is increasing faster than the production of food grains. Therefore, scientists and policymakers are increasingly concerned about how to feed the growing population in the future when the productivity of modern rice varieties has stagnated at a lower level and land and water resources are shrinking. In this situation, hybrid rice technologies could help break through the yield ceiling of semidwarf modern varieties because it has been proven that hybrid technology can give 15–20% higher yield than the best conventional rice varieties. It is expected to have positive socio-political implications for the food supply under Bangladeshi conditions. The research and development program of hybrid rice at the Bangladesh Rice Research Institute (BRRI) began in 1993. Since then, with strong collaboration from IRRI, several CMS lines and hybrids were tested under Bangladeshi conditions. Some of the rice hybrids outyielded the standard check high-yielding varieties of the same growth duration by more than 1 t ha^{-1}. BRRI hybrid dhan1 was selected for a typical boro season, primarily for Jessore and Barisal regions, with a view to subsequent cultivation in farmers' fields in other regions of the country. A specific and goal-oriented work plan has been undertaken for developing heterotic rice hybrids for both the irrigated (boro) and rainfed (T. aman) ecosystems. Efforts are also being made to develop new sources of CMS lines adapted to Bangladeshi conditions. Routine evaluation of newly developed and exotic hybrids is being carried out through preliminary, advanced, on-station, and on-farm trials. The most promising heterotic hybrids are being evaluated through multilocation trials and national hybrid rice trials conducted by the Seed Certification Agency (SCA). One hybrid for the boro season and two hybrids for the T. aman season have been evaluated in farmers' fields. Several experiments were also conducted at BRRI in both the boro and T. aman seasons during 2007 to 2009 to develop an appropriate seed production package for promising hybrids. Results suggested possibilities for raising the seed yield of F_1 hybrids by more than 2 t ha^{-1} in a large-scale commercial seed production program.

Rice occupies 77% of total cropped area in Bangladesh. At present, rice alone constitutes about 92% of the total food grain produced annually in the country. It provides 75% of the calories and 55% of the protein in the average daily diet of the people (Bhuiyan et al 2002). It is estimated that the population by 2025 will be about 21% higher than the population of 2000 (Bhuiyan et al 2002). To produce the required quantity to feed this higher population, the only option remaining is to increase production per unit area, as land is scarce. Concern is growing among scientists and policymakers about how to feed the extra millions in the future when the productivity of modern rice varieties (MVs) has stagnated at a lower level and land and water resources are shrinking. Tomorrow's technology and management must not only enhance the production of rice per unit area, with less water and less pressure on the natural resource base, but also maintain rice as an attractive crop for future generations of farmers. The increased demand for rice will have to be met with less land, less water, less labor, and less pesticide. To shift the yield frontier in rice, one of the best options available to plant breeders is hybrid rice. Hybrids offer to break through the yield ceiling of semidwarf rice, begun in 1964. China was the first country to commercially exploit heterosis in rice. The discovery of CMS in rice (Athwal and Virmani 1972) suggested that breeding could develop a commercially viable F_1 hybrid. The most promising hybrids yielded 20–30% (Lin and Yuan 1980) and 15–20% (Yuan 1998) higher than the best conventional rice varieties. A substantial increase (20–30%) in yield is possible through selective improvement of major yield components (Siddiq 1993). Hybrid rice technology offers considerable opportunity for increasing rice productivity in Bangladesh, where the labor-land ratio is high, labor costs are reasonably low, land is becoming scarcer, and population density is increasing at an alarming rate. Therefore, to go beyond the present yield ceiling of semidwarf modern varieties, hybrid rice seems to be an attractive alternative. It is expected to have positive socio-political implications for the food front under Bangladeshi conditions.

Present status of hybrid rice in Bangladesh

Starting in 1993, hybrid rice research expanded in collaboration with IRRI and several CMS lines and hybrids were tested under Bangladeshi conditions. Some of the rice hybrids outyielded the standard check high-yielding varieties (HYVs) of the same duration by at least 1 t ha^{-1}. Some promising hybrids were evaluated at different regional stations of the Bangladesh Rice Research Institute (BRRI) (Gazipur, Comilla, Bhanga, Habiganj, and Barisal) under typical boro conditions. Based on the preliminary results, two hybrids were evaluated in a multilocation trial, of which the most promising hybrid was selected for pilot testing. A pilot production program was launched in the 2000-01 boro season using more than 20 plots of each hybrid around the country. In the pilot production program, IR69690H had an average yield of 8.4 t ha^{-1} and yield ranged from 7.25 to 9.45 t ha^{-1} in four regions (Table 1). Although IR68877H had good yield in some regions, it showed shattering and sterility in most regions. The National Seed Board (NSB) recommended IR69690H as BRRI hybrid-

Table 1. Region-wide results of pilot testing of hybrids IR68877H and IR69690H.

Region	Days to maturity		Spikelet sterility (%)		Yield (t ha^{-1})	
	IR69690H	IR68877H	IR69690H	IR68877H	IR69690H	IR68877H
Jessore	150	145	24	23	9.45	8.78
Barisal	159	148	19	31	7.93	7.69
Comilla	166	161	24	30	7.25	6.37
Rajshahi	154	138	25	32	7.97	6.42
Range	150–166	138–161	11–37	14–32	7.25–9.45	6.37–8.78
Mean	157	150	23	27	8.40	7.34

dhan-1, primarily for Jessore and Barisal regions, with a view to subsequent cultivation in farmers' fields in other regions of the country.

The development of CMS lines

The CMS lines introduced from China were mostly unstable under Bangladeshi conditions in the rainfed ecosystem (T. aman) but gave good results during the boro season. We tried to identify CMS lines based on ecosystem but most of the IRRI-developed CMS lines showed a stable performance for both seasons. The wild-abortive (WA) cyto sterility system from both IRRI-developed CMS lines and China was used to develop locally adaptable CMS lines. Several selected local varieties/lines were initially identified as maintainers and were backcrossed to their respective CMS sources. Four stable CMS lines that were developed during this period were comparable with IRRI-bred CMS lines and CMS lines introduced from China. These were again evaluated for their adaptability in the respective season. Features of the stable CMS lines for T. aman and boro seasons are presented in Table 2.

Identification of restorers

Many high-yielding locally developed elite lines have been identified as good restorers. These are being purified and multiplied for use in producing experimental hybrids. The important ones are BR827-35-2-1-IR, BR736-20-3-2R, BR6723-1-1-2R, BR6839-41-5-IR, BR7013-62-1-IR, and BR7011-37-1-2R.

Evaluation of experimental hybrids

BRRI-developed experimental hybrids are being evaluated under an observational nursery. Some promising hybrid combinations have been identified that are being further tested under multilocation trials. Experimental seed production plots of those promising hybrids are being established for fine tuning of synchronization

Table 2. Features of CMS lines (developed locally and exotic ones) found to be stable in Bangladesh during T. aman 2007 and boro 2007-08 seasons.

Designation	Plant height (cm)	Days to 50% flowering	Pollen sterility (%)	Panicle exsertion rate (%)	Stigma exsertion rate (%)	Outcrossing rate (%)	Spikelet sterility (%)
T. aman season 2007							
BRRI 1A	97	70	100	78	77	45	100
BRRI 3A	113	88	100	64	66	36	100
BRRI 9A	121	84	100	77	75	42	100
IR58025A	102	86	98	60	47	33	99
IR68888A	105	79	100	69	47	39	100
IR78362A	90	74	100	76	75	35	99
IR70960A	101	94	100	65	64	38	100
IR73328A	107	86	100	61	53	32	98
Jin 23A	98.5	75	100	67	64	41	100
II32A	118.0	78	100	65	71	40	98
D. ShanA	101.6	64	100	66	72	39	100
Boro season 2007-08							
BRRI 1A	65	124	100	80	77	47	100
BRRI 3A	73	135	99	63	65	38	100
BRRI 9A	77	134	100	78	76	43	100
BRRI 10A	75	132	100	76	69	41	100
IR73328A	76	132	98	63	53	35	100
IR80156A	74	132	100	61	66	36	100
IR58025A	77	137	97	62	48	34	100
IR79155A	76	129	100	71	66	36	100
Jin23 A	67	129	100	67	64	41	100
Gan 46 A	67	112	100	70	68	42	100
D. Shan A	65	124	100	66	72	40	99
II32 A	68	134	100	66	72	41	100

and feasibility of F_1 seed production. Two combinations, BRRI 10A/BRRI 10R and IR58025A/BRRI 10R, have been selected as suitable for boro and T. aman seasons. These two combinations have already been submitted to SCA trials. The results are shown in Tables 3, 4, and 5.

Multilocation trials

Three promising hybrids were evaluated at headquarters, Gazipur, with BRRI dhan30, BRRI dhan33, and BRRI dhan39 as checks to find out the adaptability and yield potential of the identified hybrids. Twenty-one-day-old seedlings were transplanted in

Table 3. Experimental hybrids found promising in observational trials during boro season, 2006-07.[a]

Designation	Days to maturity	Plant height (cm)	Panicles m^{-2}	Spikelet fertility (%)	Yield (t ha^{-1})	Yield advantage (t ha^{-1})
IR68897A/BRRI 10 R	151	90	373	78	8.1	1.0 over BRRI dhan28
IR68888A/BR736R	155	100	440	83	9.3	1.1 over BRRI dhan29 and BRRI hybrid dhan1
Jin 23 A/IR69702-48-2-2R	149	97	340	68	8.1	1.0 over BRRI dhan28
BRRI10A/BR168R	159	97	307	94	9.9	1.7 over BRRI dhan29 and BRRI hybrid dhan1
BRRI10A/IR72887-38-1-3-2R	161	105	393	72	9.9	1.7 over BRRI dhan29 and BRRI hybrid dhan1
BRRI10A/IR65482-7-216-1-2R	154	101	353	68	10.9	2.7 over BRRI dhan29 and BRRI hybrid dhan1
BRRI10A/BRR10 R	160	107	340	85	9.5	1.7 over BRRI dhan29 and BRRI hybrid dhan1
BRRI dhan28 (check 1)	150	100	405	78	7.1	–
BRRI dhan29 (check 2)	163	104	313	78	8.2	–
BRRI hybrid dhan1 (check 3)	163	106	302	67	8.2	–

[a] Date of sowing: 2-XII-06, date of transplanting: 1-I-07, plot size = 2 m^2.

Table 4. Experimental hybrids found promising in observational trials during boro season, 2007-08.[a]

Designation	Days to maturity	Plant height (cm)	Panicles m^{-2}	Spikelet fertility (%)	Yield (t ha^{-1})	Yield advantage over check (t ha^{-1})		
						Over BRRI hybrid dhan1	Over BRRI dhan28	Over BRRI dhan29
II 32 A/M.H. 63R	148	100	310	93	8.1	1.04	2.03	0.55
Gan 46A/BRRI 10R	145	102	350	81	8.7	1.64	2.63	1.15
D. Shan A/BR7013-62-1-1(R)	145	120	325	83	8.6	1.54	2.53	1.05
BRRI 3A/IR69702-3-2-3 R	148	108	356	85	8.6	1.54	2.53	1.05
IR80156A/IR72906-24-1-3-1R	148	87	330	82	8.0	0.94	1.93	0.45
BRRI 9A/IR 69702-3-2-3 R	148	113	310	81	8.1	1.04	2.03	0.55
BRRI 1A/HP-4	145	91	415	91	8.6	1.54	2.53	1.05
BRRI 1A/BRRI 11R	148	100	383	95	8.8	1.74	2.73	1.25
BRRI 11A/BRRI 11R	148	100	277	97	8.2	1.14	2.13	0.65
BRRI 9A/BRRI 10R	145	100	316	84	9.0	1.97	2.96	1.49
BRRI 9A/BRRI 11R	142	103	330	83	9.1	2.00	2.99	1.52
II 32A/BRRI 10R	145	105	323	80	8.7	1.62	2.61	1.14
II 32A/BR7013-62-1-1(R)	147	119	376	84	8.1	1.02	2.01	0.54
D. Shan A/BR 7013-62-1-1(R)	143	115	349	79	8.9	1.82	2.81	1.34
BRRI 1A/BRRI 10R	142	98	356	80	8.2	1.16	2.15	0.68
BRRI 1A/BR 6839-41-5-1R	138	100	244	78	8.9	1.82	2.81	1.34
BRRI hybrid dhan1 (check 1)	154	111	327	81	7.1			
BRRI dhan 28 (check 2)	142	107	310	79	6.1			
BRRI dhan 29 (check 3)	158	104	366	87	7.6			

[a]Date of sowing: 3-XII-07, date of transplanting: 2-I-07.

Table 5. Performance of some promising hybrids of the observational trial during T. aman season, 2007.[a]

Designation	Days to maturity	Plant height (cm)	Panicles m^{-2}	Spikelet fertility (%)	Yield (t ha^{-1})	Yield advantage over check (t ha^{-1})		
						Over BRRI dhan30	Over BRRI dhan33	Over BRRI dhan39
D. Shan A/Gui 99 R	108	102	251	78	6.42	1.91	2.78	2.88
BRRI 9A/M.H1 63R	106	95	297	69	7.13	2.62	3.49	3.59
BRRI 9A/IR73004-7-3-3-3R	118	122	198	62	6.65	2.14	3.01	3.11
BRRI 9A/IR73013-95-1-3-2R	120	112	178	65	6.45	1.94	2.81	2.92
BRRI 9A/IR21567R	122	110	185	70	6.84	2.33	3.20	3.30
IR78355 A/M.H.77 R	108	102	257	62	6.71	2.20	3.07	3.17
IR80154[a]/BR 827R	113	104	205	86	6.85	2.34	3.21	3.31
Gan 46A/BRRI 10 R	118	102	297	65	6.98	2.47	3.34	3.44
BRRI 9A/BRRI 10 R	108	94	223	65	6.52	2.01	2.88	2.98
BRRI 9A/Gui 99 R	112	109	178	58	6.01	1.50	2.37	2.47
IR58025A/BRRI 10R	110	108	185	86	6.29	1.78	2.66	2.76
BRRI dhan30 (check 1)	132	117	207	80	4.51	–	–	–
BRRI dhan33 (check 2)	112	110	200	70	3.64	–	–	–
BRRI dhan39 (check 3)	115	108	206	78	3.54	–	–	–

[a] Date of sowing: 2-VII-07, date of transplanting: 23-VII-07.

20-m^2 plots following a randomized complete block (RCB) design with 3 replications using a single seedling per hill. BRRI 1A/BR827R and BRRI 1A/BR168R combinations outyielded by 1.30 t ha^{-1} and 1.23 t ha^{-1}, respectively, check 2 (BRRI dhan33) with similar growth duration at BRRI headquarters. During the boro season of 2007-08, three promising hybrids were evaluated at BRRI headquarters and other regional stations with BRRI hybrid dhan1, BRRI dhan28, and BRRI dhan29 as checks. The hybrid combinations (BRRI 1A/BR168R, BRRI 10A/BRRI 10R, and IR58025A/BRRI 10R) outyielded the checks by at least 1 t ha^{-1} at all locations, showing similar growth duration (Table 6).

CMS seed production

Seed production is another important constraint in hybrid rice. The effect of several seed production components on outcrossing rate and seed yield of CMS lines was studied. Eight CMS lines (IR58025A, BRRI IA, BRRI 3A, BRRI 9A, II32A, IR68888A, IR73328A, and Jin23A) along with their maintainers (B lines) were grown with a view to producing pure and good-quality seed of CMS lines for subsequent use. The seed yield of each of the A lines is shown in Table 7. Similarly, we made an attempt to produce a sufficient quantity of pure CMS seeds during the boro season of 2007-08 using 12 promising CMS lines. BRRI A/B, BRRI 9A/B, and BRRI 10A/B were found suitable for large-scale commercial multiplication. The seed yield of each of the A lines is given in Table 8.

Experiments were conducted during the boro season of 2006-07 to find out the optimum row ratio, spacing, and appropriate doses of GA_3 for hybrid rice seed production of some selected hybrid combinations. An experiment was conducted using one promising A line (BRRI 1A) along with its restorers (BR168R). Restorers were sown on three different dates at 5 days' interval and a CMS line was sown along with a second set of its respective R lines. Thirty-day-old seedlings were transplanted at a spacing of 15 × 15 cm, 20 × 15 cm, and 20 × 20 cm, with 2:8, 2:10, 2:12, and 2:14 ratios of R to A lines using an RCB design with 3 replications. Intercultural operations, irrigation, roguing, GA_3 application, and supplementary pollination were performed as recommended. Plant spacing of 20 × 15 cm with a 2:10 row ratio gave the highest seed yield (2.71 t ha^{-1}). The highest outcrossing rate was found with a 2:8 ratio with 20 × 15-cm spacing, followed by a 2:10 ratio with 20 × 15-cm spacing. Results are given in Table 9. F_1 seed production of released hybrid BRRI hybrid dhan1 was undertaken during the boro season. Seed production during the boro season of 2006-07 was not satisfactory as continuous rain occurred during flowering time. However, 1,020 kg ha^{-1} of seed yield was obtained (Table 10). Seed production of promising hybrids was also undertaken during the boro season of 2006-07. Satisfactory seed yield from promising hybrid combinations was obtained (Table 11). F_1 seed production plots were established for three promising hybrids during the boro season of 2007-08. Seed yield was very encouraging, indicating that large-scale commercial seed production is possible for those hybrids (Table 12). An experiment was conducted to find out the effective dose of GA_3 and its time of application on hybrid rice seed production during the boro season of 2006-07. In this connection, one CMS line (BRRI IA) along

Table 6. Results of multilocation trials of promising hybrids at Gazipur during T. aman 2007 and at five locations during boro season, 2007-08.[a]

Designation	Days to maturity	Plant height (cm)	Panicles m^{-2}	Spikelet fertility (%)	Yield (t ha^{-1})	Yield advantage (t ha^{-1})
T. aman 2007 (Gazipur)						
BRRI 1A/BR827R	118	114	286	75	3.74	1.30 over BRRI dhan33
BRRI 1A/BR168R	119	109	251	76	3.67	1.23 over BRRI dhan33
BG 407	126	120	209	63	3.02	
BRRI dhan30	133	128	229	81	3.17	
BRRI dhan33	118	113	204	76	2.44	
BRRI dhan39	122	114	211	74	2.71	
Boro season 2007-08 (Gazipur)						
BRRI 1A/BR168R	144	91	310	86	7.67	2.25 over BRRI dhan28
BRRI 10A/BRRI 10R	148	104	304	75	7.90	2.48 over BRRI dhan28
IR58025A/BRRI 10R	148	106	332	74	8.24	2.82 over BRRI dhan29
RP-703	150	97	303	70	6.13	
RP-704	144	104	209	71	6.24	
BRRI hybrid dhan1	154	112	343	69	6.07	
BRRI dhan28	140	103	348	91	5.42	
BRRI dhan29	156	102	352	84	7.30	
Boro season 2007-08 (Barisal)						
BRRI 1A/BR168R	140	92	337	84	7.90	1.50 over BRRI dhan28
BRRI 10A/BRRI 10R	147	109	288	87	8.40	2.00 over BRRI dhan28
IR58025A/ BRRI 10R	154	106	312	84	8.99	1.57 over BRRI dhan28
BRRI hybrid dhan1	155	108	328	86	8.30	
BRRI dhan28	139	108	308	88	6.40	
BRRI dhan29	158	119	293	81	7.42	

Continued on next page

Table 6 continued.

Designation	Days to maturity	Plant height (cm)	Panicles m^{-2}	Spikelet fertility (%)	Yield (t ha^{-1})	Yield advantage (t ha^{-1})
Boro season 2007-08 (Satkhira)						
BRRI 1A/BR168R	141	90	335	85	8.25	1.98 over BRRI dhan28
BRRI 10A/BRRI 10R	145	103	308	79	8.70	2.43 over BRRI dhan28
BRRI hybrid dhan1	154	110	332	75	7.16	
BRRI dhan28	140	118	325	82	6.27	
BRRI dhan29	156	118	325	77	6.44	
Boro season 2007-08 (Comilla)						
BRRI 1A/BR168R	144	84	409	80	7.40	2.28 over BRRI dhan28
BRRI 10A/BRRI 10R	148	96	395	75	7.14	2.02 over BRRI dhan28
IR58025A/ BRRI 10R	149	94	405	76	7.23	2.11 over BRRI dhan28
BRRI hybrid dhan1	151	102	378	67	6.16	
BRRI dhan28	141	99	388	72	5.12	
BRRI dhan29	155	100	386	73	6.17	
Boro season 2007-08 (Rangpur)						
BRRI 1A/BR168R	143	77	264	77	6.02	1.07 over BRRI dhan28
BRRI 10A/BRRI 10R	147	92	226	69	7.65	2.02 over BRRI dhan28
IR58025A/BRRI 10R	152	97	253	68	8.89	1.56 over BRRI dhan29
BRRI hybrid dhan1	156	99	336	67	7.17	
BRRI dhan28	141	94	259	70	4.95	
BRRI dhan29	162	91	292	78	7.33	

[a] Date of sowing: 4-VII-07, date of transplanting: 25-VII-07.

Table 7. CMS multiplication of some promising A lines during T. aman, 2007.[a]

Combination	Plant height (cm)		Days to flowering (50%)		Panicle exsertion rate (%)	Out-crossing rate (%)	Plot size (m^2)	Yield	
								(kg plot^{-1})	(kg ha^{-1})
	A line	B line	A line	B line	A line	A line		A line	
IR58025A/B	88	90	93	91	72	30	435	50	1,160
BRRI 1 A/B	78	90	76	73	72	35	270	60	2,200
BRRI 3A/B	105	107	88	85	64	32	8.5	1.0	1,200
BRRI 9A/B	115	118	84	81	77	37	3.5	0.5	1,350
II 32A/B	116	118	78	76	78	33	3.5	0.5	1,300
IR68888A/B	105	107	79	76	69	30	3.0	0.35	1,225
IR73328A/B	107	110	86	84	61	31	2.5	0.3	1,200
Jin23A/B	98	101	75	73	67	31	3.5	0.42	1,150

[a]Date of sowing: B1 = 4-VII-07, A/B2 = 7-VII-07, B3 = 10-VII-07; date of transplanting: A/B = 28-VII-07.

Table 8. Multiplication of some promising A lines during boro season 2007-08.

Combination	Plant height (cm)		Days to flowering (50%)		Panicle exsertion rate (%)	Out-crossing rate (%)	Plot size (m^2)	Yield	
								(kg plot^{-1})	(kg ha^{-1})
	A line	B line	A line	B line	A line	A line		A line	
IR58025A/B	74	76	137	135	72	33	395	65	1,650
BRRI 1 A/B	66	68	124	121	73	42	350	80	2,300
BRRI 3A/B	75	79	135	132	66	36	175	25	1,400
BRRI 9A/B	78	80	134	132	78	37	150	27	1,750
BRRI 10A/B	75	78	132	130	78	45	320	75	2,350
II 32A/B	68	70	135	133	78	40	100	20	2,000
Gan 46A/B	65	68	112	110	75	45	215	45	2,100
IR68888A/B	80	82	135	132	69	33	100	12	1,225
D. Shan A/B	64	66	124	122	72	41	30	5	1,850
IR73328A/B	75	77	135	133	62	32	60	8	1,400
Jin23A/B	68	70	129	126	67	35	20	3	1,500
IR78355A/B	75	77	132	130	69	33	25	3.5	1,400

Table 9. Determination of suitable row ratio and spacing for increasing seed yield in hybrid rice seed production during boro season, 2006-07.[a]

Row ratio	Spacing	PACP veg.	PACP mat.	50%F (A)	50%F (R)	PHT (cm)	Tillers hill^{-1}	Panicles m^{-2}	PER (%)	OCR (%)	Plot size (m^2)	Yield (t ha^{-1})
R0 (2:8)	S1 (15 × 15)	5	4	106	132	78	9	313	81	49	705	1.89
R0 (2:8)	S2 (20 × 15)	4	4	106	131	81	10	278	77	50	445	2.26
R0 (2:8)	S3 (20 × 20)	4	4	107	132	81	11	243	79	49	445	2.17
R1 (2:10)	S1 (15 × 15)	3	4	107	132	79	8	337	77	47	435	2.43
R1 (2:10)	S2 (20 × 15)	3	3	108	133	81	10	276	80	49	525	2.71
R1 (2:10)	S3 (20 × 20)	3	5	107	132	81	11	226	81	43	520	2.17
R2 (2:12)	S1 (15 × 15)	3	4	108	132	81	9	332	79	43	495	2.40
R2 (2:12)	S2 (20 × 15)	4	5	107	132	80	10	287	80	42	405	2.34
R2 (2:12)	S3 (20 × 20)	4	5	105	131	79	12	245	80	48	605	2.06
R3 (2:14)	S1 (15 × 15)	4	4	107	131	78	8	338	77	40	555	2.23
R3 (2:14)	S2 (20 × 15)	5	5	107	132	78	10	286	78	43	685	2.06
R3 (2:14)	S3 (20 × 20)	5	5	107	131	78	10	217	82	43	685	2.22

[a] Days to sowing: R1—2-XII-06; days to tillering: R—7-I-07; PACP = phenotypic acceptability, PER = panicle exsertion rate,
R2—7-XII-06 A—5-II-07 OCR = outcrossing rate.
R3—12-XII-06
A—2-I-07

Table 10. F_1 hybrid seed production of BRRI hybrid dhan1 during boro season, 2006-07, Gazipur.[a]

Combination	Plant height (cm)		Days to 50% flowering		Panicle exsertion rate (%)	Out-crossing rate (%)	Yield per plot (kg)	Yield (kg ha^{-1})
	A line	R line	A line	R line	A line	A line	A line	A line
IR58025A/BR827R	73.8	112	126	138	82	33	51	1,020

[a] Plot size (m^2) = 500.

with its restorers (BR168R) was grown as parental materials. A restorer was sown on three different dates at 5 days' interval and one CMS line was sown along with a second set of its respective R line. GA_3 concentrations of 0, 40, 60, and 80 g ha^{-1} at 5%, 15–20%, and 35–40% flowering stage were applied and no GA_3 was used for the control. Intercultural operations, irrigation, roguing, and supplementary pollination were performed as recommended. Among the 12 treatments, a higher seed yield (2.6 t ha^{-1}) was obtained from applying GA_3 at 60 g ha^{-1} at 5% and 15–20% flowering stage. Results are shown in Table 13. An experiment was also conducted with one promising hybrid combination during the boro season of 2007-08 to find out the optimum row ratio and spacing for hybrid rice seed production. The experiment was conducted using one A line (IR58025A) along with its restorer (BRRI 10R). The restorer was sown on three different dates at 5 days' interval and a CMS line was sown on the same date as the second set of its restorer line. Thirty-day-old seedlings were transplanted at a spacing of 15 × 15 cm, 20 ×15 cm, and 20 × 20 cm with 2:8, 2:10, 2:12, and 2:14 row ratios of R to A lines using an RCB factorial design with 3 replications. Intercultural operations, for example, irrigation, roguing, GA_3 application, and supplementary pollination, were performed as recommended. The highest seed yield was recorded from the 2:10 row ratio with 15 × 15-cm plant spacing (2.80 t ha^{-1}). The 2:8 row ratio showed the highest outcrossing rate with 15 × 15-cm spacing, followed by a 2:8 ratio with 20 × 20-cm spacing. Results are given in Table 14.

Future action plans

The government of Bangladesh has taken pragmatic steps to develop and use hybrid rice technology on a large scale by involving public, private, and nongovernment (NGO) organizations. Research, seed production, and technology transfer agencies in public, private, and NGO sectors are also interested in exploring the prospects of this technology. Government has allowed some NGOs and private seed companies to introduce and commercialize exotic hybrids for large-scale cultivation by farmers with a view to getting immediate benefit from this technology. As a result, about 0.8 million hectares of boro rice land were covered by exotic hybrid rice during boro 2007-08, mostly from China and India. It is expected that 1.2 million hectares of land will be covered by hybrid rice in the next season. Special attention has been given to

Table 11. F_1 seed production of promising hybrids during boro season, 2006-07.

Combination	Plant height (cm)		Days to 50% flowering		Panicle exsertion rate (%)	Out-crossing rate (%)	Area (m^2)	Yield per plot (kg)	Yield (kg ha^{-1})
	A line	R line	A line	R line	A line	A line		A line	A line
BRRI 1A/BR168R	71	96	96	122	79	54	300	54	1,800
BRRI 1A/BR827R	70.5	111	108	135	78	36	500	60	1,200
BRRI 10A/BRRI 10R	76	110	130	135	76	43	300	70	2,333

Table 12. F_1 seed production of promising hybrids during boro season, 2007-08.

Combination	Plant height (cm)		Days to 50% flowering		Panicle exsertion rate (%)	Out-crossing rate (%)	Area (m^2)	Yield per plot (kg)	Yield (kg ha^{-1})
	A line	R line	A line	R line	A line	A line		A line	A line
BRRI 1A/BR168R	73	98	95	121	77	53	500	85	1,700
IR58025A/BR827R	75.5	111	125	135	78	36	500	65	1,300
BRRI 10A/BRRI 10R	75	111	132	133	78	44	500	125	2,500
IR58025A/BRRI 10R	76	110	129	132	76	39	600	120	2,000

developing hybrid rice varieties within the country. The foremost constraint to overcome by researchers is to identify heterotic hybrid combinations that are adaptable under Bangladeshi conditions as well as able to outyield the most popular commercial varieties by at least 20%. Another hurdle to be overcome is to develop a cheaper seed production package, which is necessary to make this technology commercially viable. At present, hybrid rice research at BRRI is being done through a government of Bangladesh project. The program also included collaborative research with the Bangladesh Agricultural Development Corporation (BADC) and other private agencies engaged in seed production in the country. The program also has close collaboration with the Department of Agricultural Extension (DAE) for the dissemination of the technology developed. A specific and goal-oriented working plan has been made for

Table 13. Effect of different doses of GA_3 and its time of application on hybrid seed production using promising hybrid combination BRRI 1A/BR168R.

Treatment combination[a]	Plant height (cm)		Days to flowering (50%)		Panicle exsertion rate (%)	Outcrossing rate (%)	Yield A line	
	A line	R line	A line	R line	A line		(kg plot^{-1})	(t ha^{-1})
C0S1S2	78	106	104	128	74	35	3.0	1.5
C1S1S2	81	106	104	128	76	38	3.4	1.7
C2S1S2	83	106	104	128	78	60	5.2	2.6
C3S1S2	83	106	104	128	76	56	4.8	2.4
C0S2S3	77	106	104	128	72	25	3.2	1.6
C1S2S3	81	106	104	128	78	39	3.8	1.9
C2S2S3	83	106	104	128	80	55	5.0	2.5
C3S2S3	84	106	104	128	79	58	4.9	2.45
C0S1S3	79	106	104	128	76	40	2.8	1.4
C1S1S3	79	106	104	128	74	37	3.2	1.6
C2S1S3	84	106	104	128	76	65	4.85	2.4
C3S1S3	82	106	104	128	73	54	4.7	2.35

[a] C0 = control, C1 = 40 g, C2 = 60 g, C3 = 80 g, S1 = 5% flowering, S2 = 15–20% flowering, S3 = 35–40% flowering. Unit plot size: 20 m^2.

developing heterotic rice hybrids for both irrigated (boro) and rainfed (T. aman) ecosystems. Efforts are also being made to develop new sources of CMS lines adapted to Bangladeshi conditions. Routine evaluation of newly developed and exotic hybrids is being carried out through preliminary, advanced, on-station, and on-farm trials. The most promising heterotic hybrids are being evaluated through multilocation trials and national hybrid rice trials conducted by the Seed Certification Agency. One hybrid for the boro season and two hybrids for the T. aman season are being evaluated in farmers' fields. The primary goal of BRRI is to gear up hybrid rice research so that (1) hybrid rice technologies relevant to Bangladeshi conditions are generated, (2) sustainable seed production technology suitable for Bangladeshi conditions is developed, and (3) hybrid rice technologies are disseminated in the country.

Table 14. Determination of suitable row ratio and spacing for increasing seed yield in hybrid rice seed production during boro season, 2007-08.[a]

Row ratio	Spacing (cm)	50% flowering (A lines)	50% flowering (R lines)	Plant height (cm)	Tillers hill^{-1}	Panicles m^{-2}	Panicle exsertion rate (%)	Outcrossing rate (%)	Yield (t ha^{-1})
R0 (2:8)	S1 (15 × 15)	129	128	80	8	287	80	56	2.50
R0 (2:8)	S2 (15 × 20)	129	128	83	10	274	80	52	2.35
R0 (2:8)	S3 (20 × 20)	128	127	85	11	218	83	54	2.30
R1 (2:10)	S1 (15 × 15)	128	128	81	8	296	79	52	2.80
R1 (2:10)	S2 (15 × 20)	129	128	83	10	268	80	51	2.47
R1 (2:10)	S3 (20 × 20)	128	127	84	11	215	84	50	2.37
R2 (2:12)	S1 (15 × 15)	129	128	81	8	296	78	49	2.38
R2 (2:12)	S2 (15 × 20)	128	128	84	10	249	80	47	2.45
R2 (2:12)	S3 (20 × 20)	129	128	86	11	225	84	45	2.42
R3 (2:14)	S1 (15 × 15)	128	128	81	9	323	79	43	2.16
R3 (2:14)	S2 (15 × 20)	129	129	83	10	253	80	40	2.06
R3 (2:14)	S3 (20 × 20)	129	129	84	11	212	84	42	2.00

[a] Date of sowing: R1 = 3-XII-07, A&R2 = 8-XII-07, R3 = 3-XII-07; date of transplanting: R&A = 7-I-08. Unit plot size: 20 m^2.

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Notes

Authors' address: Bangladesh Rice Research Institute, Gazipur, Bangladesh, brrihq@bdonline.com.

The successful introduction of hybrid rice in Brazil

M.R. Ritter, L. Pasqualli, R. Luzzardi, and C. Saravia

Rice is a major staple food in Brazil (population 186 million), with a domestic production of 12 million tons of paddy rice and an annual consumption of 13.1 million tons. The difference is sourced with imports from Argentina and Uruguay. Rice production area in Brazil is divided into two main cropping systems: (1) irrigated rice, with a total cultivated area of 1.2 million ha, and (2) rainfed upland rice, with a total cultivated area of 1.8 million ha. About 70% of the volume is produced in irrigated fields in southern Brazil and the remaining 30% in upland fields under rainfed conditions in central Brazil. First attempts to cultivate rice date back to 1860. Around 1900, considerable investments in irrigation systems and rice milling were made. By 1940, the first dedicated rice research institute (IRGA) was created with the objective to support the development of the rice crop and to breed better adapted varieties. Nowadays, rice variety breeding is done by four public state and federal institutions.

The development of hybrid rice is undertaken by private companies such as RiceTec, Bayer, Ana Paula Farms, and AgroNorte, and by public institutions such as Embrapa and IRGA. RiceTec was the first company to launch hybrids in the Brazilian market (in 2004) and it remains the market leader, with a market share of >95%. RiceTec offers a number of hybrids for irrigated and upland rainfed conditions. The adoption rate for hybrids is growing fast as hybrids deliver a yield advantage of 2,000–3,000 kg ha^{-1} over check varieties.

RiceTec started its hybrid program in 1988 in the United States, and started to test hybrids in South America in 1992, initially as counter-season tests to support the business development in the U.S. Besides RiceTec, other companies and institutions are also developing hybrid rice.

In Brazil, the first hybrid developments actually started in 1984 in cooperation between Embrapa, Goiânia, and CIRAD (Prabhu et al 2002). Another hybrid development project was established in 2003 between IRGA (Rice Research Institute of Rio Grande do Sul), Ana Paula Farm, and the Hunan Rice Research Institute. Other private hybrid development programs in Brazil are conducted by the companies Bayer and AgroNorte.

RiceTec established its legal entities in South America in 2000 and launched its first commercial hybrid (TUNO CL) on a small test-marketing scale in 2003-04.[1] Since then, RiceTec has launched at least one new hybrid every year and has been doubling its sales year after year.

Last season's sales reached a hybrid adoption rate of 1.5% within the total Mercosur irrigated market. RiceTec is developing standard hybrids and Clearfield hybrids with IMI herbicide tolerance. In the 2008-09 season, the first hybrid for upland rice will be introduced in the market.

The purpose of this paper is to explain the advances made in hybrid development in South America, namely, in Brazil, Argentina, and Uruguay. RiceTec's hybrid program is the most advanced commercial hybrid program in the subtropical and temperate climate in the southern hemisphere. The paper will examine several critical issues and solutions adopted in establishing a commercial hybrid program from development to production and marketing.

The rice markets in the Mercosur region

The common economic market Mercosur is composed of four countries—Argentina, Brazil, Paraguay, and Uruguay. In terms of rice consumption and rice production, Brazil is the most important market, making up 83% of total production estimated at 14.8 million tons (Cogo 2008). In terms of rice cultivation, Brazil produces 2.9 million ha. The irrigated rice area makes up 1.2 million ha; of this, 85% is dry sown and 15% is water seeded with pregerminated seed. The remaining 1.7 million ha are cultivated under rainfed upland conditions.

Irrigated rice is concentrated in the state of Rio Grande do Sul (RS), with 1.0 million ha in southern Brazil. This state produced in 2008 a total of 12.3 million tons of paddy rice, which is 61% of the country's production. The average productivity in RS is 7.0 t ha^{-1}. The second most important state for irrigated rice is Santa Catarina (SC), with crop area of 160,000 ha and a productivity of approximately 7.5 t ha^{-1}.

Upland rice is mainly cultivated in the states of Maranhão (MA), Piauí (PI), and Mato Grosso (MT). During the 1980s and 1990s, while the agricultural front was opened in the Brazilian Highlands (Cerrado), rice was usually planted as the first crop after deforestation. Upland farmers tend to be very large farmers cultivating mainly soybeans, with farm sizes mostly above 1,000 ha for each farm. Upland rice as a first crop after deforestation is usually cultivated with low inputs, taking advantage of the remaining fertility from the rain forest and low weed infestations. Rice helps to restructure the soil for future soybean cultivation. For that reason, average yields of the 1.7 million ha are only 3.6 million tons of paddy rice, with an average productivity of 2.1 t ha^{-1}. Upland rice in the past decades reached a maximum cultivation area of 5.3 million ha in 1986-87. Environmental regulation and control have limited the

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[1] The predominant planting period is September to November and harvest is from February to April; the seasons are therefore expressed as follows: 2003-04.

further expansion of new agricultural land into the Amazon region. Land that had been planted with upland rice as a first crop normally will be turned into soybean cultivation. For these reasons, the area of upland rice has declined drastically; however, upland rice is now increasingly being incorporated into a crop rotation with soybean and pasture mainly in the areas that have been under soybean monoculture for one or two decades. Those farmers that adopt a crop rotation with soybeans or pasture land are increasingly using a higher amount of inputs such as fertilizers and pesticides. They are now obtaining an average yield of 3.5 t ha^{-1} and maximum yields of 5.0 t ha^{-1}.

The other Mercosur countries cultivate dry-sown irrigated rice: Argentina, 170,000 ha; Uruguay, 170,000 ha; and Paraguay, 40,000 ha. The total Mercosur production reaches 14.8 million tons of paddy rice, which covers well the regional demand of 13.6 million tons. Excess production from the Mercosur region is exported to other markets such as Europe, the Middle East, and several countries in Africa.

The seed market

Almost all commercial varieties have been developed through the national public research system, that is, Embrapa in Brazil, INTA in Argentina, and INIA in Uruguay. In addition to the national institutions, two state institutions have also successfully bred varieties, namely, IRGA for RS and Epagri for SC. The use of certified seed varies from region to region considerably. While in Uruguay and in SC the use of certified seed is very high at >90%, in Argentina and in RS, the use of certified seed is <40%. Varieties are maintained by breeding institutions, which also sell foundation seed to seed multipliers.

Hybrids are currently commercialized only by RiceTec and Bayer Seeds, out of which 95% is by RiceTec.

IMI herbicide tolerance technology developed by Louisiana State University and INTA-Argentina is being licensed through BASF and has been on the market since 2003, commercialized under the trademark Clearfield.[2] The Clearfield technology (CL) proved to be very efficient for red rice and general weed control and was very quickly adopted by farmers. In CL varieties, for which the seed multiplication effect is much faster than with hybrids, the CL adoption rate already reached 40% to 50% of the market in the third year.

The technology owner and its license partners, however, confronted big difficulties in protecting intellectual property and selling certified seed through seed channels because farmers tried to avoid CL royalty payments. Farmers switched to farm-saved seed and purchased cheaper generic IMI herbicide. As a consequence of the use of farm-saved CL seed, contaminated with red rice, outcrossing of IMI tolerance into red rice is now being observed.

Companies developing new traits (mutagenic and transgenic) are now recognizing the importance of hybrids as a secure technology delivery system to protect intel-

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[2] Clearfield is a BASF trademark.

lectual property, ensure royalty collection, avoid misuse, and therefore also prolong the life cycle of technologies.

Developing adapted hybrids

The initial strategy was to test the adaptability of hybrids developed in the U.S. program and to transfer these in South America. However, we recognized quickly that hybrids, though planted in similar latitudes in the north as in the south, delivered different results. This was best exemplified by the companies' first commercial hybrid, XL6, that showed good yield performance in the U.S. and in Brazil, but it had a tendency to lodge in the U.S. and a tendency to shatter in Brazil. Also, the cooking requirements are different. While the U.S. market demands soft fluffy cooking type of rice, the Brazilian consumer prefers harder cooking rice with strong emphasis on nonstickiness. Brazilians have an annual consumption of 40 kg per capita and this is among the highest on the American continent. As can be observed in general in countries with high consumption rates, consumers tend to be more specific about their preferences with rice quality than in countries with low consumption rates.

In the selection and advancement process of potential hybrids, a yield advantage target of 1.5 t ha^{-1} over the predominant varietal checks was defined next to good straw strength and tolerance for shattering, the disorder straighthead, iron toxicity, and diseases.

RiceTec implemented a rigorous development system with narrowly defined hybrid advancement criteria at each development stage. Hybrids that have successfully passed the initial testcrossing evaluation are then tested from stage I to IV during four years in an increasing number of testing locations in replicated small-plot trials. Successful hybrids then enter stage V (product development), and are evaluated in strip trials with a plot size of 300 m^2. This system has proved to be a very efficient way to get a big number of trials conducted in farmers' fields. The strip-trial system is efficient in terms of seed amount that is required, and operational efficiency in terms of planting and harvest evaluation. Each development agronomist has a small-plot drill and an electronic balance that can hold the grain weight of the 300-m^2 plots. Both pieces of equipment can easily be transported by small pick-up vehicles. This approach of customer close development with large-plot trials is commonly used by hybrid maize companies. Some varietal breeding institutions are now adopting similar approaches for precommercial varieties.

RiceTec had an interesting learning experience working together with leading farmers. This approach has the advantage that innovators know two to three seasons ahead about the new products being launched. The strip-trial approach proved to deliver reliable product data. All launched hybrids performed according to expectations and all launched hybrids did fit with the customer segments toward which they had been positioned.

Data for Avaxi were published for the first time by Ritter et al (2002). The hybrid Avaxi (research code XSP116) delivered an average yield advantage of 0.93 t ha^{-1} in 29 regional strip trials. In the 2006-07 and 2007-08 seasons, improvements

Table 1. Strip trial data with standard hybrids.

Combined 2-year strip trial data, 2006-07 and 2007-08, total of 93 locations				
Tested genotypes	Type	Yield (t ha^{-1})	Yield advantage (t ha^{-1})	Heterosis % over check
Avaxi	RT hybrid	12.128	2.356	24.1
Inov	RT hybrid	11.937	2.165	22.2
Irga417	Check variety	9.772		

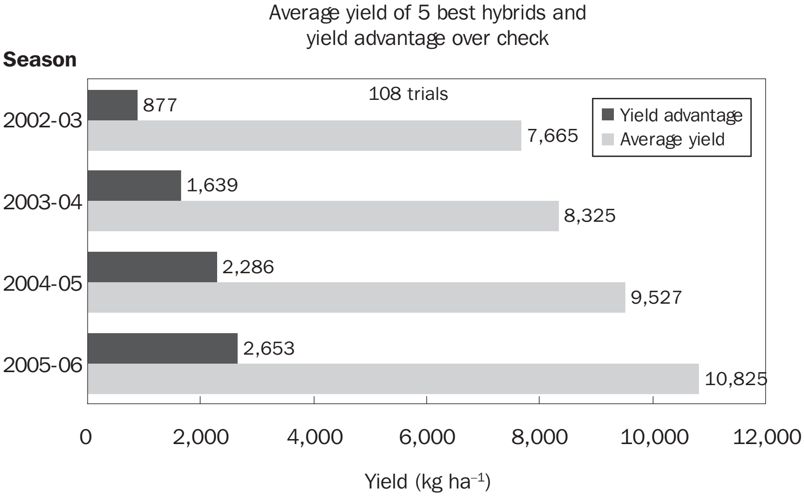

Fig. 1. Improving yield potential and heterosis over the years.

in seed quality, seed purity, and learning in agronomic management led to an average yield improvement of 2.36 t ha^{-1} (93 regional strip data of two seasons) with the same hybrid against the same check variety (see Table 1).

Inov, a new hybrid with improved grain quality characteristics, delivered in the 2006-07 and 2007-08 season a yield advantage of 2.16 t ha^{-1}.

The combination of improved management, higher seed quality, and improving genetics has resulted in a continuous improvement of the hybrids that reach the final testing stage before commercial market introduction (see Fig. 1 published by Luzzardi et al 2006).

The development of CL hybrids has been a priority; this technology is providing a substantial benefit for farmers. This has helped to overcome some preconceptions farmers had about hybrids such as high seed price and lower seed density. CL hybrids showed good tolerance and very good heterosis compared with available CL varieties

Table 2. Strip-trial data with Clearfield hybrids.

Combined 2-year strip-trial data, 2006-07 and 2007-08, total of 22 locations

Tested genotypes	Type	Yield (t ha^{-1})	Yield ha^{-1}	Heterosis % over check
Avaxi CL	RT CL hybrid	12.144	2.372	34.5
Sator CL	RT CL hybrid	11.581	1.809	28.3
Irga422 CL	Check variety	9.028		

(Table 2). Two years of strip-trial data from 22 locations showed that Avaxi CL has a heterosis of 34.5% and Sator CL 28.3%.

Satisfying millers' and consumers' requirements

RiceTec could identify relatively quickly several hybrids that had a reasonable yield advantage and a good overall fit with regard to agronomic requirements. Since RiceTec in South America has not been a traditional breeding institution with a long history, it has been a considerable challenge to develop hybrids to meet millers' and consumers' grain quality requirements.

Grain quality is evaluated within three broad categories: (1) milling quality (total and whole-grain milling, chalky grains), (2) functional quality (alkali spreading value, RVA, and amylose), and (3) sensorial quality (grain appearance, stickiness, softness, aroma, and taste). A rigid application of these quality criteria on hybrid advancement decisions has led to fast improvement.

The first item, milling quality, is now being analyzed by the Isuzuki Image Analyzer S21. This equipment allows analysis of larger samples and work is much faster than when analysis is done manually. The equipment has a software that interprets video images of the grain sample and provides data on chalky grain, white belly, and total white area as well as grain dimensions. The use of this equipment helped to make more rigorous selection on hybrid advancement. Figure 2 shows the reduction in chalk of hybrids advanced to stage V during three years.

Low stickiness has become a market driver and has narrowed the industry's preference on genotypes to very few varieties and hybrids. This trend is leading a shift toward high-amylose rice with low gelatinization temperature. Most varieties were bred for intermediate amylose, around 25%, with the objective of meeting consumers' preference of loose and dry grain appearance after cooking and soft when warmed up a second time (Ferreira et al 2006).

The most recently launched varieties are high-amylose varieties in order to meet this new quality trend of very low stickiness. Examples are IRGA423 with 30% amylose as described by Lopes et al (2007) and IRGA424 with 29% amylose as described by da Cruz et al (2007), both varieties with low gel temperature.

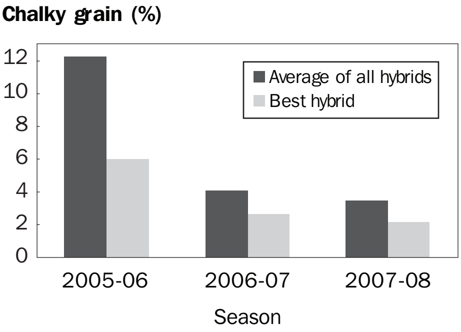

Fig. 2. Improvements in chalk data.

Table 3. Cup test data for the irrigated market.

Genotype	Hybrid			Check varieties		
	Avaxi	Tiba	Inov	El Paso	Epagri 107	IRGA417
Degree of stickiness	2.2	2.1	3.8	2.3	2.8	4.1
Stickiness	Medium	Medium	Low	Medium	Medium	Low

Work at RiceTec showed that amylose and gel temperature explained only partially cooking behavior as it is perceived by consumers. For that reason, complementary to the grain chemistry, a cooking test has been developed to measure stickiness objectively. This test has been called the "cup test." Defined quantities of cooked rice are spooned into a cup, the cup is turned over on a glass plate, and the cup is carefully removed vertically. Sticky rice will retain the cup-shape, whereas nonsticky rice will separate and the shape may collapse. The ratio of final width of the collapsed rice to the remaining height is a measure of a stickiness index.

As shown in Table 3, the first hybrids launched, Avaxi and Tiba, had a stickiness similar to that of variety El Paso 144, which had been defined as our lower quality benchmark. In the breeding and development program, an increased effort toward low stickiness was made. As a result, the latest hybrid launched on the market under the brand Inov shows a stickiness index very close to that of the upper benchmark, IRGA417.

Developing genetics and methodologies for systematic analysis of hybrids with improved grain quality that are equal or close to the preferred varieties is crucial to making hybrids widely accepted by millers and consumers. Farmers must feel secure that their grain produced with hybrid seed will be accepted by millers without restrictions.

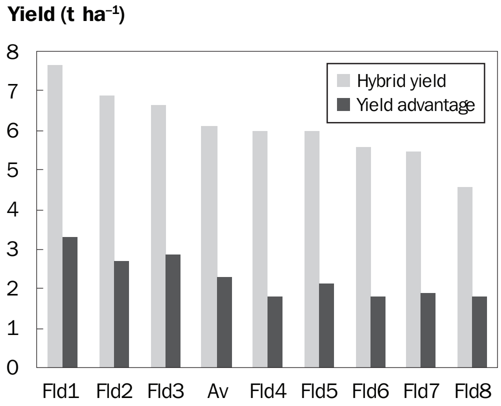

Fig. 3. Farm tests with hybrid Ecco.

Table 4. Blast incidence rating for upland hybrid Ecco.

Genotype	Blast rating	
	Leaf	Panicle
Ecco hybrid	1	4
Check variety Primavera	6	7

Rating 0 to 9 (meaning 0 = no infection and 9 = totally infected)

Hybrid development for rainfed upland cultivation

In the testcrossing program, germplasm with a tropical background has also been included and tested. In this screening process of hybrids, we could identify a very interesting hybrid combination that is now being introduced under the trademark Ecco. This hybrid has attracted our attention because of superior blast tolerance compared with that of the most commonly used check variety, Primavera (see Table 4), and its very high yield performance. Ecco has been tested during 4 years in strip trials and, in the 2007-08 season, marketing tests were done on eight farms under real farm conditions, planting in each case on an area superior to 10 ha. The data of these marketing tests are shown in Figure 3. This hybrid delivered in the trials an average yield of 6.1 t ha^{-1} and has a yield advantage over check variety Primavera of 2.275 t ha^{-1}, a heterosis of 60%.

The choice of a hybrid system

RiceTec works with the CMS (cytoplasmatic male sterility) system as well with the TGMS (temperature-sensitive genetic male sterility) system. Thanks to the high degree of combinability, the TGMS system delivers relatively fast first results in a new hybrid program. The wide combinability of TGMS S lines allows using at least one parent from the region, providing some initial success. Multiplication of the S line was quickly mastered by production in the south, the coolest rice production regions below 33° south latitude. However, it continues to be difficult to find suitable production locations at which S lines remain sterile for safe two-line hybrid seed production. Cold fronts from the Antarctic, which can occur at any time during the summer and the critical flowering period, have caused a real challenge in finding suitable production locations. For the selection of a production location, the following criteria were defined: (1) temperature conditions that allow seed production at low selfing risk, (2) red-rice-free land, (3) water and land availability that allow seed production on a large scale, (4) isolated fields to avoid cross-pollination from neighboring fields, and (5) other production cost–related criteria. Several tests were done initially in core rice production fields in Argentina and Brazil between 26° and 28° south latitude. Later tests were done in lowland rice production fields at 20° south latitude in Miranda, Mato Grosso do Sul, at 10° south latitude in Lagoa da Confusão, Tocantins, and at 3° north latitude (in Boa Vista, Roraima). The more we moved into the inner tropics, the less selfing was observed with the TGMS two-line system. Currently, we produce seed regularly in Boa Vista without selfing problems. The Boa Vista production site, however, proved to be expensive because of its remote location and high input costs caused by the distance. More recently, new regulations on the preservation of territories for the native indigenous population have limited expansion. The other tested locations proved not to be completely safe and, in addition to other limitations such as environmental regulations, land availability, and excess rain at harvest, led to the abandonment of these other tentative production areas. The difficulties in identifying suitable production locations for efficient large-scale seed production led RiceTec to concentrate on the CMS system for new product development.

Seed production

At the previous Fourth International Hybrid Rice Symposium, the complexity of hybrid rice production was reported by different authors (Mao and Virmani 2002). The outcrossing rate, that is, the net seed yield, obtained after drying and conditioning, is the most important factor affecting seed costs.

Hybrid seed production yield is being reported as 2.7 t ha^{-1} in China and 1–1.5 t ha^{-1} in other Asian countries (Mao and Virmani 2002). In South America, RiceTec has made good progress in average net female yields. After seed conditioning, net yields are now in the range of 2.0 t ha^{-1}, almost twice what was achieved 3 years ago. Certain hybrids with good outcrossing females are reaching female seed yields of > 3 t ha^{-1}. Seed production under upland conditions is reported by Taillebois and

Maronezzi (2002) to have an objective of 1.5 t ha^{-1} using parents that do not require split planting. RiceTec achieved a net female seed yield of 1.65 t ha^{-1} under upland conditions with complementary pivot sprinkler irrigation and this with a hybrid that requires a 3-week split planting. Experience has shown that rice is very sensitive if irrigation water is kept short, as can happen when farmers try to achieve economies in water management.

In South America, RiceTec recommends seed densities of 40 kg ha^{-1} in upland cultivation and 45 to 50 kg ha^{-1} in irrigated cultivation. Seed production needs to be highly efficient and cost-effective to economically justify these seed amounts. To achieve this, seed production is done in mechanized agriculture using seed drills, with possibilities for aerial spraying, pollen movement by helicopter if required, and combine harvest. Seed drying is done in intermittent or continuous-flow dryers commonly used for the drying of grain and varietal seed. This equipment is suitable for hybrid seed, although several adjustments are required to make this equipment gentler to the hybrid seed, thus reducing the risk of mechanical damage.

Market introduction

Close involvement of leading farmers during the product development phase allows early exposure of new products to potential customers. Product introduction is done in a gradual process that allows farmers to become familiarized with hybrid cultivation and learn about new products. The first and most drastic change perceived and questioned by farmers was the seed density reduction from 150 kg ha^{-1} with varieties to 50 kg ha^{-1} with hybrids. New hybrids are also regularly presented to the leading rice mills, giving samples to ensure acceptance of the rice produced with hybrid seed.

Farmers have been initially skeptical toward hybrids, although good yields, acceptance at the rice mills, and the huge benefit of IMI tolerance for red rice control helped to overcome these initial difficulties. Now, the demand for CL hybrid seed with good grain quality exceeds its availability.

Conclusions

Hybrid rice has become a reality in South America for both irrigated and upland rice. The major factors that have led to its acceptance are high yield performance, grain quality within the standard of currently commercialized varieties, and availability of IMI tolerance.

However, new grain quality trends toward low chalk and low stickiness are causing additional challenges for hybrid breeders. A better understanding of the grain chemistry is essential for breeding and measurement of stickiness. Also, the chalk issue needs further research to better understand the interactions of chalk with genetics and environment.

The expansion of hybrids is now a question of building up adequate seed production infrastructure and capability. The two-line system of TGMS has proved to be somewhat limited, though technically feasible. Production at suitable locations is limited for a major expansion because of surface, regulatory, and cost limitations. However, a stable photoperiod-sensitive genetic male sterility system would have wide opportunities in a country with geographical extension from 5° north latitude to 33° south latitude.

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Authors' addresses: M.R. Ritter, business director; L. Pasqualli, development manager; R. Luzzardi, research manager; and C. Saravia, quality manager, RiceTec Sementes LTDA, Rua 18 de Novembro # 341, Porto Alegre, Brazil, CEP 90240-040.

Developing indica-type hybrid rice in China

(Country Report)

Chang-Xiang Mao

Hybrid rice has made great contributions to China's food security since 1976. Indica is the main type of hybrid rice in China and research and development on it began in 1964 by professor L.P. Yuan and his group. The crop has passed through four stages of development: the initial stage, the fast-growing stage, the strategic adjustment stage, and the new developing stage. Work has covered three-line and two-line breeding systems; breeding heterotic hybrids with high yield, good grain quality, and resistance to diseases and insect pests; breeding intra- and intersubspecific and super-high-yielding hybrids; agronomic management packages; seed production techniques; and related basic studies. Since hybrid rice was first commercialized in China in 1976, the total hybrid rice-growing area is now up to around 60–65% of the total rice-growing area, and indica-type hybrid rice always makes up more than 90% of the total hybrid rice in the country.

Increasing breeding efficiency and seed production yield through innovations in applied and basic studies to increase growing area is the major challenge for the further development of indica hybrid rice in China.

China is the second largest rice-growing country (in area) and the largest rice-producing country in the world. But, before the foundation of the new China in 1949, the rice-growing area was 25.33 million ha, with average yield of 1.91 t ha^{-1} that could not feed half of the nation's 450 million people. Because of the use of hybrid rice and improved inbred varieties, modified cultural management, and other supporting techniques, today, the average yield of rice in China is about 7 t ha^{-1} and the total growing area is 22–23 million ha, which is less than that in 1949 but still enough to feed the 1.3 billion population (Yuan and Chen 1988).

Indica-type hybrid rice has been the core of hybrid rice in China since the very beginning of rice research and development. More than 90% of the hybrid rice grown in China every year is indica hybrid rice. In terms of breeding methods, indica-type hybrid rice involves both three-line and two-line systems. The development of indica hybrid rice in China has passed through four major stages. For many reasons, from 1976 to 2006, the annual hybrid rice-growing area was around 60–65%, and never

Table 1. Total rice and hybrid rice growing area (million ha) in China (1988 to 2006).[a]

Year	Total rice area	Hybrid rice area	Hybrid rice in total rice area (%)
1988	23.793	12.143	51.0
1989	23.658	11.932	50.4
1990	26.929	15.643	58.1
1991	21.839	13.687	63.0
1992	25.045	14.761	58.9
1993	21.853	12.801	58.6
1994	22.151	12.858	58.0
1995	19.801	13.456	68.0
1996	23.401	14.636	62.5
1997	24.373	15.316	62.8
1998	24.419	14.969	61.3
1999	22.663	13.923	61.4
2000	23.026	13.727	59.6
2001	22.513	14.441	64.2
2002	22.059	14.311	64.9
2003	20.105	13.223	65.8
2004	22.318	14.637	65.6
2005	22.837	14.951	65.5
2006	24.283	15.199	62.6

[a] Only rice hybrids with annual growing area of more than 6,666.7 ha are included.

more than 70% of the total rice-growing area in China (Table 1) (China National Agricultural Service Center 1988-2006).

Recently, rice hybrids have been diversified, with an increased portion of ones with good grain quality and super-high-yielding two-line hybrids. Market-oriented breeding and seed production are featured, with an increasing number of hybrids developed by the private sector. Most seed companies for hybrid rice seed production have been part of the private sector since just before 2000. The major challenges for further developing indica hybrid rice are how to increase breeding efficiency, how to provide perfect agronomic management for super-high-yielding hybrids to exploit their heterosis, how to further increase seed production yield and reduce its cost, how to help rice growers increase their income by growing more hybrid rice in traditional rice-growing areas, innovation with new MS (male sterile) lines and R lines in breeding, basic studies on predicting heterosis, and biotechnological approaches to increase the genetic diversity for biotic and abiotic stress breeding to overcome environmental constraints.

Initial stage

From the beginning of hybrid rice research in 1964 and up to 1975, hybrid rice was widely demonstrated and showed great yield potential in China. In 1964, the father of hybrid rice, Professor Long-Ping Yuan, and his group started research on heterosis of rice with indica-type rice. Then, in November of 1970, abortive plants in a wild rice were discovered in Hainan Province, which was a breakthrough in breeding male sterile and maintainer lines in China. A set of stable sterile lines and maintainer lines was successfully bred in 1972, including Zhengshan 97A, 71-72A, V41A, Erjiunan A, etc., and a set of strong heterotic restorer lines was also developed, mostly by using varieties from the tropics, such as IR661, IR665, IR24, IR26, Thai-yin No. 1, Gu-154, etc. In 1973, the so-called three lines, that is, CMS lines and their maintainer and restorer lines (A, B, and R), had been accomplished. Since then, indica hybrid rice was declared a success in China as well as in the world (Lin and Yuan 1980).

From 1974 to 1975, indica rice hybrids were bred for demonstration, and different rice hybrids were grown on more than 373.3 ha in more than 10 provinces in China in 1975. Of these 373.3 ha, there were 13.3 ha of early-cropping rice, 93.3 ha of mid-cropping rice, and 266.7 ha of late-cropping rice, and all of them showed very high yield compared with high-yielding inbred cultivars widely grown at that time. The year 1976 was a landmark for hybrid rice commercialization in China as well as worldwide, with a total growing area of 138,000 ha (CAAS and HNAAS 1991).

The fast-growing stage

The period of 1976 to 1990 was called the fast-growing phase of hybrid rice in China, featuring a fast increase in growing area, and the so-called three-line method (i.e., using A, B, and R lines) became technically mature. The total growing area of hybrid rice (more than 90% was indica hybrids) jumped from 0.138 million ha in 1976 to a first peak of 15.643 million ha in 1990.

The major factors that were crucial for the fast development of hybrid rice in China were (1) government support, (2) nationwide cooperation and collaboration, (3) free sharing of breeding materials and information, (4) combination of intensive basic and applied research, (5) encouraging policies from the government, (6) international cooperation, and (7) dedication from all related scientists and people nationwide.

The exploitation of heterosis in rice was formally declared as a strategic research thrust in China by the Ministry of Agriculture in the early stage of hybrid rice R&D. A nationwide network and many regional networks for hybrid rice research, information and germplasm exchange, training, and extension were established and put under general coordination by the Chinese Academy of Agricultural Sciences (CAAS) and the Hunan Hybrid Rice Research Center (HHRRC), directed by the father of hybrid rice, Prof. L.P. Yuan. A national hybrid rice consultancy group was also established to nationally coordinate important activities related to hybrid rice R&D in the country. Nine regional and six national meetings for planning and monitoring were held from 1972 to 1987, which played a very important role for hybrid rice R&D in China. A Special Innovation Prize for indica hybrid rice was awarded by the central government in 1981 (Lou and Mao 1994).

Meanwhile, through efforts to improve seed production techniques, the nationwide average yield of hybrid rice seed production in China increased from 0.27 t ha^{-1} in 1976 to 2.72 t ha^{-1} in 1997, with a record of 7.39 t ha^{-1} (Yuan and Fu 1995).

The strategic adjustment stage

This stage lasted from about 1990 to 2000. The growing area of hybrid rice in those 10 years straggled. The growing area for the most widely grown hybrid, Shan You 63, dropped from a peak of 6.81 million ha in 1990 to 0.76 million ha in 2001, the last year its growing area covered more than 0.67 million ha in its history (Table 2).

Though a natural male sterile plant was discovered by Ming Son Shi in a field of Nongken 58, a late japonica rice variety, in 1973, which was the foundation of two-line hybrid rice in China, an innovative strategy for hybrid rice research was developed by L.P. Yuan and his colleagues in the late 1980s, when the yield and efficiency of three-line intravarietal hybrids could not be further increased. That strategy included developing two-line hybrid rice by using thermo-sensitive genetic male sterile (TGMS) and photo-sensitive genetic male sterile (PGMS) lines to increase the efficiency of breeding and seed production; developing intersubspecific hybrids to increase heterosis; and developing interspecific hybrids by using apomixis, the so-called one-line method, through distance hybridization that theoretically would not need hybrid seed production any more when heterosis was fixed by apomixis (Yuan 1992, Mao 1994). Prof. Yuan proposed a grain yield of 100 t ha^{-1} day^{-1} as the goal for breeding super high-yielding rice in a super hybrid rice breeding program in China. He expected to achieve this by 2002 (Yuan 2001). Super hybrid rice has morphological features such as tall erect-leaf canopy, lower panicle position, and larger panicle size. Grain weight per panicle is around 5 g, the number of panicles is about 300 m^{-2}, and theoretical yield is 15 t ha^{-1} (Yuan 2003).

Research on two-line and intersubspecific hybrid rice was smoothly carried out and the hybrid rice successfully commercialized in 1995 (Yuan 2005). One-line breeding after about ten years of practice showed that it was impossible to get 100% of apomixis from existing germplasm or by cumulative hybridization or even by transgenic approaches, so this was halted in the mid-1990s.

Table 2. Development trend of rice hybrids commercialized in China (1988 to 2006).[a]

Year	No. of hybrids grown >6,666.7 ha	No. of hybrids grown >666,666.7 ha	Name of hybrids grown on more than 670,000 ha and their area (million ha)
1988	41	5	Shan You 63 (4.623), Wei You 64 (1.369), Shan You 64 (1.099) D You 63 (0.952), Wei You 6 (0.701)
1989	39	4	Shan You 63 (5.311), Wei You 64 (1.151), Shan You 64 (1.355), D You 63 (12.50)
1990	63	6	Shan You 63 (6.813), Shan You 64 (1.905), Wei You 64 (1.351), D You 63 (1.114), Shan You Gui 33 (0.767), Bo You 64 (0.671)
1991	58	5	Shan You 63 (5.771), Shan You 64 (1.413), Wei You 64 (1.090), D You 63 (0.865), Shan You Gui 33 (0.735)
1992	78	4	Shan You 63 (6.088), Shan You 64 (1.263), D You 63 (0.874), Wei You 64 (0.828)
1993	81	3	Shan You 63 (5.021), Shan You 64 (0.996), D You 63 (0.713)
1994	97	2	Shan You 63 (4.455), Shan You 64 (0.772)
1995	104	3	Shan You 63 (4.076), Shan You 64 (0.861), Gang You 22 (0.716)
1996	130	3	Shan You 63 (3.564), Gang You 22 (1.280), Shan You Duo Xi No. 1 (0.687)
1997	160	2	Shan You 63 (2.940), Gang You 22 (1.598)
1998	186	2	Shan You 63 (2.306), Gang You 22 (1.613)
1999	175	2	Shan You 63 (1.439), Gang You 22 (1.151)
2000	199	3	Shan You 63 (1.159), II You 838 (0.791), Gang You 22 (0.786)
2001	217	1	Shan You 63 (0.761)
2002	228	1	Pei S 9311 (0.825)
2003	243	1	Pei S 9311 (0.731)
2004	267	2	Jinyou 207 (0.719), Pei S 9311 (0.671)
2005	379	0	–
2006	458	1	Pei S 9311 (0.771)

[a] Only rice hybrids with annual growing area of more than 6,666.7 ha are included.

The new developing stage

This period goes from 2000 to now. This is market-oriented development with more guidance from market demand. The growing area of two-line hybrids increased gradually, and the growing area of the famous two-line hybrid Pei S 9311 reached 0.86 million ha in 2002, replacing Shan You 63, which had occupied first place in growing area in China for more than 20 years. The growing area of hybrids with either high yield or good grain quality has expanded very fast. The growing area of the series of hybrids derived from Zhengshan 97 A, the longest-lasting CMS line in China, as a female parent declined from 9.57 million ha in 1992 to 0.45 million ha in 2006, and its occupation of total hybrid rice area also decreased from 64.8% in 1992 to only 2.9% in 2006, whereas the growing area of another series of hybrids using Jin 23 A, with good grain quality, as a female parent increased from only 0.3% in total hybrid rice area in 1994 to 17.3% in 2006, which became the series of hybrids with the largest growing area in China now because of their good grain quality and stable high yield. More and more new hybrids are released and the number of hybrids grown on more than 0.67 million ha dropped to nearly zero (Table 2).

On the other hand, the growing area of a series of two-line hybrids using Pei S as a female parent was 0.095 million ha in 1996, but jumped to 1.067 million ha and occupied 7.0% of the total hybrid rice-growing area in China because of their super high yield and good grain quality. The proportion of two-line hybrid rice and super high-yield hybrids is increasing gradually. Some yield records were set recently. From Table 5, we can see that, from 1997 to 2006, the total number of commercialized two-line hybrids and their growing area increased greatly. The number of TGMS or PGMS lines has increased too. Now, two-line hybrid rice-growing area surpasses 15% of the total hybrid rice area in China.

Another feature of the new developing stage is that initiatives of hybrid rice breeding have reached a peak. The public and private sector have equal capability to do breeding. For example, after the first hybrid was named as part of a company's series, Xian Nong in 2003, this kind of hybrid increased quickly year by year. In 2004, 13 hybrids came from Xian Nong, and 18 in 2005 and 23 in 2006. Other companies showed their series of hybrids such as Long Ping, De Nong, and Shen Nong in their national annual statistics in 2005 and 2006, which means that more and more hybrids will be named as part of a company's series, no longer showing parentage as did previous hybrids, which included an MS line's name combined with an R line's name. For example, from the name Shan You 63, we know that its MS line is Zhengsha 97A and the R line is MH 63 R. Now, if hybrids have only a company's series number, no one will know their parentage.

The major hybrid rice-growing area has changed as economic development has changed in some provinces. From Table 3, the change and trend of hybrid rice-growing area in different provinces from 1997 to 2006 are clearly seen. After a few years, Jiangxi Province became the largest hybrid rice-growing province in China, with a fast increase in the number and growing area of hybrids. In the past ten years, Jiangxi, Hunan, Sichuan, Hubei, Anhui, Guangxi, Guangdong, Fujian, Guizhou, and

Table 3. Number and area (million ha) of indica rice hybrids commercialized in different provinces from 1997 to 2006 in China.[a]

Province	Year	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Hunan	No.	40	46	43	71	52	59	51	60	96	78
	Area	1.749	2.143	2.089	2.342	2.186	2.279	1.929	2.291	2.468	2.069
Jiangxi	No.	27	37	49	45	60	40	41	66	112	118
	Area	1.864	1.824	1.744	1.701	3.137	1.996	1.737	2.303	2.691	3.049
Guangxi	No.	49	46	46	49	48	53	56	57	59	74
	Area	1.749	1.606	1.710	1.181	1.353	1.219	1.262	1.257	1.197	1.428
Sichuan	No.	23	25	25	38	25	18	22	13	47	92
	Area	1.948	1.885	1.761	1.765	1.679	1.705	1.723	1.667	1.872	1.775
Anhui	No.	13	17	21	39	18	36	36	37	43	51
	Area	0.779	1.239	1.190	1.151	1.285	1.629	1.311	1.571	1.319	1.436
Hubei	No.	25	24	22	57	26	32	35	45	46	50
	Area	1.241	1.337	1.183	1.298	1.133	1.365	1.200	1.525	1.625	1.545
Guangdong	No.	39	38	26	48	23	39	32	35	37	44
	Area	1.196	1.096	0.659	0.479	0.472	0.877	0.777	0.805	0.773	0.878
Fujian	No.	21	27	27	27	28	27	30	32	34	34
	Area	0.751	0.704	0.771	0.643	0.633	0.669	0.683	0.627	0.649	0.565
Guizhou	No.	16	13	17	19	21	23	19	27	19	25
	Area	0.565	0.485	0.492	0.433	0.423	0.443	0.452	0.485	0.383	0.663
Yunnan	No.	7	12	9	12	12	16	14	13	10	12
	Area	0.219	0.273	0.205	0.248	0.200	0.234	0.205	0.190	0.156	0.153
Zhejiang	No.	15	13	30	33	17	14	12	17	22	16
	Area	0.567	0.519	0.585	1.075	0.453	0.341	0.324	0.359	0.379	0.327
Jiangsu	No.	6	7	10	8	10	12	13	17	17	13
	Area	0.618	0.582	0.523	0.365	0.387	0.335	0.341	0.323	0.298	0.319
Chongqing	No.	10	18	21	32	7	11	16	20	28	33
	Area	0.725	0.620	0.518	0.503	0.613	0.621	0.613	0.612	0.595	0.486
Henan	No.	7	6	7	13	6	9	13	14	15	19
	Area	0.340	0.327	0.203	0.309	0.291	0.307	0.363	0.336	0.301	0.287
Shanxi	No.	5	5	7	6	3	9	9	9	3	2
	Area	0.126	0.193	0.082	0.086	0.038	0.101	0.090	0.090	0.030	0.022
Hainan	No.	4	5	7	10	6	7	6	4	7	5
	Area	0.141	0.138	0.211	0.149	0.151	0.153	0.170	0.173	0.184	0.165

[a] Only rice hybrids with annual growing area of more than 6,666.7 ha are included.

Table 4. Comparison of average growing area of rice hybrids and inbred varieties from 1988 to 2006 in China.[a]

Year	Hybrid rice			Inbred rice			Hybrid/ inbred (times)
	No. of hybrids	Area (million ha)	Average (000 ha)	No. of inbred varieties	Area (million ha)	Average (000 ha)	
1988	41	12.143	296.16	246	11.650	47.36	6.25
1989	39	11.932	305.95	248	11.726	47.28	6.47
1990	63	15.643	248.29	255	11.257	44.15	5.62
1991	58	13.687	235.99	193	8.031	41.61	5.67
1992	78	14.761	189.24	251	10.285	40.97	4.62
1993	81	12.801	158.03	239	9.053	37.88	4.17
1994	97	12.858	132.49	259	9.332	36.03	3.68
1995	104	13.456	129.39	206	6.345	30.80	4.20
1996	130	14.636	112.59	243	8.765	36.07	3.12
1997	160	15.316	95.73	273	8.991	32.93	2.91
1998	186	14.969	80.48	282	9.451	33.51	2.40
1999	175	13.923	79.56	250	8.740	34.96	2.28
2000	199	13.727	68.98	256	8.394	32.79	2.10
2001	217	14.441	66.55	261	8.072	30.93	2.15
2002	228	14.311	62.77	251	7.748	30.87	2.03
2003	243	13.223	54.42	245	6.881	28.09	1.94
2004	267	14.637	54.82	238	7.681	32.27	1.70
2005	379	14.951	39.45	258	7.886	30.57	1.29
2006	458	15.199	33.19	264	9.084	34.42	0.96

[a]Only rice hybrids with annual growing area of more than 6,666.7 ha are included.

Chongqing are the top-ten hybrid rice-growing provinces, with more than 90% of the total hybrid rice area in China. Though the total number of commercialized rice hybrids increased greatly, the average area of each hybrid decreased year by year, and, compared with inbred varieties, the use efficiency of rice hybrids has declined a lot (Table 4).

Now, both research institutes and the public or private seed sector have the capability to breed and release heterotic rice hybrids. Developing new rice hybrids is not as difficult as in the past. If any new MS or R line is bred, a series of hybrids using the new MS or R line as parents will be developed and widely tested in a short period.

Constraints

Though hybrid rice is quite successful in China, its total growing area from the initial stage to now has never surpassed 70% of the total rice-growing area in the country. The major reasons are that japonica hybrids do not have enough heterosis and seed production yield is relatively low, so the japonica rice-growing area is mainly occupied by inbred varieties. Inbred rice varieties have been improved a lot in yield and grain quality, so many farmers are still willing to grow inbred rice varieties, and some rice-growing areas with poor conditions can achieve high yield by planting hybrid rice. The major constraints to its further development follow:

1. The efficiency of breeding and use of indica hybrid rice has decreased greatly. Recently, the total number of newly bred rice hybrids increased very fast year by year, but the average area of each hybrid grown decreased, and was even lower than that of inbred varieties in the entire country. The total number of hybrids in 2006 was 458, but only 41 in 1988, while the average area of each hybrid in 1988 was nearly 9 times that in 2006. Meanwhile, the average area and total number of each inbred variety had no big change during the same period (Table 4). The average life-span of each hybrid became shorter than in the early stages of the hybrid rice program in China. This means that the variety use efficiency of hybrid rice is decreasing.
2. Seed production techniques have not been further improved. Through efforts to improve these techniques, the nationwide average yield of hybrid rice seed production in China increased from 0.27 t ha^{-1} in 1976 to 2.72 t ha^{-1} in 1997, with a record of 7.39 t ha^{-1} (Yuan and Fu 1995). But, since then, the techniques for hybrid rice seed production have not been improved much, so seed yield did not increase greatly.
3. A lack of basic research to support technological innovation. Very little basic or applied-basic research has been carried out by scientists compared with the initial stage and fast development stage, so no significant discoveries have been reported.
4. No more effective national, regional, and international cooperation and collaboration. The national or regional cooperation and collaboration organized in the initial and fast development stages by the government disappeared; instead, competition has increased among research institutions.

Future outlook

1. For food security in China, hybrid rice, especially indica-type hybrid rice, should be properly developed by improving breeding, seed production, and cultural management. The growing area should be increased or at least should once again be what it was at its peak in 1990. Some provinces had good conditions for growing hybrid rice in the past, and should increase their area and inputs for hybrid rice production, such as Guangxi, Guangdong, and

Table 5. Major two-line hybrids commercialized and growing area in China (1997 to 2006).[a]

Year	No. of hybrids	Area (million ha)	Area in total hybrids (%)	No. of S lines	Name of S lines
1997	4	0.12	0.8	2	Pei S, Ba S
1998	5	0.23	1.5	2	Pei S, Ba S
1999	8	0.37	2.7	3	Pei S, Ba S, Xiang S
2000	16	0.86	6.3	4	Pei S, Ba S, Xiang S, An S
2001	18	1.35	9.4	5	Pei S, Ba S, Xiang S, An S, Tian S
2002	19	1.55	10.8	5	Pei S, Ba S, Xiang S, Feng S, Zhu S
2003	17	1.30	10.0	7	Pei S, Ba S, Xiang S, Feng S, Zhu S, Tian S, An S
2004	19	1.30	9.0	6	Pei S, Xiang S, Feng S, Zhu S, Tian S, Jin S
2005	32	1.46	9.7	11	Pei S, Xiang S, Feng S, Zhu S, Tian S, Jin S, Yang S, Zhun S, An S, Xin S, Lu S
2006	26	2.16	14.2	10	Pei S, Feng S, Zhu S, Tian S, Yang S, Zhun S, An S, Xin S, Lu S, Ben S

[a]Only rice hybrids with annual growing area of more than 6,666.7 ha are included.

Fujian provinces, which have large potential to grow hybrid rice to increase overall rice production, especially by growing super hybrid rice.

2. To increase hybrid rice-growing area, rice hybrids should have both high yield potential and good grain quality, and resistance to major diseases and insect pests. Farmers' initiative to grow more rice should be encouraged by some policies from the government; otherwise, the low output-input ratio for rice growers will continuously affect their enthusiasm for growing regular rice or hybrid rice.
3. Agronomic research on a technical package for hybrid rice, especially for super high-yielding hybrid rice, should be given more attention, and how to save labor, water, fertilizer, herbicide, and pesticide should be considered and studied while new rice hybrids are being released.
4. As cropping systems or rice-growing methods change, and rice planting and harvesting are mechanized, agronomists should work with breeders to develop suitable rice hybrids to adopt for changes in rice cultivation.
5. The high yield of seed production should also be studied to increase seed yield and reduce the cost so that farmers can increase their income by lowering the production costs of hybrid rice.

6. Some basic studies, such as on biotechnology for breeding, seed production, seed purity testing, heterosis prediction, etc., should be carried out by research groups with good facilities and experts, which could help to increase breeding efficiency.
7. The spirit of cooperation that existed for a long time in the hybrid rice research and development program in the initial and fast development stages in China should be recovered and a national, regional, provincial, and institutional cooperation network should be re-established, and breeders' rights should be fully protected.
8. To avoid too much repetition in breeding and to increase variety use efficiency, and to create new breeding methods and materials, such as new cytoplasmic sources for CMS, TGMS, or PGMS lines, or new approaches for high seed production, some breeders or rice scientists should be encouraged into innovative research.

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Notes

Author's address: Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, 530007, China, email: cxmao@gxaas.net.

Hybrid rice technology in Egypt

H.F. El-Mowafi, A.O. Bastawisi, A.F. Abdekhalek, K.A. Attia, and R.A. El-Namaky

One of many options available, hybrid rice is a proven technology to increase rice productivity by 15–20%. The success story of commercial rice cultivation in China has encouraged Egypt to adopt this technology. The hybrid rice research program in Egypt began in 1995, supported by collaborative projects involving the United States, FAO, and IRRI. By 2004, almost all components of the hybrid rice technology program were completed upon producing certified hybrid rice seed, which was made available for commercial cultivation of hybrid rice in farmers' fields during the 2005, 2006, and 2007 seasons.

The six main components of the hybrid rice program involve (1) breeding, (2) seed production, (3) agronomic practices, (4) training, (5) extension, and (6) hybrid rice economics, and are discussed in detail. The program resulted in the development of two promising hybrid rice combinations—SK 2034H (HR1) and SK 2046H (HR2)—that outyielded current commercial cultivars by 15–20%. A similar cultivar (Giza 178) possessed tolerance of biotic and abiotic stresses, and good grain quality expressed through grain appearance, milling recovery, nutrient value, and a panel taste test. Hybrid rice cultivation in Egypt began on a commercial basis in 2005.

In addition to these hybrids, second- and third-stage hybrid combinations are in the development pipeline. However, the two-line method with thermo-sensitive genic male sterility (TGMS) and photoperiod-sensitive genic male sterility (PGMS) is used also. The environment in Egypt appears to favor high outcrossing, seed setting, and seed quality. The average seed yield under experimental plots ranged from 2.0 to 3.0 t ha^{-1}.

Keywords: Egypt, hybrid rice, breeding, seed production, CMS, EGMS

After wheat, rice is considered the second most important cereal crop as a main food for the Egyptian population. Rice is also the second export crop after cotton. Rice area in Egypt ranges annually from 0.5 to 0.6 million ha, all of it under irrigation.

During the past 15 years, Egypt's Rice Research Program has succeeded in raising the national average rice yield more than 66%, from 5.71 t ha^{-1} for the base period (1984-86) by a steady increase annually to reach its maximum (9.84 t ha^{-1}) in

Table 1. Average national yield of inbred and hybrid rice in commercialized hybrid rice by country.

Country	Hybrid variety	Inbred variety	Yield advantage (%)	Source
China	6.80	5.40	25.9	Yuan et al (2003)
India	6.33	5.22	21.3	
Vietnam	6.30	4.84	30.2	Virmani (2002)
Philippines	7.80	6.50	20.0	Virmani (2002)
Bangladesh	7.48	5.79	29.2	Virmani (2002)
Egypt	12.18[a]	9.45	Commercial av. projection under hybrid rice, 10.90 t ha^{-1} (15%)	Virmani (2002)

[a] Average of multilocation yield trials.

2007 (RRTC 2008). This achievement allowed Egypt to occupy the first rank with the highest average yield worldwide in the past nine years. Several factors contributed to this achievement, the most important of which were the development and spread of new modern inbred varieties, which covered almost 100% of the total rice area in Egypt.

In spite of the great progress achieved in rice productivity in Egypt, we need to make another breakthrough to increase rice yield per unit area and unit time by raising the national average yield more than 15%. This is hard to achieve through inbred varieties because we have improved commercial varieties with superior genetic background.

Of the many options available, hybrid rice is a proven technology to increase yield by 15–25% under normal and saline soils. The success story of commercial hybrid rice cultivation in China, India, Vietnam, the Philippines, and Bangladesh encouraged Egypt to adopt this technology (Table 1).

Preliminary hybrid rice research began in Egypt in 1995 to determine the natural fertility restoration of Egyptian varieties on some Chinese CMS lines, evaluate certain CMS lines, do yield tests of some hybrid varieties, and evaluate yield and determine seed-setting percentage of some CMS lines under Egyptian conditions. The hybrid program was retained to provide more chance for improving rice productivity through the development of new improved inbred varieties (Maximos and Aidy 1994).

A systematic hybrid rice research program was developed as a national program at RRTC (Bastawisi et al 1998). After two years, a trilateral project titled "Enhancement of hybrid rice research and development" sponsored by the government of Egypt, the International Rice Research Institute (IRRI), the USDA, and ARS University of California began. The project resulted in the generation of valuable breeding materials and good hybrid combinations. The program gained further strength through the Food and Agriculture Organization (FAO)–sponsored project "Training in hybrid rice technology through technical cooperation between developing countries" (TCP/EGY/8923 & 2801 (T))," which resulted in developing human resources inside and outside the country and accelerated hybrid rice technology in Egypt.

Table 2. Relative performance and salient features of two hybrids chosen for on-farm and verification yield trials from 2001-05 seasons.

Hybrid	Parentage	Ecology	Yield (t ha^{-1})		Standard heterosis (%)	Accessory characters		
			Range	Mean		DDG[a] (days)	Stature	Grain type
SK 2034 H	EMS1A/ Giza 178 R	Normal soil	10.1–15.3	11.27	22.6	135	Semi-dwarf	Short grain with low amylose
		Saline soil	5.1–6.6	5.65	31.7	136		
SK 2046 H	EMS1A/ Giza 181 R	Normal soil	10.2–14.9	11.62	26.4	135	Semi-dwarf	Medium fine indica grain with low amylose
		Saline soil	5.1–6.1	5.60	30.5	140		

[a]DDG = days to heading.

By 2004, all the main hybrid rice technology components—(1) breeding, (2) seed production, (3) hybrid rice cultivation, (4) training, (5) extension, and (6) a study of hybrid rice economics—had been accomplished. Two hybrid rice combinations, SK 2034 H and SK 2046 H, have been found to consistently outperform the best leading inbred varieties, Sakha 101 and Giza 178, by 20–30% under normal and saline soils. These two hybrid combinations were evaluated in verification yield trials and on-farm demonstrations during the 2003 and 2004 seasons. SK 2034 H is characterized by relatively short grain and low amylose content and was the first hybrid rice to be released for commercial cultivation in 2005, whereas SK 2046 H has medium-fine indica grain type with low amylose content. The salient features of these hybrids appear in Table 2. In addition to these hybrids, second- and third-stage hybrid combinations are in the pipeline. The yield range and average yield under normal and saline soils, percentage of standard heterosis over the local inbred check (Giza 178), and accessory characteristics are presented in Table 3. By releasing the first hybrid rice in commercial cultivation, a hybrid rice program in Egypt was established. Hybrid rice technology components and the future prospects of hybrid rice in Egypt are discussed.

Hybrid rice breeding

In Egypt, the three-line breeding method for hybrid rice is used; however, the two-line method (TGMS and PGMS) can be used as well, but it is in its initial stage (Bastawisi et al 2002, 2003, El-Mowafi 2006). Hybrid rice breeding components and procedures are presented in Table 4. The hybrid rice breeding program in Egypt includes

- Identification and development of parental lines, which includes a source nursery, testcross nursery, CMS maintenance and evaluation nursery, highly adapted CMS lines under Egyptian conditions (Table 5), backcross nursery,

Table 3. Promising hybrid entries in the pipeline tested from 2004-07 seasons.

Hybrid	Parents	Ecology	Yield		Standard heterosis	Accessory characters	
			Range	Mean		DDG[a]	Grain type
SK 2035 H	SKMS9 A/	Normal	12.43–14.93	12.8	21.90	134	Medium
	Giza 178 R	Saline	6.68– 6.80	6.74	31.90	135	
SK 2029 H	SKMS4 A/	Normal	10.10–15.37	11.57	22.14	130	Medium
	Giza 178 R	Saline	5.30– 6.61	5.96	16.63	132	Slender
SK 2010 H	SKMS10 A/	Normal	10.63–12.95	11.34	25.16	130	Short
	Giza 182 R	Saline	3.63– 3.75	3.69	20.00	133	Bold
SK 2058 H	SKMS8 A/	Normal	11.12–12.51	12.11	20.54	130	Medium
	Giza 182 R	Saline	5.76– 6.34	6.05	18.40	132	Slender
SK 2074 H	SKMS8 A/	Normal	11.73–12.80	12.18	18.45	134	Medium
	Giza 5121 R	Saline	5.39– 7.13	6.17	18.90	135	
SK 2003 H	SKMS10 A/	Normal	12.15–12.95	12.43	16.04	130	Short
	Giza 178 R	Saline	4.63– 5.59	5.07	18.26	132	

[a] DDG = days to heading.

Table 4. Hybrid rice breeding components and nurseries.

	Component/material	Remarks
I	*Evaluation of experimental hybrids*	
	* On-farm trials	Demonstration
	* Multilocation yield trial (MYT)	Replicated
	* Advanced yield trial (AYT)	Replicated
	* Preliminary yield trial (PYT)	Replicated
	* Observational yield trial (OYT)	Augmented design
	* IRHON	Augmented design
II	*Identification and development of parental lines*	
	* Source nursery (SN)	Hybridization
	* Testcross nursery (TCN)	Identification
	* CMS evaluation nursery (CMSN)	Maintenance
	* Backcross nursery (BCN)	Pedigree selection for A and B lines
	* Combining ability nursery (CAN)	Identification
	* Restoring ability nursery (RAN)	Selection of R lines
	* Improvement of parental lines	Improvement of B and R lines
	* TGMS nursery	Evaluation and pedigree selection
	* PGMS nursery	Evaluation and pedigree selection
	* Wide compatibility nursery (WCN)	Development
III	*Seed production of experimental hybrids*	
	* CMS multiplication (A/B)	Seed increase
	* Hybrid seed production (A/R)	Increase for experiments
	* Purification of A, B, and R lines	Purification

Table 5. Evaluation of experimental hybrids.

Materials	Entries/ cross	Remarks
Multilocation yield trial (MYT)	14	Replicated at 4 locations
Advanced yield trial (AYT)	20	Replicated at 4 locations
Preliminary yield trial (PYT)	30	Replicated at 1 location
Basmati preliminary yield trail (BPYT)	6	Replicated at 1 location
Observational yield trial (OYT)	91	Augmented design

combining ability nursery, restoring ability nursery, improvement of parental line nursery, TGMS and PGMS nurseries, and wide compatibility nursery. The time line of hybrid rice breeding in Egypt is presented in Figure 1.

- Evaluation of experimental hybrids was done through observational, preliminary, advanced, and multilocation yield trials. Five hybrid rice combinations were identified in both normal and saline soils.

Hybrid rice seed production

The tasks of the hybrid rice program are to (1) produce enough seeds to maintain promising CMS lines (A/B) and for experimental yield trials (A/R), and (2) produce nucleus and breeder seeds from parental lines for commercial production of hybrid seeds.

To maintain the purity and good quality of hybrid seeds, many steps have to be considered such as isolation, flowering synchronization for parental lines, roguing, GA_3 application, flag-leaf clipping, and supplementary pollination.

Hybrid rice seed production requires a high amount of accuracy and tedious work to be cost-effective. The environment in Egypt appears to be highly favorable for high outcrossing, seed set, and seed quality. The average seed yield under experimental plots is now 2.9 t ha^{-1}, ranging from 2.0 to 3.8 t ha^{-1}.

Based on experiments on seed production under research and at commercial levels, an optimum seed production package has been developed (Table 6).

Hybrid rice cultivation

Any promising hybrid combination cannot express its potential productivity unless an ideal package of recommended practices is adopted. Necessary information is being generated on optimum seed rate, seedling density in a nursery, seedling age, number of seedlings per hill, planting dates, planting geometry, fertilizer requirement, and irrigation. An optimum package of recommendations is presented in Table 7.

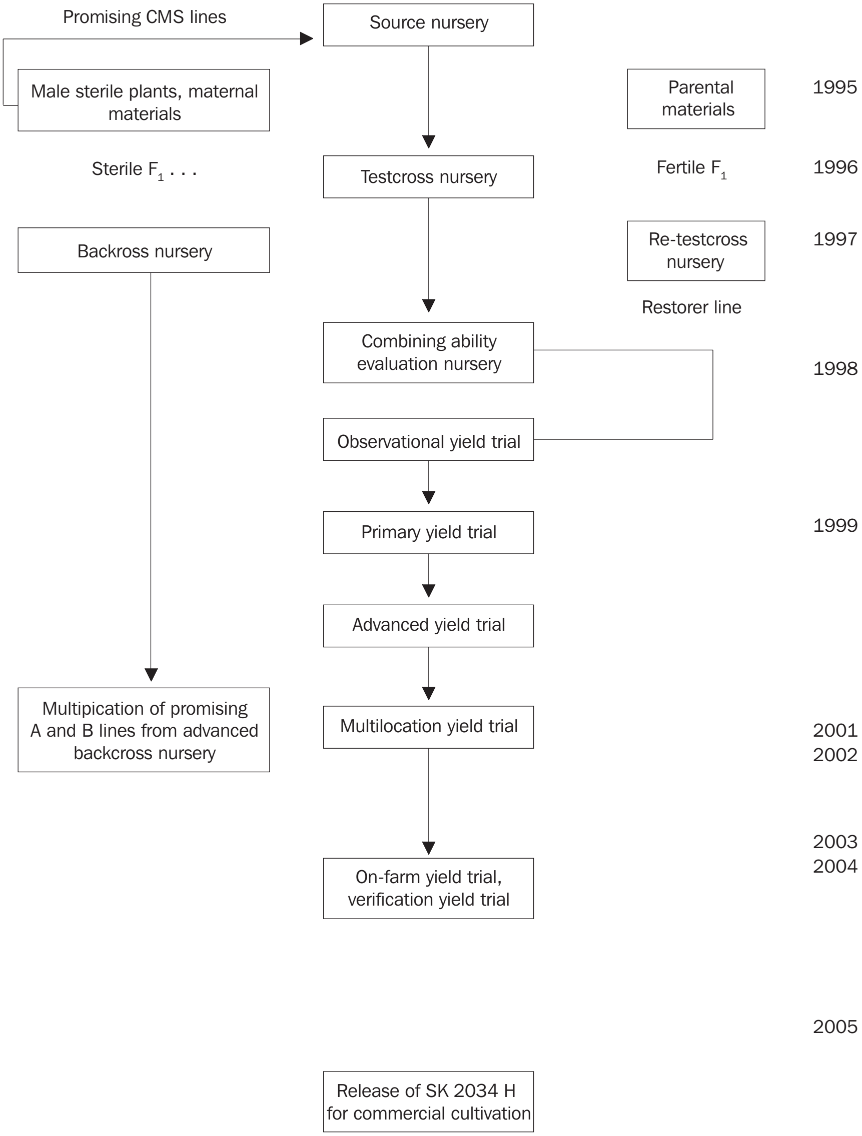

Fig. 1. Time line of hybrid rice breeding in Egypt.

Table 6. Promising hybrids identified in advanced yield trials (AYT) under normal conditions at three locations (Sakha, Gemmiza, and Zarzoura), 2007 season.

Hybrids/ check	Yield ($t\ ha^{-1}$)	Yield advantage ($t\ ha^{-1}$)	% over check (Sakha 101)	Remarks	Rank
SK 2034 H	12.55	2.16	20.80	Released	3
SK 2046 H	12.67	2.29	22.01	Under release	2
SK 2035 H	11.97	1.58	15.23		7
SK 2058 H	11.42	1.03	9.90		8
SK 2003 H	12.21	1.82	17.54	Short grain	6
SK 2010 H	12.88	2.49	23.99		1
SK 2007 H	12.55	2.16	20.80		4
SK 2107 H	10.68	0.30	2.85	Aromatic	9
SK 2074 H	12.44	2.05	19.74	Salt tolerance	5
Giza 177 (inbred)	9.55				
Giza 178 (inbred)	10.17				
Giza 182 (inbred)	10.32				
Sakha 101 (inbred)	10.39				
Sakha 104 (inbred)	9.91				

Table 7. Promising hybrids identified in multilocation trials under normal conditions at three locations (Sakha, Gemmiza, and Zarzoura), 2007 season.

Hybrids/ check	Yield ($t\ ha^{-1}$)	Yield advantage ($t\ ha^{-1}$) over Sakha 101	% over S. 101	Yield advantage over E. Yasmine	% over E.Y	Remarks
SK 2034 H	12.46	2.14	20.73			Released
SK 2046 H	12.54	2.22	21.51			Under release
SK 2003 H	12.60	2.28	22.09			Short grain
SK 2037 H	11.75	1.43	13.85	2.03	20.88	Aromatic
SK 2058 H	11.15	0.83	8.04			
SK 2107 H	10.43			0.71	7.30	Aromatic
SK 2096 H	10.88			1.16	11.93	Aromatic
SK 2122 H	12.53	2.21	21.41	2.81	28.91	Aromatic
SK 2108 H	10.89			1.17	12.04	Aromatic
SK 2065 H	10.99	0.67	6.49			
SK 2029 H	12.14	1.82	17.63			
SK 2121 H	12.61	2.29	22.19	2.89	29.73	Aromatic
SK 2010 H	12.65	2.33	22.58			
SK 2007 H	12.90	2.58	25.00			
Giza 178	10.05					
Sakha 101	10.32					
E. Yasmine	9.72					Aromatic

Table 8. Promising hybrids identified in yield trials during 2007 season.

Rank	Hybrids/ check	Yield (t ha^{-1})	Yield advantage (%)	DDG (days)	Ht (cm)	1,000-grain weight (g)	Grain type
1	SK 2010 H	12.77	23.27	130	118	28.3	M
2	SK 2007 H	12.73	22.88	132	119	28.0	M
3	SK 2046 H	12.61	21.72	135	116	26.3	M-Short
4	SK 2034 H	12.51	20.76	134	112	24.6	Short
5	SK 2003 H	12.41	19.80	135	120	26.0	M-Short
6	SK 2074 H	12.20	17.76	128	112	26.9	M-Short
Check	Sakha 101	10.36	–	140	100	25.9	Short

Hybrid rice training

The success of a hybrid rice program largely depends on the availability of trained personnel. In-country training was organized at three levels: (1) training of a core group, associated exclusively with the hybrid rice breeding and seed production program at RRTC, Sakha, which is the main center for hybrid rice research in Egypt; (2) a short-term training course of 2 weeks for young national scientists on hybrid breeding and seed production technology; the trainees included rice breeders, agronomists, and plant protection scientists (Table 8); (3) a 1-week training for persons associated with commercial seed production for seed growers and seed production specialists in both the public and private sector, including seed specialists from private seed companies; (4) training of extension officers on hybrid rice cultivation for 3 days; and (5) training of farmers from hybrid rice target areas on hybrid rice cultivation for 2 days.

Hybrid rice extension

On-farm (15 sites per year) and verification yield trials (20 sites per year) were conducted in farmers' fields from 2002 to 2004. A package of recommendations for hybrid rice cultivation was applied and the results confirmed the superiority of hybrids over inbreds by 20–30%.

Hybrid rice economics

Using the experimental data of hybrid rice yield trials and on-farm and verification yield trials, cost-benefit analysis was conducted for both hybrid rice seed production and hybrid rice cultivation (Sabaa 2002).

Cost-benefit analysis of hybrid rice seed production (F_1)

Table 9 presents the average yield of hybrid rice seeds: F_1 is 1.2 t ha^{-1}, with 2.3 t ha^{-1} from restorer lines, and the net return is about 14,987 LE ha^{-1} and the annual return to investment reached 268% (134% in 6 months). Assuming that marketing margin

Table 9. Promising aromatic hybrids identified in HR yield trials during 2007 season.

Rank	Hybrids/ check	Yield ($t\ ha^{-1}$)	Yield advantage (%)	Days to heading	Ht (cm)	1,000-grain weight (g)	Grain type
1	SK 2121 H	12.61	29.73	130	121	27.5	L
2	SK 2122 H	12.53	28.91	133	126	26.3	L
3	SK 2037 H	11.75	20.88	138	116	26.3	L
4	SK 2108 H	10.89	12.04	135	118	27.1	L
5	SK 2107 H	10.88	11.93	135	123	29.6	L
Check	E. Yasmine	9.72	–	140	117	28.0	L

Table 10. Promising hybrids identified in HR yield trials under saline condition during 2007 season.

Rank	Hybrids/ check	Yield ($t\ ha^{-1}$)	Yield advantage (%)	Days to heading	Ht (cm)
1	SK 2007 H	1.60	0.72	135	90
2	SK 2029 H	1.50	0.62	130	96
3	SK 2121 H	1.43	0.55	132	101
4	SK 2122 H	1.30	0.42	135	94
5	SK 2107 H	1.30	0.42	136	101
6	SK 2058 H	1.30	0.42	130	82
Check	Giza 178	0.88	–	132	84

will be 33%, farmers can get hybrid rice seeds at a price of 1 LE kg^{-1} (US\$1.60 kg^{-1}), about 4 times the cost of inbred seed.

Cost-benefit analysis of hybrid rice cultivation

Table 10 presents a cost-benefit analysis of hybrid rice cultivars compared with conventional cultivars (inbreds). The average yield of hybrid rice cultivars (based on a yield average of multiplication, on-farm, and verification yield trials) was 12.18 t ha^{-1} compared with 10.21 t ha^{-1} for conventional cultivars, with a yield advantage of 19%. The net benefit was 44% higher than that of inbred varieties and the return to investment reached 630% (315% in 6 months) compared with 508.4% for conventional cultivars. The only difference between hybrid and inbred cultivars is the cost of seed (66.5% higher for hybrids).

Hybrid rice grain quality

Grain quality, including grain appearance and milling recovery, eating and nutrient quality, and a panel taste test for two promising hybrids, SK 2034 H and SK 2046 H, are at the same level as those of the popular commercial cultivar Giza 178 (Tables 11, 12,

Table 11. Promising Basmati hybrids identified, 2007 season. All have a strong aroma.

Hybrid/check	Yield (t ha^{-1})	Yield advantage (%) Over E.Y.	Yield advantage (%) Over PB.1	Duration (days)	Milling (%)	Head rice (%)	Amylose (%)
SKPusa H1 (SKPH1)	11.6	36.5	48.7	138	71	62.0	22.0
SKPusa H2 (SKPH2)	11.3	32.9	44.9	140	68	55.0	22.5
Egyptian Yasmine	8.5	–	–	150	65	49.0	24.0
Pusa basmati	7.8	–	–	145	69	51.0	24.0
PR1 (restorer)	9.0	–	–	137	70	55.4	22.2
PR2 (restorer)	8.6	–	–	136	71	56.2	22.8

Table 12. TGMS parental lines and gene transfer in Sakha during 2007 season.

Generation	Planted		Selected	
	Crosses	Lines	Crosses	Lines
TGMS				
F_1	3	–	3	20
F_2	9	271	8	260
PGMS				
Anther culture	1	271	1	86
New PGMS	8	20	6	70

and 13). Data in Table 11 recorded some physical and milling quality characters of four rice entries. With grain shape expressed as the ratio between grain length and width, SK 2034 hybrid rice and variety Giza 178 have short grain type, whereas SK 2046 and Giza 181 have long grain. Grain weight of the tested rice entries was heavy, except for Giza 178, which was lower (1.9 g). Milling recovery of long-grain rice recorded a higher percentage than short-grain rice, which might be due to higher grain filling. Nonetheless, short-grain rice entries recorded the highest head-rice percentage.

Cooking and eating quality characters—amylose content, gel consistency, grain elongation, and chalkiness of grain—are presented in Table 12. The two hybrid rice lines, SK 2034 and SK 2046, had medium amylose content (22.5% and 23.7%, respectively), whereas varieties Giza 178 and 181 had low amylose percentage. All the tested rice entries had soft gel consistency, of 80–100 mm. These two characters are the main factors of gel viscosity of milled rice and softness after cooking.

Table 13. List of stable and highly adapted CMS lines under Egypt environment.

Entry	Cytosterility source	Grain type[a]	Amylose content (%)
IR58025 A	WA	L	15.8
IR68888 A	WA	L	23.8
IR69625 A	WA	M	22.9
IR70368 A	WA	L	24.2
G 46 A	Gambiaca	MB	21.2

[a]L = long, M = medium, MB= medium bold.

The highest elongation % was recorded by Giza 181, followed by Giza 178, whereas the two hybrid rice cultivers had the same value.

The amount of chalkiness directly affects the translucency of milled rice, which affects milled rice marketing. Giza 181 recorded the lowest chalkiness (0.1%), whereas SK 2034 recorded the highest value (14.4%) among the tested entries.

Palatability characters are the main indication of consumers' demand for different rice entries. This differs according to consumers' nutrient habits.

Data recorded in Table13 revealed that hybrid rice line SK 2046 and variety Giza 178 have better grain shape than SK 2034 and Giza 181 because of the hybrid rice high breakage % after cooking, and Giza 181 has long grain. Egyptian consumers prefer the short medium grain, bold to medium-high translucency, low breakage, and stickiness after cooking.

Giza 181, followed by SK 2034, was the best in other panel test traits such as hardness, stickiness, odor, and taste.

The total scores of different tested rice line entries were close and above 70% of the maximum scores. These results revealed that hybrid rice can be accepted easily by Egyptian consumers.

Hybrid rice biotechnology: progress and prospects

Egypt has successfully released its first indica hybrid rice. However, research is conducted in the rice biotechnology lab of RRTC to explore the most heterotic effects by using biotechnology.

Hybrid rice biotechnology achievements

Anther culture or pollen culture provides advantages of accelerating the breeding process and increasing selection efficiency in the breeding of hybrid rice over conventional techniques. Some Egyptian CMS and TGMS lines have been developed through anther culture and tested extensively in the field under normal conditions. These lines have been selected, and they were new hybrid lines with stable sterility,

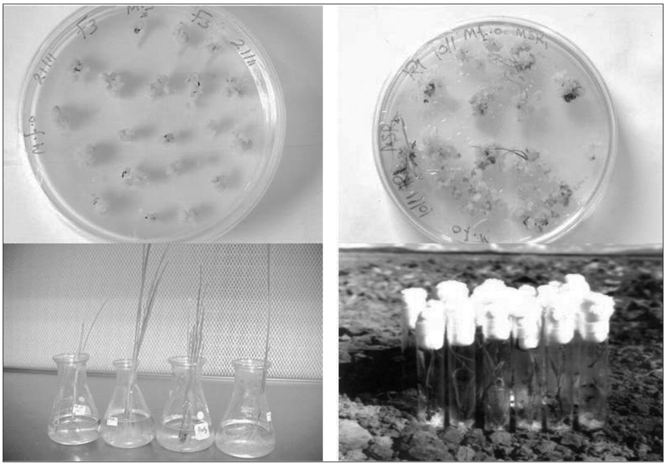

Fig. 2. Development of different stages of anther culture hybrid lines.

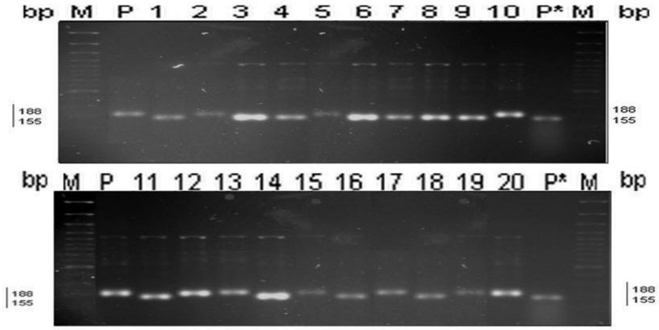

Fig. 3. SSR primer pair RM412 with the two parents and 20 lines selected from (Giza 178 × Dular). M: marker, P: Dular, P*: Giza 178, 1–20: lines selected, bp: base pair.

clear fertility alteration, heterobeltiosis, and competitive superiority (Fig. 2). Another set of Egyptian hybrid rice lines is under evaluation via anther culture.

Regarding molecular-assisted selection (MAS), most traits that are related to hybrid rice are difficult to select under field conditions. Recently, some DNA-based markers became available. It has already been established that traits such as restorer genes and wide compatibility genes are identified in some Egyptian hybrid rice restorer lines (Fig. 3). In addition, as is known, there are more than six genes for restoring ability in rice; thus, evaluating different R genes in a single individual plant is hard to do. But, with a known simple sequence repeat marker that is tightly linked to an R-gene, this will be very useful. We have accumulated both WC genes and R-genes in a single Egyptian line of an F_4 population derived from a cross between Dular and

Table 14. Optimum package for hybrid rice seed production and CMS multiplication.

Operation	Particulars
Sowing date	25 April-10 May
Seed rate	Seed parent : 27–36 kg ha^{-1} Pollen parent: 9–12 kg ha^{-1}
Nursery	Sparse seeding (42–57 g m^{-2}) to ensure seedlings with 3–5 tillers in 25 days
Row ratio	8 A lines:2 B lines 10 A lines:2 R lines or 12 A lines:2 R lines
Number of seedings/hill	1–3 for seed parent 2–3 for pollen parent
Spacing	Male:male 20 cm Male:female 25 cm and 30 cm Female:female 15 cm Plant:plant 15 cm
GA_3 application A local GA_3 (BERLEX)	300 g ha^{-1} in 500 liters of water at 10% of heading in two split doses of 40% and 60% on consecutive days
Supplementary pollination	3–4 times a day at peak anthesis time with 30-minute interval
Roguing	- At vegetative phase based on morphological traits - At flowering before anthesis - At maturity based on grain characteristics and seed set rate after removing R line
Seed yield	- A × B: 2.5–3.0 t ha^{-1} - A × R: 2.0–3.8 t ha^{-1}

Giza 178 (Table 14). Data showed that line no. 5 carries both genes, which would be of great use in developing indica/japonica hybrids.

Assessing hybrid rice seed purity

Many markers have been developed for hybrid rice seed purity, including morphological, biochemical, and molecular markers. Hybrid rice seed can have an outstanding yield if it is completely pure. But, if the seeds are contaminated, yield will fall. So, the seed must be tested before distribution to farmers. To do this, a year is needed for evaluation, along with land, water, and effort. Molecular marker technology solves this problem, thus saving time, effort, and money. Primer RM212 can differentiate between pure and contaminated lines of F_1 seeds (Fig. 4).

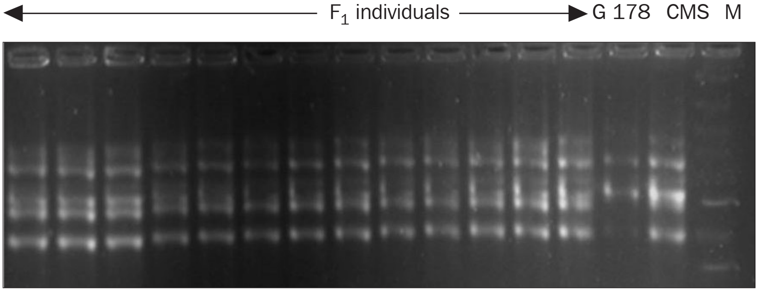

Fig. 4. Hybrid rice purity test using marker ISJ9.

Future prospects

The following represent future prospects for hybrid rice:

- Premium grain quality. The grain quality of promising hybrid combinations is at the same level as that of the leading inbred variety, Giza 178. However, premium grain quality is needed to improve both inbred and hybrid varieties.
- Development of parental lines for resistance to biotic and abiotic stresses.
- Introduction of wide compatibility genes to both two-line and three-line systems to raise yield potential.
- Use biotechnology to facilitate breeding and hybrid rice seed production.
- Introduce hybrid Basmati rice to commercial production.

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Authors' address: Rice Program, Field Crops Research Institute, ARC, Egypt. Email: hfelmowafi@gmail.com.

Hybrid rice research and development in India

B.C. Viraktamath, M.S. Ramesha, M. Ilyas Ahmed, A.S. Hari Prasad,
N. Shobha Rani, C.N. Neeraja, and R.M. Sundaram

Hybrid rice technology plays a pivotal role in increasing rice production and productivity in India and is one of the components of the national food security mission launched in 2007 to boost rice production. A total of 33 hybrids have been released for commercial cultivation and, during 2007, around 1.1 million hectares were planted to hybrid rice. Hybrid rice is being cultivated predominantly in the states of Uttar Pradesh, Jharkhand, Bihar, Punjab, Haryana, and Chhattisgarh. Some popular hybrids grown in the country are PA 6444, PHB-71, KRH-2, Pusa RH-10, PA 6201, Suruchi, JKRH-2000, PSD-3, Sahyadri, and DRRH-2. Many promising hybrids have been identified for abiotic stress situations such as rainfed upland, salinity/sodicity, and aerobic conditions. An array of hybrids with higher heterosis, better grain quality, and multiple disease and insect pest resistance are in the advanced stage of testing. Specific breeding efforts involving recombination breeding and genetic male sterility facilitated population improvement, resulting in the development of a large number of genetically diverse parental lines with improved grain quality and floral and agronomic traits.

To increase breeding efficiency, molecular markers are being used in genetic diversity analysis, the introgression of fertility restorer genes, prediction of heterosis, and use of wide compatibility genes in the exploitation of intersubspecific heterosis. Molecular identities of hybrids and parental lines of released hybrids have been developed. DNA-based genetic purity testing of hybrids and parental lines is being used extensively.

A suitable package for hybrid seed production was developed and popularized with seed growers and every year more than 18,000–20,000 tons of hybrid rice seed are being produced in the country. Large-scale hybrid seed production and marketing of public-bred hybrids is being facilitated through public-private partnership based on a memorandum of understanding (MOU) between eight public-sector research institutes/universities and more than 12 private companies. An aggressive transfer of technology efforts through compact block frontline demonstrations and training programs resulted in a large-scale adoption of the technology. More than 9,000 compact block frontline demonstrations were conducted in 16 states and, in these demonstrations, the hybrids have outyielded the best inbred check varieties to the tune of 1.5–2.0 t ha^{-1}. In order to impart knowledge and necessary skills on hybrid rice technology, more than 400 training programs were organized and around 12,000 people were trained.

Many constraints are being examined through ongoing research projects and through aggressive transfer of technology efforts. The national food security mission envisages increasing annual rice production by at least 10 million tons by the end of 2012 and hybrid rice technology is going to play a very significant role in contributing to national food security.

Rice is the staple food for more than 70% of Indians and is grown on 44 million hectares, with an average annual production of about 90 million tons. With the current growth of population, the rice requirement by 2011-12 is estimated to be around 100 million tons. It is a challenging task to meet this demand in the background of shrinking land, dwindling water resources, and nonavailability and skyrocketing costs of labor. Therefore, enhancing rice productivity through novel genetic approaches such as hybrid rice was believed necessary.

The Hybrid Rice Program in India was launched in 1989 through a systematic goal-oriented and time-bound network project with financial assistance from the Indian Council of Agricultural Research (ICAR). Technical support from the International Rice Research Institute (IRRI), Philippines, and the FAO, Rome, and additional financial support from the UNDP, ICAR, NATP, and Barwale Foundation were the major factors contributing to the remarkable success of hybrid rice technology in India.

The national food security mission launched in 2007 envisaged increasing annual rice production by at least 10 million tons by 2011-12. Hybrid rice technology is likely to play a pivotal role in achieving the targeted production increase in the near future. Therefore, it is included as one of the components of the National Food Security Mission. This country report briefly covers the significant progress made during 2002-07.

System of evaluating hybrids

Multilocation evaluation of promising experimental hybrids at 25–30 locations representing different agro-climatic zones of the country is the major activity of the coordinated evaluation in the Hybrid Rice Network, through which hybrids are being tested in replicated trials. A three-tier system of testing—Initial Hybrid Rice Trial (IHRT), Advanced Variety Trial-1 (AVT-1), and Advanced Variety Trial-2 (AVT-2)—is being followed in the country. Based on maturity duration, three groups of trials, early (<120 days), mid-early (121–130 days), and medium (131–140 days), are being conducted at different stages of testing. Test hybrids that record more than a 5% yield advantage over the best hybrid check and a 10% yield advantage over the best varietal check are promoted to the next stage of testing. At the AVT stage, hybrids will also be tested for agronomic performance, disease/insect pest resistance, and grain quality traits. Those entries with a consistent yield advantage and other desirable traits will be identified for release at the time of the Annual Rice Workshop by a specially constituted varietal identification committee. The proposals for identified hybrids are placed before the Central Subcommittee on crop standards and for notification of varieties for deliberation and final approval.

In total, 496 hybrid entries were evaluated during the period, out of which 357 were in IHRTs and 139 were in AVTs. Some 118, 167, and 193 hybrid entries were tested in the early, mid-early, and medium-duration groups. Among the hybrids tested in these trials, 51% were from the private sector and 49% were from public-sector research institutes.

Hybrids released

So far, 33 hybrids, 25 from the public sector and eight from the private sector, have been released. During 2002-07, five hybrids—RH-204, Suruchi-5401, DRRH-2, JKRH-2000, and PA 6129—were released by the Central Variety Release Committee and nine hybrids were released by the respective State Varietal Release Committees (Table 1). Among the released hybrids, the most popular ones are PA 6444, PHB-71, Pusa RH-10, KRH-2, PA 6201, Sahyadri, JKRH-2000, DRRH-2, and PSD-3.

Development of aromatic hybrids

In the Basmati rice hybrid development program at the Indian Agricultural Research Institute, New Delhi, a series of isocytoplasmic Basmati-quality restorer lines, Pusa 1238, Pusa 2502, Pusa 2503, Pusa 2504, Pusa 2511, and Pusa 2512, that were comparable with the check variety Pusa Basmati-1 in quality and matured 15–20 days early with 10–25% higher yield were developed. Of these, three, Pusa Sugandh-2 (Pusa 2504-1-3-1), Pusa Sugandh-3 (Pusa 2504-1-26), and Pusa Sugandh-5 (Pusa 2511), were released for commercial cultivation as varieties by virtue of their 15–20% higher yield, short duration, and excellent grain quality.

By using one of these restorer lines and a CMS line, Pusa RH-10, an aromatic rice hybrid was developed and it was released by the CVRC in 2001 for commercial cultivation in the irrigated ecosystems of Haryana, Delhi, and Uttaranchal. It is an early-maturing hybrid (115 days) as against 135 days taken for the best check variety, Pusa Basmati-1. With the release of Pusa RH-10, the Indian Agricultural Research Institute and the Indian Council of Agricultural Research have the distinction of developing the world's first superfine-grain aromatic rice hybrid. Being of early maturity, it suits well cropping systems such as rice-wheat, rice-rice-wheat, rice-potato, rice-chickpea, and rice-brassica. It has yielded from 6 to 10 tons per hectare in farmers' fields. This hybrid is in great demand and several public and private seed companies have taken up its seed production.

Table 1. List of recently released hybrids in India (2002-07).

Hybrid	Year of release	Duration (days)	Yield advantage over check (%)	Released for the state of	Developed by
RH-204[a,b]	2003	120–126	22.6	Andhra Pradesh, Karnataka, Tamil Nadu, West Haryana, Uttarakhand, and Rajasthan	Parry Monsanto India Ltd.
Suruchi 5401[a,b]	2004	130–135	19.5	Haryana, Andhra Pradesh, Gujarat, Orissa, Chhattisgarh, Karnataka, and Maharashtra	Mahyco Seeds Ltd.
DRRH-2[b]	2005	112–116	24.9	Haryana, Uttarakhand, West Bengal, and Tamil Nadu	Directorate of Rice Research (DRR), Hyderabad
Rajlakshmi	2005	130–135	27.9	Irrigated areas of Orissa	Central Rice Research, Institute (CRRI), Cuttack
Ajay	2005	130–135	35.9	Irrigated areas of Orissa	
Sahyadri-2	2006	115–118	25.0	Maharashtra	Balasaheb Sawant Konkan Krishi Vidyapeeth (BSKKV), Karjat
Sahyadri-3	2006	123–126	17.0	Maharashtra	
HKRH-1	2006	135–139	15.2	Haryana	Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hissar
CORH-3	2006	130–135	25.5	Tamil Nadu	Tamil Nadu Agriculture University (TNAU), Coimbatore
JRH-4	2007	110–115	12.8	Madhya Pradesh	Jawharalal Nehru Krishi Vishwavidhyalaya (JNKVV), Jabalpur
JRH-5	2007	110–115	16.0	Madhya Pradesh	
Indira Sona	2007	125–130	24.7	Chhattisgarh	Indira Gandhi Krishi Vishwavidhyalaya (IGKV), Raipur
JKRH-2000[a,b]	2007	135–140	13.5	West Bengal, Bihar, Orissa	J.K. Agri Genetics, Hyderabad
Hybrid 6129[a,b]	2007	85–90	39.4	Punjab, Tamil Nadu, Pondichery	Bayer Bio-Science, Hyderabad

[a]Private hybrids. [b]Central release

Table 2. Promising hybrids for abiotic stress and aerobic conditions.

Abiotic stress	Promising hybrids
Rainfed upland	DRRH-2, PSD-3, PSD-1, KJTRH-4
Salinity	DRRH-28, PSD-3, KRH-2, HRI-148, JRH-8, PHB-71
Alkalinity	Suruchi (MPH-5401), PHB-71, JKRH-2000, CRHR-5, DRRH-2, DRRH-44
Aerobic rice	PSD-3, KRH-2

Multilocational evaluation of released hybrids

To make a comparative evaluation of hybrids released in the country and to get information on their adaptability in different states, two sets of multilocational trials were conducted at 45–50 locations per year. In the trials conducted during 1999 and 2000, based on overall performance pooled over 125 locational means (3 seasons of data), the hybrid KRH-2 was ranked first, followed by PHB-71 and Sahyadri. In the trials conducted during 2006 and 2007, three hybrids, DRRH-2, PSD-3, and CORH-3, in the early-maturity group and three more, PA 6444, Suruchi (MPH 5401), and JKRH 2000, in the medium-duration group were found to be promising in many states.

Evaluation of hybrids under abiotic stress conditions

In general, hybrids are known to have more tolerance of abiotic stresses because of their genetic plasticity. To find out the suitability of hybrids for abiotic stresses such as moisture stress (rainfed upland) and aerobic and saline/alkaline soil conditions, many released hybrids were tested at hot-spot locations. Based on preliminary studies, the hybrids in Table 2 were found to be promising under different abiotic stress conditions.

Grain quality

The large-scale adoption of hybrid rice depends on the profitability of the technology, which in turn depends on the amount of heterosis and market price of the produce as determined by region-specific grain/cooking quality requirements. Hence, quality considerations are of paramount importance for the popularization and large-scale adoption of hybrid rice in India. Grain quality features of recently released and popular hybrids grown in the country appear in Table 3. It is evident that most of the hybrids have grain quality features either better than or on a par with those of popular varietal checks such as Jaya, IR64, and Annada, which are grown in different parts of the country.

Table 3. Grain quality characteristics of recently released hybrids and inbred variety checks.[a]

Hybrids and checks	Grain type	Milling (%)	HRR (%)	AC (%)	ASV	GC (mm)
Hybrids						
DRRH-2	LS	73	63	26	7.0	70
KJTRH-2	LS	70	52	24	7.0	58
JKRH-2000	LB	72	67	23.2	5.0	49
CRHR-7 (Ajay)	LS	75	68	24.9	4.0	55
KRH-2	LS	74	56	23.5	5.0	60
PA-6444	LS	74	74	–	4.5	–
PSD-3	LS	70	63	20.0	6.5	–
Sahyadri-3	LS	74	60	24.8	–	–
Sahyadri-2	LS	70	56	22.7	6.0	66
Suruchi 5401	MS	72	68	23.6	5.3	44
Rajalaxmi	LS	69	60	24.3	5.0	–
Checks						
IR64	LS	68	59	24.2	5.0	60
Annada	MB	69	54	25.4	7.0	50
Jaya	MB	73	62	25.6	7.0	36

[a] HRR = head-rice recovery, AC = amylose content, ASV = alkali spreading value, GC = gel consistency.

To develop and release medium-slender-grain hybrids with better cooking quality on a par with popular variety BPT 5204, a separate trial for medium slender grain type was conducted for three years. Among these hybrids, DRRH-44, developed by DRR, Hyderabad, was found to be superior for both yield advantage and grain quality traits, on a par with BPT 5204.

Screening for resistance to biotic stresses

The incorporation of resistance to major insect pests and diseases is one of the major objectives of the hybrid rice project. In addition to developing parental lines with high resistance to biotic stresses, hybrids in the coordinated trials are being regularly screened for resistance to major insect pests and diseases through national hybrid rice screening nurseries. Table 4 lists the recently released hybrids with resistance to or tolerance of insect pests and diseases.

Table 4. Resistance/tolerance of recently released hybrids for major insect pests and diseases.

Hybrid	Resistant to	Tolerant of
PA 6444	Neck blast, RTD, and gall midge	BLB, sheath rot, BPH, WBPH
Pusa RH-10	–	BLB, BPH, RTD, LF
DRRH-2	Leaf blast, neck blast	RTD, brown spot, WBPH, sheath rot
JKRH-2000	–	Leaf blast, RTD
KJTRH-2	Neck blast	Leaf blast, RTD
Narendra Usar Sankar Dhan-3	–	WBPH, BPH
PSD-3	Neck blast	Leaf blast, RTD, brown spot, BPH
Sahyadri-3	Neck blast	Leaf blast
Sahyadri-2	Neck blast	Sheath rot, WBPH, RTD, BLB
Suruchi-5401	–	Leaf and neck blast, RTD, brown spot
Rajalaxmi	–	Blast, WBPH, BLB
Ajay	–	Blast, WBPH, LF
HKRH-1	–	Neck blast, WBPH
PA 6129	Blast	Brown spot, BPH, WBPH

[a] RTD = rice tungro disease, BLB = bacterial leaf blight, BPH = brown planthopper, WBPH = whitebacked planthopper, LF = leaffolder.

Development and evaluation of CMS lines

The development of commercially usable CMS lines with desirable traits is an important activity of the hybrid rice network project in India. Work on developing region-specific CMS lines is actively done by many centers. Presently, 48–50 promising maintainers are at various stages of conversion in a backcross nursery. Table 5 lists the promising CMS lines developed by different centers in India.

Genetic improvement of restorers and maintainers

A parental line improvement program was taken up by recombination breeding and by resorting to genetic male sterility–facilitated population improvement. In the recombination breeding, single, three-way, and incomplete backcrosses among restorers/outstanding partial restorers as one group and maintainers/outstanding partial maintainers as the other divergent group were made and desirable segregants were selected in different generations. In the restorer breeding program, the major selection criteria were relatively tall stature, improved plant type with synchronous tillering, lower panicle position, sturdy culm, long/heavy panicles, moderate to heavy tillering, MS/LS grain type with intermediate amylose content, delayed leaf senescence, better

Table 5. Promising CMS lines developed.

Center	Promising CMS lines
DRR, Hyderabad	DRR 6A, 9A, 10A, 12A, 14A, 15A
IARI, New Delhi	Pusa 3A, 4A, 5A, 6A, 10A, 11A
PAU, Ludhiana	PMS 3A, 10A, 12A, 17A
RARS, Karjat	KJTCMS 1A, 2A, 3A, 4A
ARS, Ratnagiri	RTN 8A, 10A, 13A, 14A
APRRI, Maruteru	APMS 6A, 8A, 9A
CRRI, Cuttack	CRMS 32A, CRMS 31A

pollen production, and dispersal ability. However, in the maintainer breeding program, selections were carried out based on desirable traits: relatively short stature, improved plant type, high rate of exserted stigma (> 30%), MS/LS grain type with better grain quality, moderate to heavy tillering, lower panicle position and synchronous tillering, and slow leaf senescence with a high number of opened spikelets. Through this program, more than 1,500 restorers and 500 maintainers were developed. Among the restorer breeding material, the highest frequency of restorers was found in lines derived from crosses among restorers (61–64%) compared with that of restorer × partial restorer crosses. Among the maintainer breeding lines, crosses involving all maintainers gave a higher frequency of maintainers (56–65%) than maintainer × partial maintainer crosses (35–44%). Through genetic male sterility–facilitated recurrent selection, four new composite populations of restorers—DRCP 101, DRCP 102, DRCP 103, and DRCP 140—and two of maintainers—DRCP 104 and DRCP 105—were developed at DRR, Hyderabad. A large number of productive segregants with many desirable traits were being handled by the pedigree method and 500 new parental lines were developed.

The large number of parental lines developed from these programs is being regularly used in developing hybrids. The status of use of the new parental lines is given in Table 6.

Applying biotechnological tools in hybrid rice

Molecular marker technology has tremendous potential for improving hybrid rice. Since selection can be done at the seedling stage, molecular markers now offer opportunities to breeders for manipulating multiple traits. They can be deployed for hybrid rice improvement and thereby help in increasing breeding efficiency. The progress of research related to the application of DNA marker technology for hybrid rice improvement in India is given below.

Table 6. Status of use of new parental lines.

Status of use	No. of entries
No. of hybrids released/in the advanced stage of release	3
No. of hybrids in national trials	20
No. of hybrids in station trials	250
No. of restorers in experimental hybrid seed production	120
No. of new CMS lines developed	17
No. of B lines in CMS conversion program	50
No. of breeding lines released as varieties	5

Molecular marker-based characterization of genetic diversity among hybrid rice parental lines

In a study carried out by Sundaram et al (2008) at DRR, Hyderabad, 48 simple sequence repeat (SSR) markers uniformly spread across the rice genome were used for characterizing 10 each of WA-CMS and restorer lines used for hybrid rice breeding in India. Twenty-seven SSR markers showed amplification of an allele, which was very specific and unique to a particular parental line and not amplified in any other rice genotype tested. Through multiplex PCR, SSR marker combinations that were unique to a particular parental line or hybrid were identified. EST-SSR markers were used to study the genetic diversity among nine WA-CMS lines and 32 restorer lines. These markers differentiated the CMS lines from the maintainer lines clearly and showed a high correlation ($R^2 = 0.8$) with per day grain yield heterosis of the hybrids derived from the parental lines analyzed (Jaikishen et al 2007).

Marker-assisted identification of fertility restorer genes and their introgression into parental lines

At the Indian Agricultural Research Institute (IARI), New Delhi, studies of fertility restoration in two rice hybrids showed a dominant monogenic inheritance in both F_2 crosses, IR58025A/IR40750 and IR62829/MTU9992. Mapping studies identified RM6100 linked to the *Rf4* gene on chromosome 10 at a distance of 8.7 cM and 7 cM in the above hybrids. The marker RM6100 identified restorer and maintainer lines with 97.5% efficiency. At the Directorate of Rice Research (DRR), studies of fertility restoration of two hybrids showed a dominant digenic control in F_2 (IR58025A/KMR3) and BC_1F_1 populations (IR62829A/IR10198//IR62829A). RM6100 was very close to the gene at a genetic distance of 1.9 cM. The accuracy of marker RM6100 in predicting fertility restoration was validated in 21 restorers and 18 maintainers with 94.9% efficiency.

DNA marker-based assessment of genetic purity of seeds of rice hybrids and parental lines

The conventional method used for hybrid seed purity estimation, the grow-out test (GOT), has many limitations. In collaboration with the Center for Cellular and Molecular Biology, Hyderabad, a novel microsatellite marker–based strategy for purity testing of rice hybrids and their parental lines was developed. The methodology is simple and involves DNA isolation from rice seeds, PCR using rice microsatellite markers, resolution of PCR amplified fragments through agarose gel electrophoresis, and visualization and documentation of gel images under UV light. The entire methodology starting from DNA isolation to gel documentation takes about 6–8 hours and an approximate expenditure of US$0.30–0.40 per seed sample is involved in the assay (Yashitola et al 2002). To effectively use the microsatellite marker–based assay in hybrid seed purity assessments, a survey was carried out among the elite parental lines used for hybrid breeding in India using 48 hyper-variable microsatellite markers spread uniformly across the rice genome (Sundaram et al 2008). With a set of 10 SSR markers, all the public-bred Indian rice hybrids along with their parental lines could be clearly distinguished. To use these SSR markers effectively for detecting impurities in parental lines, a two-dimensional bulked DNA sampling strategy involving a 20 × 20 grow-out matrix was designed by Sundaram et al (2008) and used for detecting contaminants in a seed lot of the popular CMS line IR58025A. A multiplex PCR strategy involving single tube analysis using two to three markers has also been designed for hybrid seed purity assessments. We have also developed an assay for detecting impurities among seed lots of hybrid rice parental lines using an SSR marker that is specific for the mitochondrial genome of rice (Rajendrakumar et al 2007). This assay can effectively discriminate all the WA-CMS lines from their isonuclear maintainer lines and can efficiently detect even 1% contamination in CMS seed stocks.

Molecular tagging and mapping of wide-compatibility gene loci

Although pronounced heterosis in intersubspecific hybrids was known in rice for a long time, its use for hybrid rice breeding has been limited due to hybrid sterility (HS). For an effective use of WCG, it is necessary to find molecular markers linked to WCG of practical importance. Singh et al (2006) analyzed a set of SSR markers located in the vicinity of *S-5* (an important WCG locus) in a population derived from the three-way cross IR36/Dular//Akihikari. Two markers, RM253 and RM276, were observed to be closely linked to *S-5* at a distance of 3.0 and 2.8 cM, respectively. The study also indicated a predominant role of HS alleles at the *S-5* locus.

Molecular marker-assisted introgression of biotic stress resistance genes into hybrid rice parental lines

In an effort to improve the elite parental lines IR58025A, IR68897A, APMS6A, BCW56, and EPLT109 for bacterial blight and blast resistance, the molecular markers pTA248 and RM206 are being used for introgression of the genes *Xa21* and $Pi\text{-}k^h$ in a backcross breeding program at DRR, Hyderabad. The lines are currently at the BC_2F_2 generation and are being screened for the presence of target biotic stress resistance genes using linked markers and the presence/absence of the major fertility restoration gene locus *Rf4* with the help of the gene-linked SSR marker RM6100.

Hybrid seed production

Through extensive trials on different components such as suitable locations, seasons, planting time, planting geometry, row ratios, GA_3 application, and supplementary pollination, etc., a package for the production of hybrid seed was optimized. This package was effectively demonstrated in farmers' fields and seed growers are following this package with some modifications to suit local conditions.

Large-scale seed production

India has a strong seed sector, both public and private. However, the private sector has taken the lead in producing hybrid seed. More than 50 private seed companies are undertaking large-scale seed production and about 10 of them possess their own R&D setup. The leading private-sector seed companies are Hybrid Rice International (Bayer Bio-Science), PHI Seeds Ltd., Mahyco, Syngenta India Ltd., Nath-Biogene Ltd., Advanta India Ltd., Indo-American Hybrid Seeds, J.K. Agri Genetics, Metahelix Life Sciences, etc. A few public-sector-funded State Seed Corporations of Maharashtra, Karnataka, and Uttar Pradesh are also taking up hybrid seed production on a small scale. More than 90% of the hybrid rice seed is being produced during the dry season and large-scale seed production is being done in Karimnagar, Warangal, and Nandyal districts of Andhra Pradesh.

The sale of hybrid rice seed produced by the private sector ranges from INR 175 to 200 per kg of seed (US$4.50–5), whereas that of public-sector hybrid seed ranges from $2.50 to $4 per kg. Average seed yield is 1.5 to 2.5 t ha^{-1}. With the very active participation of the private sector, the area under hybrid rice seed production is increasing steadily over the years, with a proportionate increase in hybrid seed yield. Every year, 18,000–20,000 tons of hybrid rice seed are being produced in the country.

Economics of hybrid seed production

Hybrid rice seed production is fast becoming a lucrative proposition for entrepreneurial farmers. With an average seed yield of 1.5 to 2.5 t ha^{-1} and an average procurement price of $0.75–1.00 per kg, the gross returns are $1,500–2,000 per hectare. Seed production costs around $625–750 per hectare. Hence, the net profit by undertaking hybrid rice seed production is $875–1,250 per hectare and hybrid rice growers benefit substantially. In addition, hybrid rice seed production generates additional employment for 60–80 person-days per hectare, particularly for rural women in activities such as supplementary pollination, etc.

Hybrid rice seed production through public-private participation

The public sector is strong in technology generation in terms of releasing hybrids and optimizing technologies for seed production. However, its capabilities in large-scale

Table 7. MOUs with private seed companies.

Hybrid	Developed by	MOU with
DRRH-2	DRR, Hyderabad	Sampoorna Seeds, Pratham Biotech Limited, Neo Seeds, Vikky's Agri Sciences Pvt. Ltd.
Pusa RH-10	IARI, New Delhi	Indian Foundation Seed and Services Association, J.K. Agri Genetics, Nath Biogene (I) Ltd., Devgen Seed and Crop Technology Pvt. Ltd., Zuari Seeds Limited, Advanta India Limited, Yashoda Seeds Pvt. Ltd., Namdhari Seeds Pvt. Ltd., Amareshwara Agri Tech Ltd., Bhavani Seeds Pvt. Ltd.
PSD-1 and PSD-3	GBPUAT, Pantnagar	Syngenta India Ltd., Pune
CORH–3	TNAU, Coimbatore	Rasi Seeds (P) Ltd., Attur, Tamil Nadu
Ajaya, Rajalakshmi	CRRI, Cuttack	Annapurna Seeds, Vikky's Agri Sciences Pvt. Ltd., Hyderabad
KRH-2	UAS, Mandya	Namdhari Seeds Pvt. Ltd., Hyderabad
Sahyadri-1	BSKKV, Karjat	Syngenta India Ltd., Pune
JRH-4, JRH-5	JNKV, Jabalpur	Vikky's Agri Sciences Pvt. Ltd., Hyderabad

seed production and marketing are rather limited. On the other hand, the private sector is quite strong in large-scale seed production and marketing. Therefore, harnessing the mutual strengths of the public and private sector in a partnership mode is the key to popularizing public-bred hybrids, which are as good as or even better in some cases than private hybrids. Public-private partnerships for hybrid seed production have been fortified in India recently by signing a memorandum of understanding (MOU) by some companies with a few public-sector research institutes and some examples appear in Table 7.

Besides this, private-sector companies are also active in exporting hybrid rice seed to Indonesia, the Philippines, Vietnam, Nepal, Myanmar, and Bangladesh. It is estimated that 800–1,000 tons of hybrid rice seed are exported to those countries annually. As a result of all these efforts, the area under hybrid rice is increasing steadily and, in 2007, it is estimated that area under hybrid rice exceeded 1 million hectares.

Technology transfer

Hybrid rice, being an innovative and new technology for Indian agriculture, needs intensive efforts to popularize it among the farmers. To create awareness about the advantages of taking up hybrid rice cultivation and seed production among the various stakeholders (extension personnel, farmers, and seed production executives of public and private agencies, etc.), efforts were made to conduct training and organize demonstrations, etc.

Compact block frontline demonstrations

To create awareness about the advantages of taking up hybrid rice cultivation among rice farmers, a large number of compact block frontline demonstrations were organized in all the rice-growing states of India for the last nine years. So far, almost 9,000 frontline demonstrations on hybrid rice have been conducted in as many as 16 states. This is an ongoing activity and transfer of technology efforts are being intensified and a large number of demonstrations are being organized in many more states under the macro-management scheme of the Ministry of Agriculture, New Delhi, which is being coordinated and implemented by the Directorate of Rice Research in Hyderabad. In 90–95% of demonstrations organized, the hybrids have outyielded the best inbred check varieties to the tune of 1.5–2.5 t ha^{-1}, thus convincing farmers about the profitability of hybrid rice cultivation. Frontline demonstrations have proved to be a very effective tool for popularizing hybrid rice technology.

Training programs

To impart the knowledge and necessary skills for hybrid rice cultivation and hybrid rice seed production, more than 400 training programs with a duration of 1 to 21 days were organized for farmers, farm women, seed growers, seed production personnel of public and private seed agencies, extension functionaries of state departments of agriculture, agricultural universities, NGOs, etc., and more than 12,000 people were trained.

The major challenges

Despite having great potential to enhance production and productivity of rice in India, hybrid rice has not been adopted on a large scale as was expected. This is due to several constraints. Some of the major constraints are

- A lack of acceptability of hybrids in some regions such as southern India, due to region-specific grain quality requirements.
- A few hybrids are reported to have stickiness and the presence of a mild aroma, which is not liked in southern India.
- The moderate (15–20%) yield advantage in hybrids is not economically very attractive and there is a need to further increase the magnitude of heterosis.
- The lower market price offered for hybrid rice produce by millers/traders acts as a deterrent for many farmers to begin growing hybrid rice.
- The higher seed cost is another deterrent to large-scale adoption and hence there is a need to enhance the seed yield in hybrid rice seed production plots.
- Efforts to create awareness and for technology transfer were inadequate in the initial stages.
- The involvement of public-sector seed corporations in large-scale seed production has been less than expected.
- Nonavailability of hybrids for the boro season and long-duration hybrids for shallow lowland conditions.

Most of the constraints mentioned are being addressed with earnestness through the ongoing research projects and through aggressive transfer of technology efforts.

Future outlook

A good beginning has been made to usher in an era of hybrid rice in the country. The development of heterotic hybrids by researchers, large-scale production of hybrid seeds by various seed agencies, and transfer of this technology to end users by extension agencies must go hand in hand for this technology to make a real impact in Indian agriculture. Though hybrid rice technology has been introduced to Indian agriculture, the successful large-scale adoption of this innovative technology, in the future, primarily depends on its economic attractiveness. Rice hybrids with still higher heterosis coupled with better grain, cooking, and eating quality and possessing resistance to major pests and diseases are being developed.

Many promising parental lines with better floral traits have been developed. Seed production technology has to be further refined to obtain average seed yields of 2.5–3.0 t ha^{-1} on a large scale, so that the cost of hybrid rice seed can be reduced to \$2 kg^{-1}. Top priority has to be given to maintaining the purity of parental lines and to producing high-quality hybrid seed. The involvement of seed agencies in the public sector, NGOs, and farmers' cooperatives along with the private seed sector will be crucial to meeting the increased demand for hybrid seed in the years to come. The transfer of hybrid rice technology from research farms to farmers' fields is as important as developing the hybrids. Extension agencies have to play a greater role in creating much-needed awareness among farmers about the advantages of cultivating hybrid rice through various innovative approaches.

Policy decisions to provide a subsidy to meet the higher seed cost and giving a minimum support price for rice hybrids for the next 4–5 years would be very helpful in bringing more area under hybrid rice. Despite the few minor problems faced in the initial stages, timely and favorable decisions by policymakers and the active involvement of researchers, seed producers, and extension workers would certainly lead to successful hybrid rice cultivation on a large scale in India during the coming decades. The national food security mission launched recently envisages increasing annual rice production by at least 10 million tons by the end of the eleventh five-year plan (2011-12). Hybrid rice technology is likely to play a major role in increasing rice production in the country. It is expected that, by 2012, hybrids will be cultivated in India on 3 million hectares and by 2015 hybrids are expected to cover at least 5 million hectares of India's rice area, thereby contributing significantly to national food security.

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Notes

Authors' address: Directorate of Rice Research, Hyderabad 500030, India. Email: pdrice@drricar.org.

Progress in hybrid rice research and development in Indonesia

Satoto and Hasil Sembiring

Indonesia relies heavily on hybrid rice to break the plateauing trend of rice production and to mimic the success in the development and commercial use of hybrid rice technology in China. Therefore, Indonesia has encouraged more intense research and development of hybrid rice since 2000. Up to now, 35 hybrid rice varieties, including 6 public and 29 private ones, have been released. Data taken from front-line demonstrations showed that Maro, Rokan, and Intani 1 yielded –11% to 46% higher than a check inbred variety. The hybrids are susceptible to brown planthopper, bacterial leaf blight, and rice tungro virus, and may not always express their heterosis across locations. Cultivation of hybrids should be followed by the application of integrated pest management. Cultivation is not recommended in endemic areas without intense supervision by an agronomist. The breeding program for developing better hybrids conducted by the Indonesian Center for Rice Research emphasized improving the resistance of hybrid and parental lines to major pests and diseases, enhancing heterosis through indica × tropical japonica hybrids, improving grain quality of hybrids by appropriate breeding and selection of parental lines, and improving cultivation and pest management strategies for consistent performance of hybrids. As a result, four other hybrid rice combinations have been released: Hipa3 and Hipa4 in 2004 and Hipa5 Ceva and Hipa6 Jete in 2007. Some promising hybrids and CMS lines resistant to major pests and diseases have been developed. Some private institutions also conduct a breeding program. Thirteen companies are involved in hybrid rice and some have their own breeding program. However, most of them import seed directly from outside the country. Support from the government is good, especially during the past five years. A dissemination program for hybrid rice technology has begun in 16 districts identified as having potential for hybrid rice cultivation under the national rice production increasing program (P2BN). The total area of hybrid rice cultivation under the P2BN in the past year was about 130,000 ha and will increase gradually to at least 500,000 ha in the wet season of 2009. A seed system suitable for hybrid rice, including seed production, inspection, and certification, is being established.

Increasing rice production to boost the food security of the increasing population has a high priority in agricultural development in Indonesia. Agricultural land for rice cultivation is limited in meeting the challenge of producing 70 million tons of rice for the estimated population of 265 million by 2025 from the present 220 million people. Rice yield during the past decade with available varieties and cultivation technologies has exhibited a plateauing trend. Hybrid rice technology, which has been commercially used to increase rice yield by 15–20% over that of high-yielding inbred varieties in China and India (Mishra et al 2003), Vietnam (Hoan et al 1998), the Philippines (Redoña et al 2003), Bangladesh (Julfiquar et al 2003), and some other countries (Mackill and Rutger 1994, Moon et al 1994), has been selected as an option in integrated efforts to increase national rice production in Indonesia.

Research on hybrid rice in Indonesia began in 1983 with the major activity being the evaluation of hybrids and other breeding materials introduced from IRRI and other countries. The success of the commercial use of hybrid rice technology in India, Vietnam, and the Philippines encouraged the government of Indonesia to intensify the research and development of hybrid rice since 1999. Activities were not only evaluating materials from IRRI but also developing hybrid rice by using local genetic materials. The main target was to obtain hybrid rice more suitable for the Indonesian environment and yielding about 20% higher than existing rice varieties. More intensive activities involving various disciplines have been carried out since 2001, with the main target of increasing heterosis, providing cultural technologies suitable for particular hybrid rice, and developing technology packages for hybrid rice seed production. Overall activities from 2000 to 2005 were reported at the International Rice Conference in September 2005 in Bali, Indonesia (Satoto et al 2006). This paper aims to describe the progress of hybrid rice research and development in Indonesia.

Current status of hybrid rice commercialization

The government of Indonesia also encouraged private companies to develop hybrid rice varieties and produce seed in Indonesia. Since rice is a strategic commodity, as a staple food and source of income for millions of farmers, seed importation should tightly follow quarantine procedures.

Thirty-five hybrid rice varieties, consisting of 6 public and 29 private varieties, have been released officially for commercial cultivation in Indonesia (Table 1). However, the area cultivated with hybrid rice has not increased as fast as expected. Some constraints to the rapid development of the cultivated area of hybrid rice were a shortage of hybrid rice seed, susceptibility of most released hybrid rice to major insects and diseases, and instability of heterotic expression. Some programs to overcome the constraints are in progress, including breeding to develop hybrid rice varieties more adapted to local conditions and resistant to major pests and diseases, research on agronomy and pest and disease management to improve the technology package for hybrid rice cultivation as well as seed production, and strengthening hybrid rice seed by involving the private sector.

Table 1. Hybrid rice varieties released in Indonesia.

Hybrid	Nature	Breeding institution	Year
Intani 1	Proprietary	PT BISI	2001
Intani 2	Proprietary	PT BISI	2001
Miki 1	Proprietary	PT Kondo	2001
Miki 2	Proprietary	PT Kondo	2001
Miki 3	Proprietary	PT Kondo	2001
Maro	Public	ICRR	2002
Rokan	Public	ICRR	2002
Longping Pusaka 1	Proprietary	PT Bangun Pusaka	2002
Longping Pusaka 2	Proprietary	PT Bangun Pusaka	2002
Hibrindo R1	Proprietary	PT Bayer Crop Science	2003
Hibrindo R2	Proprietary	PT Bayer Crop Science	2003
Batang Kampar	Proprietary	PT KNB Mandiri	2003
Batang Samo	Proprietary	PT KNB Mandiri	2003
Hipa 3	Public	ICRR	2004
Hipa 4	Public	ICRR	2004
Manis 4	Proprietary	PT Kondo	2004
Manis 5	Proprietary	PT Kondo	2004
Segara Anak	Proprietary	PT Makmur Sejahtera	2005
Brang Biji	Proprietary	PT Makmur`Sejahtera	2005
Adirasa 1	Proprietary	PT Tri Usaha Saritani	2005
Adirasa 64	Proprietary	PT Tri Usaha Saritani	2005
PP 1	Proprietary	PT Dupont	2006
PP 2	Proprietary	PT Dupont	2006
Mapan P02	Proprietary	PT Primasid Andalan Utama	2006
Mapan P05	Proprietary	PT Primasid Andalan Utama	2006
Bernas Super	Proprietary	PT SAS	2006
Bernas Prima	Proprietary	PT SAS	2006
SL-8-SHS	Proprietary	SI Agritech-SHS	2006
SL-11-SHS	Proprietary	SL Agritech-SHS	2006
Hipa5 Ceva	Public	ICRR	2007
Hipa6 Jete	Public	ICRR	2007
Sembada B3	Proprietary	PT Biogene Plantation	2008
Sembada B5	Proprietary	PT Biogene Plantation	2008
Sembada B8	Proprietary	PT Biogene Plantation	2008
Sembada B9	Proprietary	PT Biogene Plantation	2008

Commercialization of hybrid rice in Indonesia has bright prospects because some key ingredients for commercialization are already present: (1) the food security challenge, (2) large irrigated areas and low cost of labor, (3) government policy support for hybrid rice technology development and promotion, (4) 35 hybrids already released, (5) seed production yields of up to 3 t ha^{-1} with 1 t ha^{-1} being regularly attained, (6) integrated crop management for hybrid rice, (7) increased national technical capacity in different sectors, (8) vibrant private-sector participation, and (9) a plan for 2005-09.

Performance of released rice hybrid varieties

Under the *P3T* program, three released hybrid rice varieties (Maro, Rokan, and Intani 1) were tested in several provinces for at least two seasons (DS 2002 and WS 2002-03). Demonstration plots were used in districts with potential for hybrid rice technology adoption based on the following criteria: the district has a wide area of irrigated lowland; it is not an endemic area of BPH, BLB, and RTV; and rice yield is high and stable across years. The government provided seed, fertilizer, and chemical pesticides for the demonstration plots, which were placed in farmers' fields. Workshop training was held for key farmers, extension workers, and local agricultural officers in each district prior to the implementation of demonstrations.

The results of the demonstration plots indicated that the hybrids could not always outyield the best inbred check variety IR64, Ciherang, Way Apo Buru, or Memberamo across locations (Table 2). Hybrid rice technology should therefore be used only for appropriate locations unless hybrids expressing more stable heterosis and resistance to the major pests and diseases could be developed.

In Sumatera, hybrid rice yielded from 4.1 (Intani-1) to 7.48 t ha^{-1} (Rokan). In comparison with a check variety, standard heterosis ranged from –11.8% compared with Cisokan shown by Intani-1 in Tanah Datar District to 46.1% compared with Ciherang shown by Rokan in South Lampung District (Table 2). In Java, the trials also showed good results. For grain yield, hybrid rice varieties yielded higher than in Sumatera; however, in terms of standard heterosis, hybrid rice varieties showed lower heterosis, because the check varieties also gave a high yield. Grain yield ranged from 6.03 t ha^{-1} (achieved by Intani-1 in the Subang WS 2002-03) to 11.06 t ha^{-1} (achieved by Rokan in the Blitar DS of 2002) (Table 2). Similar results were found in Sulawesi, Kalimantan, and Bali.

Other demonstration plots were used in Sukamandi during the DS of 2007 and DS of 2008, and in Kuningan DS of 2007 and the results appear in Table 3. In Sukamandi DS of 2007, Mapan P02 is the only hybrid yielding significantly higher than Ciherang; in Kuningan, eight hybrid varieties yielded significantly higher than Ciherang. Moreover, in Sukamandi DS of 2008, five hybrid varieties (Maro, Hipa3, Hipa5, Intani 2, and SL-8-SHS) yielded >1 t ha^{-1} higher than Ciherang.

Table 2. Average yield of Maro and Rokan and standard heterosis (%) in comparison with the best check variety at several locations, DS 2002 to DS 2003.

District	Season	Hybrid	Yield (t ha^{-1})	Check variety	Standard heterosis (%)
Subang	DS 2002	Maro	7.20	Ciherang	2.8
		Rokan	7.90	Ciherang	12.8
Majalengka	WS 2002-03	Maro	8.30	–	–
	DS 2003	Maro	6.80	–	–
Sragen	DS 2002	Maro	7.83	–	–
		Rokan	9.57	–	–
Cilacap	DS 2002	Rokan	6.05		
Bojonegoro	DS 2002	Rokan	7.52	WA Buru	7.3
	WS 2002-03	Maro	8.90	Ciherang	22.0
	DS 2003	Maro	8.40	Ciherang	8.4
Blitar	DS 2002	Maro	8.84	Ciherang	–7.5
		Rokan	11.06	Ciherang	15.7
	WS 2002-03	Maro	10.30	Ciherang	15.7
		Rokan	9.40	Ciherang	5.6
Asahan	DS 2002	Maro	6.08	Ciherang	21.6
		Maro	6.89	Ciherang	9.0
Simalungun	DS 2002	Maro	5.04	IR64	–5.3
		Rokan	6.27	IR64	17.8
		Maro	5.72	–	–
			6.21	–	–
Tanah Datar	DS I 2002	Maro	5.60	Cisokan	1.8
		Rokan	5.52	Cisokan	0.4
Lampung	DS I 2002	Maro	7.30	IR64	32.7
		Rokan	7.40	IR64	34.5
	DS II 2002	Maro	7.48	Ciherang	46.1
		Rokan	6.27	Ciherang	22.5
Gowa	DS 2002	Maro	7.09	Ciliwung	8.9
		Rokan	7.53	Ciliwung	15.7
	WS 2002-03	Maro	8.32	–	–
Maros	DS 2002	Maro	9.80	Ciliwung	33.2
		Rokan	9.20	Ciliwung	25.0
	WS 2002-03	Maro	7.73		39.3
Pontianak	DS 2002	Maro	5.30	Ciherang	–10.2
		Rokan	5.60	Ciherang	–5.1
	DS 2002-03	Maro	6.63	IR64	10.7
Musirawas	DS 2002	Maro	6.34	Ciherang	8.9
		Rokan	7.30	Ciherang	25.4
	DS 2002-03	Maro	6.08	Ciherang	2.2
		Rokan	6.22	Ciherang	4.5

Source: Indonesian Central Food Crops for Research and Development (2003).

Table 3. Grain yield of 20 hybrid rice varieties and check variety Ciherang in Sukamandi during DS of 2007 and DS of 2008, and Kuningan DS of 2007.[a]

Hybrid rice variety	Grain yield (t ha^{-1}) at 14% moisture content		
	Sukamandi DS 2007	Kuningan DS 2007	Sukamandi DS 2008
Maro	7.40	6.90	7.47*
Rokan	6.81	6.40	7.19
Hipa3	7.74 ns	8.11**	7.42*
Hipa4	7.23	6.66	7.00
Hipa5 Ceva	7.87 ns	6.74	7.55*
Hipa6 Jete	6.47	7.35*	6.17
Arize R1	6.99	6.91	6.98
Arize R2	6.94	6.05	5.89
Intani-2	7.25	7.64*	7.56*
PP1	7.12	5.56	6.72
PP2	6.98	7.36*	7.10
Bernas Prima	6.37	7.13*	–
Bernas Super	7.18	7.92*	6.94
Batang Kampar	5.89	7.82*	–
Batang Samo	7.55	8.10**	7.13
Brang Biji	6.78	6.50	–
Segara Anak	–	–	7.24
Adirasa-64	6.04	6.90	7.04
SL-8-SHS	6.59	6.10	7.80*
Mapan P-02	8.89*	6.97	7.16
Ciherang	8.06	6.06	6.39

[a]The same treatment was given to all varieties. * = significant at the 5% level, ** = significant at the 1% level, and ns = nonsignificant.

The breeding program

Improvement of parental lines

Hybrid rice varieties released in Indonesia were developed by using the three-line method. By using this method, hybrid rice varieties resistant to pests and diseases can only be developed if a resistant parental line CMS and/or restorer line is available. Three approaches can be used to obtain resistant parental lines: improving the resistance of parental lines, developing resistant parental lines, and introducing a resistant

Table 4. Indonesian restorer lines and their reaction to *Bph3*, BLB IV, BLB VIII, and RTV.

No.	Restorer	Reaction score[a] to			
		Bph3	BLB IV	BLB VIII	RTV
1	S4124F	3	4	5	5
2	B9645E	7	3	4	4
3	B4070D	9	4	4	5
4	B10373E	3	4	4	3
5	B9775B	5	4	4	3
6	B2791B	9	4	4	4
7	B8239G	9	4	4	4
8	S4325D	3	3	4	3
9	S4424F	3	4	5	5
10	B8049F	5	4	4	6
11	BI09	3	3	4	2
12	B5960	9	4	4	3
13	BP51	9	3	4	2
14	BI012-2	3	3	4	4
15	B10214F-2	5	5	4	4
16	B10214F-1	5	4	4	3

[a] 3 = resistant; 4 and 5 = moderately resistant; 6, 7, and 9 = susceptible.

parent from imported germplasm. The first approach can be carried out by crossing among maintainers, among restorers, or between a maintainer or restorer and resistant donor varieties. The second approach is carried out by selecting breeding materials so that the lines used in a parental line development program are the lines having a gene with resistance to bacterial leaf blight (BLB) only. The third approach has been followed by introducing a number of CMS and restorer lines from IRRI. Results of the hybrid rice parental line improvement showed that 12 of 19 introduced restorer lines were moderately resistant to BLB, and 16 of 68 established restorer lines were also moderately resistant (Table 4).

From a test-cross nursery, six moderately resistant restorer line candidates have emerged (Table 5). In addition, from the parental line improvement program, two CMS (maintainer) lines and ten restorer lines have been improved. A maintainer line improvement program also resulted in five F_4 populations, whereas the restorer line improvement program produced three F_4 populations.

Table 5. Restorer line candidates and their reaction to *Bph3*, BLB IV, BLB VIII, and RTV.

Restorer lines	Reaction score[a] to			
	Bph3	BLB IV	BLB VIII	RTV
S4850-9F-6	3	4	4	6
S4653-164	3	5	5	7
B8974B	3	5	5	5
B10277D-1	3	5	5	6
BP1368-1D	3	5	5	6
BP1088-2E	3	5	5	7

[a] See values in Table 4.

The development of hybrid rice resistant to BPH, BLB, and RTV

Based on the genetic materials available in Indonesia, the strategies applied to develop hybrid rice were (1) to evaluate and select introduced hybrid combinations in order to obtain introduced hybrid varieties adaptable to the local climate and preferences; (2) through a domestic breeding program, develop restorer lines suitable for CMS introduced from outside. The expected result was hybrid rice obtained from crosses between the introduced CMS and domestic restorer lines; (3) provide CMS and restorer lines obtained from the available domestic gene bank. The expected result was hybrid rice obtained from crosses of genetic materials available in the country, having high adaptability to the Indonesian ecosystem; and (4) develop new plant type (NPT) hybrids having yield potential 15–20% higher than that of the best existing NPT rice, with resistance to major pests and diseases, and possessing good cooking quality.

To identify hybrids resistant to the major pests and diseases, screening has been conducted in the laboratory and greenhouse by artificial inoculation of BPH, BLB, and RTV. Table 6 lists some hybrids resistant to BPH, BLB, and/or RTV.

Check variety IR64 is the predominant variety grown by farmers in Indonesia although it is susceptible to BLB strain VII and RTV. All of the selected hybrids have better resistance to the major pests and diseases than the inbred check. Among 71 hybrid rice combinations tested for their resistance, 28 hybrid combinations have resistance to one of the three major pests and diseases. Two of the resistant hybrids have been released commercially, whereas the remaining combinations were still in advanced yield trials and multilocation trials. From these data, we can say that hybrid rice varieties with resistance to BPH, BLB, and RTV would be possible in Indonesia.

The performance of some promising hybrids

Several hybrid combinations resulted from crosses between introduced CMS and local varieties having an average yield 1 t ha^{-1} higher than that of elite rice variety IR64 and resistant to BPH, BLB, and RTV. These hybrids were undergoing further evaluation.

Table 6. Reaction of some hybrid rice combinations to *Bph3*, BLB IV, BLB VIII, and RTV.

Hybrid	Reaction score[a] to				Status
	Bph3	BLB IV	BLB VIII	RTV	
Hipa3	7	4	4	6	Released
Hipa4	5	3	4	5	Released
H13	3	4	4	5	AYT
H14	3	3	4	5	AYT
H17	3	4	4	5	MLT
H27	3	3	3	7	MLT
H29	5	4	4	8	MLT
H30	5	3	4	7	MLT
H33	3	3	4	5	MLT
Hipa5 Ceva	3	4	4	5	Released
Hipa6 Jete	9	4	4	7	Released
H39	3	5	5	2	AYT
H42	5	5	4	2	MLT
H48	3	5	5	4	AYT
H51	9	3	4	7	MLT
H54	5	4	4	5	AYT
H55	7	4	4	3	AYT
H57	5	4	5	2	MLT
H58	7	5	4	2	AYT
H59	5	5	5	3	AYT
H60	5	5	5	3	AYT
H63	5	4	4	3	MLT
H50	9	5	5	3	AYT
H64	5	5	5	2	AYT
H67	5	5	5	3	AYT
H68	3	5	5	5	MLT
H70	3	5	5	4	MLT
H71	3	5	5	5	MLT

[a]See Table 4 for explanation of scores.

Promising hybrids resistant or moderately resistant to BPH, BLB, and RTV have been identified. Among the tested hybrids, H35, H42, H55, H56, H58, and H68 averaged high yield with standard heterosis of more than 20% (Table 7).

The heterosis, except for H42, was less than that of released hybrid Maro; however, those hybrids are more resistant to the major pests and diseases. The selected hybrids are now being tested at more locations.

Table 7. Grain yield and standard heterosis of promising hybrids tested in an AYT in Muara, Sukamandi, and Cianjur, WS of 2003-04.

Hybrid/ check	Muara		Sukamandi		Cianjur		Average	
	Yield (t ha^{-1})	IR64[a] (%)	Yield (t ha^{-1})	IR64[a] (%)	Yield (t ha^{-1})	IR64[a] (%)	Yield (t ha^{-1})	IR64[a] (%)
H30	5.14	21.88	6.11	7.19	7.61	18.72	6.28	15.5
H35	4.94	17.06	6.16	8.07	9.61	49.92	6.90	26.8
H42	5.79	37.24	7.05	23.68	9.62	50.07	7.48	37.5
H50	4.83	14.38	5.80	1.75	8.02	25.12	6.21	14.2
H55	5.08	20.36	5.80	1.75	9.21	43.68	6.69	23.0
H56	5.50	30.28	6.25	9.65	8.02	25.11	6.58	21.0
H58	5.61	32.98	6.16	8.07	8.42	31.25	6.72	23.6
H59	5.12	21.36	6.10	7.02	7.63	18.75	6.27	15.3
H65	5.08	20.48	5.97	4.74	8.40	31.25	6.48	19.2
H68	4.72	11.74	7.06	23.86	8.02	25.12	6.59	21.2
H70	4.71	11.68	6.74	18.25	8.03	25.27	6.48	19.2
Maro	5.77	36.72	5.12	–10.18	10.01	56.16	6.96	27.6
IR64	4.22	–	5.70	–	6.41	–	5.44	–

[a] With standard heterosis to IR64.

Some hybrids selected from observation yield trials conducted during the WS of 2003-04 are listed in Table 8. The hybrids had better agronomic characters, higher yield, and more resistance to natural infection of BLB than inbred check variety IR64 as well as released hybrid Maro. BIO-12 is an inbred restorer line bred for BLB resistance, containing the gene *Xa7*. The restorers must have good combining ability since good hybrids were obtained from combinations with different CMS lines. Other data were obtained from advanced yield trials (AYTs) during the DS of 2007 at four locations (Table 9).

Results of multilocation trials conducted at ten locations from the DS of 2001 to the DS of 2003 are listed in Table 10. From the trials, we can conclude that the performance of IR62829A/MTU9992, IR58025A/RHS-412, IR58025A/B10277, IR58025A/Maro, and IR58025A/Cisokan hybrids was stable across locations and was better in terms of the adaptability and yield stability indicated by their genotype × environment (G × E) interaction value, which was not significantly different from 1. On the other hand, IR58025A/MTU9992 and IR58025A/IR65515 hybrids were adapted in only favorable growing environments, while IR58025A/C20R, IR58025A/IR68, and IR58025A/B10373E were still adapted even in suboptimal areas (Satoto et al 2007).

Table 8. Performance of some selected hybrids from OYTs conducted in the WS of 2003-04.

Hybrids/check varieties	Days to flowering	Plant height (cm)	No. of tillers	BLB[a]	Yield (t ha^{-1})	Standard heterosis (%)
IR68897A/BP165	90	99	13	5	7.34	50.1
IR68885A/BP154E	95	95	11	5	7.44	48.9
IR68885A/BIO-12	98	72	18	3	6.51	34.9
IR68885A/IR68077	89	88	13	5	6.56	34.3
IR62829A/IR59544	90	93	15	5	6.68	33.6
IR68888A/BIO-12	92	97	12	3	6.49	32.1
IR68886A/BIO-12	89	94	15	3	6.35	31.8
IR62829A/BP1028F	96	97	14	5	6.58	31.5
IR68888A/IR68078	95	85	15	5	6.54	30.7
IR68885A/IR25912	99	73	16	3	6.41	28.1
IR64 (Check)	97	89	11	7	4.89	–
Maro (Check)	104	70	12	7	5.60	14.5

[a] Disease score with natural infection on a 1–9 scale, where 1 = highly resistant and 9 = highly susceptible.

Table 9. Grain yield (t ha^{-1}) of some promising hybrids during the DS of 2007.

Hybrid	Location			
	Bantul-Yogya	Cianjur-West Java	Klaten-Central Java	Salatiga-Central Java
H17	9.92*	7.18*	8.28	7.15
H22	3.73*	7.25*	10.60	7.36
H25	10.23*	6.77	9.73	6.41
H27	8.59*	7.56*	10.07	8.23
H51	7.64	7.27*	11.11	7.32
H53	8.49*	6.83	10.30	5.10
H72	10.59*	6.90	11.28	7.34
H73	8.06	6.52	9.73	7.26
H88	9.89*	7.61*	10.44	6.60
H90	9.97*	7.95*	10.92	6.33
Maro	8.03	7.37*	10.32	7.21
Ciherang	7.64	5.00	9.79	6.88
LSD (5%)	0.69	1.98	2.73	2.05
CV (%)	7.02	15.80	18.60	18.40

* = significantly different from Ciherang at the 5% level.

Table 10. Regression coefficient and standard deviation of yield.

Hybrids	Yield (t ha^{-1})	Standard heterosis for IR64 (%)	Bi[a]	SD[b]
IR58025A/MTU9992	8.63	19.7	1.20 ns	1.96 ns
IR58025A/C20R	7.59	5.3	0.83 ns	1.37 ns
IR58025A/IR65515	7.99	10.8	1.22 *	1.83 ns
IR62829A/MTU9992	8.01	11.1	1.09 ns	1.91 ns
IR58025A/RHS-412	7.65	6.1	0.96 ns	1.57 ns
IR58025A/B10277	7.68	6.5	0.95 ns	1.39 ns
IR58025A/Maros	7.39	2.5	0.99 ns	1.62 ns
IR58025A/Cisokan	7.46	3.5	1.01 ns	1.51 ns
IR58025A/IR68	7.16	–0.7	0.78*	1.44 ns
IR58025A/B10373	6.92	–4.0	0.88 ns	1.30 ns
IR64	7.21	–	1.05 ns	1.60 ns

[a]ns = not significantly different from 1. [b]ns = not significantly different from 0. Bi = regression coefficient, SD = deviation of regression * = significantly different from IR64 at the 5% level.

Seed production

The government of Indonesia has also encouraged the private sector to develop hybrid rice varieties and produce seed in Indonesia. Since rice is a strategic commodity, as a staple food and source of income for millions of farmers, seed importation must strictly follow quarantine procedures.

On-the-job training on hybrid rice seed production technology was conducted in Sukamandi during the dry season of 2004. The participants were 10 provincial agricultural officers of NAD Province. The participants worked together with station technicians to produce hybrid rice seed on about 1 ha. They practiced all activities of seed production from land preparation up to harvesting and seed processing. Supervision by hybrid rice breeders and a seed production specialist was conducted on critical and important activities such as seedbed preparation, seed sowing, transplanting, roguing, GA3 application, supplemental pollination, harvesting, and seed processing. Class lectures and discussion were also provided by researchers and a seed production specialist from the Indonesian Center for Rice Research (ICRR).

A seed system for inbred rice has been established for a long time in Indonesia. The system, which includes ICRR, provincial seed farms, district seed farms, public seed companies, and seed inspection and certification officers, has capability to produce, distribute, and market quality seed, especially for public varieties. Production and distribution of hybrid rice seed were integrated into the system, with some adjustment of seed classes and purity requirements. In order to improve the capacity of the seed farms, training on hybrid rice seed production technology was held more than

Table 11. F_1 hybrid seed yield achieved at some locations from the DS of 2002 to the DS of 2008.

Location	Hybrid	Season	Area (ha)	Total yield (kg)	Yield (kg ha^{-1})
Pusakanegara	Maro	DS 2002	4.0	5,050	1,488
Pusakanegara	Rokan	DS 2002	3.0	1,382	466
Pusakanegara	Rokan	DS 2003	1.0	550	550
Pusakanegara	Maro	WS 2003-04	1.0	520	520
Pusakanegara	Rokan	WS 2003-04	1.0	730	730
Sukamandi	Maro	DS 2002	0.90	250	278
Sukamandi	Rokan	DS 2002	0.60	90	150
Sukamandi	Hipa5 Ceva	DS 2006	0.25	150	600
Sukamandi	Hipa6 Jete	DS 2006	0.25	150	600
Sukamandi	Maro	MK 2002	3.0	660	220
Sukamandi	Hipa3	DS 2007	1.0	1,200	1,200
Sukamandi	Hipa5 Ceva	DS 2008	1.0	1,340	1,340
Sukamandi	Hipa6 Jete	DS 2008	2.0	4,580	2,290
Muara	Maro	MK 2002	1.0	4,640	464
Muara	Maro	MK 2006	0.25	275	1,100
Muara	Rokan	MK 2006	0.25	215	860
Tegalgondo	Maro	MH 2003-04	1.0	320	320
Tegalgondo	Rokan	MH 2003-04	1.0	710	710
Tegalgondo	Hipa6 Jete	MK 2006	0.25	150	600

10 times in Sukamandi and at other institutes. The training was attended by persons from provincial seed farms with good potential for hybrid rice seed production.

Training-by-doing on hybrid rice seed production was conducted in Tegalgondo Seed Farm (Central Java), Sang Hyang Seri state-owned seed company (West Java), and PT Makmur Sejahtera seed company (West Nusa Tenggara). Seed production for hybrid rice was also conducted at the Tegalgondo seed farm, Pusakanegara research station, Sukamandi research station, and Muara. ICRR provided technical guidance and intensive supervision during the critical or important activities, including seedbed preparation, seeding, transplanting, selection, supplemental pollination, harvesting, and seed processing. The results of the seed production activities are listed in Table 11.

The government of Indonesia selected hybrid rice as one of the technologies to be disseminated for increasing rice production in the country. The government encouraged the public and private sector to participate in developing and disseminating hybrid rice technology. Research on hybrid rice became more intensified in 1999 and procedures for releasing hybrid rice varieties were developed in 2001.

Promotion and dissemination of hybrid rice technology began in 2002 under an integrated program for increasing rice production. The capacity of the public seed company, Sang Hyang Seri, and seed farms was improved by conducting group training, on-the-job training, and training-by-doing on hybrid rice seed production technology.

The government provided extension services, monitoring of pest and disease incidence, and credit for seed, fertilizers, and pesticides for commercial hybrid rice cultivation. However, the development of hybrid rice cultivation area was not as fast as was expected due to the constraints of seed shortage, inconsistent expression of heterosis, and the susceptibility of hybrids to the major pests and diseases. Breeding to develop better hybrids resistant to BPH, BLB, and RTV has been given high priority in the national rice research and development program, and some promising hybrids have been obtained.

Hybrid rice seed was produced by public seed companies and seed farms on a limited area (5–15 ha), because of the low seed yield of less than 1 t ha^{-1}. Although the capacity of public seed companies and seed farms is being improved, the government offers seed production of public hybrids to private seed companies. Syngenta obtained a license to produce seed of Hipa3 and intended to obtain a license for Hipa4, and Dupont had also obtained a license to commercialize Maro and PT SAS for Rokan. Seed production for Hipa3 began in the DS of 2006 on 5 ha and a 1.2 t ha^{-1} seed yield was obtained. Seed production of variety Maro by Dupont began on a 50-ha area during the DS of 2007 and the company proposed to produce hybrid seed on 100 ha in 2008.

Issues identified for future research and technology development

- Unstable expression of heterosis
  Hybrids could not always express their superior yield over inbred check varieties across locations, indicating instability of heterosis. Increasing the number for testing for evaluation of early hybrids at observation yield trials might identify hybrids with more stable expression of heterosis.
- Resistance to major pests and diseases
  Hybrid rice varieties released in Indonesia are susceptible to major pests and diseases, especially BPH, BLB, and RTV. This susceptibility could contribute to the instability of heterosis expression and constrain the rapid adoption of hybrid rice technology.
- Hybrids of new plant type rice
  Inbred varieties of new plant type rice with higher yielding capacity have been released in Indonesia. A further increase in yield capacity could be obtained by developing hybrids of new plant type rice.
- Biotechnology for hybrid rice
  Anther culture to develop doubled-haploid lines has been applied in hybrid rice breeding. The technology needs to be improved since some genotypes showed very low regeneration. The development of molecular markers associated with desirable characters difficult to be selected conventionally such as combining ability will be useful for hybrid rice breeding.

Future plans for research and development on hybrid rice and their source of funding

Most rice research in Indonesia is conducted by ICRR since the institute, as a national institute, has the mandate on rice research and development in Indonesia. Hybrid rice research was considered a high-priority program and given high priority in the next five-year program. The major objectives of the research on hybrid rice are to develop better hybrids with (1) high and stable heterosis; (2) resistance to BPH, BLB, and RTV; (3) and good grain quality; to improve seed production technology; and to develop integrated crop management technology.

An inbred variety of the new plant type with higher yield potential has been released in Indonesia. New plant type breeding material has also been used in hybrid rice breeding to improve yield potential. The development of breeding materials through B × B crosses for B-line breeding and A × R and R × R crosses for R-line breeding will be continued. A gene pool with recurrent random mating and recurrent selection facilitated by male sterility character has begun for R-line improvement.

The Indonesian Center for Agriculture Biotechnology and Genetic Research and Development (ICABIOGRAD) has a program to support hybrid rice breeding by applying biotechnology. The program includes the development of doubled-haploid breeding lines of B and R through anther culture applied on F_1 plants of B × B, A × R, and R × R crosses; marker-aided selection to identify R and B lines; and molecular analysis to study genetics related to combining ability.

Promising hybrids will be tested at more locations to identify hybrids with more stable heterosis. A network for testing inbred breeding lines has been developed between ICRR, some private companies, and Assessment Institutes of Agricultural Technology (AIAT) present in all provinces. Testing of rice hybrids will be conducted through the network.

Research on hybrid rice seed production will study flowering synchronization between parental lines at different potential locations. The research will also include refinement of cultivation technology for seed production. Research on hybrid rice cultivation to develop appropriate integrated crop management suitable for hybrid rice will be continued. Superimposed trials to adjust fertilizer rate, plant spacing, and seedling age suited to local conditions will be conducted on demonstration plots in different districts.

Strengthening the capacity of public seed farms will be continued by conducting training, followed by training-by-doing on hybrid rice seed production technology. More private seed companies and growers will be allowed to participate in seed production of public hybrids. A breeder seed production unit that was established in ICRR for inbred varieties is being strengthened to include breeder seed production of parental lines of hybrid rice.

Conclusions

Thirty-five hybrid rice varieties have been released in Indonesia; however, the development of hybrid rice cultivation area has been slow due to the constraints of hybrid seed shortage, inconsistent expression of heterosis, and the susceptibility of some released hybrids to major pests and diseases. Seed production of hybrid rice in public seed companies and on seed farms was conducted in a limited area, with low seed yield. A public-private-sector partnership has been developed on hybrid seed production to accelerate the adoption of hybrid rice technology and some private seed companies began to produce seed of public hybrids.

Breeding to develop hybrid rice resistant to BPH, BLB, and RTV has high priority in the national rice research program and some promising hybrids were obtained. A network between ICRR and AIATs was developed to evaluate hybrids at more locations and to identify those with stable heterosis. Breeding materials, including new developed parental lines and genetic sources for desirable characters from IRRI, are still needed.

The government of Indonesia supports not only research and development but also the dissemination and adoption of hybrid rice technology. The government provided demonstration plots, extension services, monitoring of pest and disease incidence, and credit for seed, fertilizer, and pesticides to promote the adoption of hybrid rice technology.

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Notes

Authors' address: Indonesian Center for Rice Research.

Development and commercialization of hybrid rice in Pakistan

Muhammad Akhter, Muhammad Afzal Zahid, Syed Sultan Ali, Mushtaq Ahamd,
and Muhammad Ramzan

Hybrid rice research in Pakistan began at the Rice Research Institute, Kala Shah Kaku, in the late 1990s. Local and exotic genetic materials have been evaluated for their use with this technology. The Institute has 23 CMS lines, including four CMS lines in basmati background. Twenty-five restorers and 50 maintainers have also been identified for commercial use. Local germplasm has a greater frequency of maintainers than restorers. In the three-line system, LH-1 (IR58025A/Basmati 385), LH-18 (IR75596A/IR73014-59-2-2-2R), and LH-19 (IR68897A/IR69714-28-1-2-6-2R) had higher yield and better grain quality than check varieties. For two-line hybrids, 51 have been evaluated in yield trials. Of these, 25 hybrids outyielded the check variety Super Basmati. Milled rice grain length ranged from 6.19 to 7.54 mm. The seed production technology of IR73834-21-26-15-25-4 and IR75589-31-27-8-33 TGMS lines was also developed. Seven exotic seed hybrids have been approved for general cultivation in Pakistan. Hybrid rice was cultivated on about 63,000 ha in 2007-08. About 2,500 t of hybrid rice seed was imported in 2008. It is expected that, in 2012, the area under hybrid rice will expand to 350,000 ha.

Keywords: hybrid rice, restorers, maintainers, Pakistan

Rice, an important food and cash crop, is the third largest crop of Pakistan in terms of area, after wheat and cotton. It is also one of the major sources of foreign exchange earnings in the country. Rice is grown annually on more than 2.5 million ha, with production averaging 5.5 million t. Pakistan is famous for producing and exporting long-grain aromatic basmati rice. In addition, a substantial quantity of coarse rice is exported. Annual exports amount to around 3 million t. Pakistan ranks 14th in rice production in the world. The provincial shares of Punjab, Sindh, NWFP, and Balochistan are as follows—overall rice area, 69%, 22%, 3%, and 6%, and total production, 58%, 30%, 3%, and 9%, respectively.

The Chinese experience and the dire need to increase rice productivity and production encouraged rice scientists to develop and disseminate hybrid rice technology in the tropics. The hybrid rice breeding program at the Rice Research Institute, Kala Shah Kaku, started in 1999. In 2000, this program was strengthened by the government with the approval of a research project titled *"Development of Hybrid Rice in the Punjab."* Hybrid rice is becoming popular in Pakistan because of its yield advantage over inbred varieties. The development of genetic tools essential for producing hybrid rice—cytoplasmic male sterile (CMS), maintainer, and restorer lines—began at the Institute (Akhter et al 2007). McWilliam et al (1995) found a higher frequency of restorers (21%) than maintainers (11%) from an evaluation of 6,000 test crosses in India. On the other hand, less restorer frequency and higher maintainer frequency were observed in the local germplasm of Pakistan (Sabar and Akhter 2003). Ali and Khan (1996) also observed that the frequency of maintainers (63%) was much higher than that of restorers among the 76 hybrids tested. In the last few decades, another type of male sterility, which is sensitive to the environment and induced by the interaction of environmental factors with nuclear genes, has also been used to develop rice hybrids (Virmani and Ilyas-Ahmed 2001). The discovery and application of thermosensitive genic male sterility (TGMS) offer great potential for revolutionizing hybrid seed production technology in rice. TGMS lines have great potential in tropical countries such as India. Knowledge of the male sterility mechanism in TGMS lines is essential for hybrid rice development through the two-line breeding approach. In the tropics, TGMS is more practical to use since daylength differences are small (Virmani and Ilyas-Ahmed 2001). The objectives of this study were to evaluate local and exotic genetic material and its use in hybrid rice breeding; to evaluate existing hybrids for yield, adaptability, and grain quality; and to develop parental lines.

Development of rice hybrids

Identification of restorers and maintainers

The establishment of a test-cross nursery to identify restorers and maintainers is the first step in three-line heterosis breeding. For this purpose, a source nursery comprising uniform rice genotypes was transplanted on three different dates each year. Standard agronomic and plant protection measures were adopted during the years. From 2000 to 2007 kharif, 912 test crosses were made among different elite lines and CMS lines to categorize existing germplasm as either a restorer or a maintainer line. The criteria used for classifying parental lines as maintainers or restorers were those proposed by Virmani et al (1997).

Out of 912 test crosses, 54 lines were identified as restorers. Of these restorer lines, 21 belong to the basmati group and the rest fall in the coarse rice category. Similarly, 65 maintainers were found. Among the 54 restorers, Basmati 370, Basmati 385, Shaheen Basmati, Basmati pak, and Basmati 198 have been approved and commercialized as varieties having good cooking quality.

Evaluation of CMS lines from diverse male sterility sources

For CMS evaluation, 23 CMS lines coming from five different male sterility sources were used. Most of the CMS lines were received from the International Rice Research Institute. These were evaluated for morphological, floral, and agronomic traits. Complete pollen sterility was observed in all lines. Better tillering ability was observed in all CMS lines. The number of spikelets per panicle ranged from 68 to 170. IR69616A ranked first with 170 spikelets per panicle, whereas IR79128A was at the bottom. Six CMS lines were late-maturing (>100 days to 50% flowering). IR68897A had the maximum outcrossing rate (46%), followed by IR70369A (40%). On the basis of their better floral and agronomic characteristics, IR58025A, IR79156A, IR68897A, IR68886A, IR73328A, IR75596A, IR70369A, and IR79128A were identified as potential CMS lines for hybrid rice seed production.

Evaluation of TGMS lines

Six TGMS lines were sown on three different dates to study the fertility-/sterility-inducing temperature regimes. IR73834-21-26-15-25-4 had the shortest plant height (63 cm) with the minimum number of tillers per plant (8.0). Phenotypic acceptability was highest for IR73834-21-26-15-25-4. Therefore, it has potential for commercial use in hybrid seed production. During the sterility phase, the highest temperature was between 39.5 °C in May and 31.6 °C in October, whereas the lowest temperature ranged from 27.6 °C in May to 21.6 °C in October. However, the critical fertility phase was observed in the last week of October, with maximum and minimum temperatures of 28.5 and 19.6 °C, respectively. This phase may be extended up to the first week of November. Four lines—IR73827-23-26-15-7, IR73834-21-26-15-25-4, IR75589-31-27-8-33, and IR76761-4-3-17-34-1—showed TGMS behavior at Kala Shah Kaku. IR68301-11-6-4-4-3-6-6 and IR76753-41-6-34-13 did not produce any seed and remained unproductive throughout the growth period. TGMS lines IR68301-11-6-4-4-3-6-6 and IR76753-41-6-34-13 showed complete sterility. The temperature was higher than 35 °C. These lines could not produce any seed, even during the critical fertility phase when maximum and minimum temperature ranged between 28 and 19 °C. TGMS lines IR73827-23-26-15-7, IR73834 -21-26-15-25-4, and IR75589-31-27-8-33 showed complete sterility during the critical sterility phase with temperature greater than 30 °C and showed spikelet fertility during the critical fertility phase when temperature ranged between 28 and 19 °C.

Evaluation of exotic rice hybrids

From 1993 to 2006, different IRRI hybrids received from the International Hybrid Rice Observational Nursery (IRHON) were evaluated for yield and other economic characteristics. The IRRI *Standard evaluation system for rice* was adopted for data collection (IRRI 2002). Some 800 hybrids were evaluated for yield, adaptability, and standard heterosis. Standard heterosis of hybrids varied from 8% to 142% over KS282 (local check variety). The maximum yield of 11.76 t ha^{-1} was given by hybrid IR76715H (Table 1). The data showed rice hybrids with yields higher than that of approved check variety KS282. Grain quality characteristics are very important parameters to determine consumer acceptance of any hybrid. It has been found that the grain quality of hybrids was not at all comparable with that of premier-quality varieties such as Basmati 385 and KS282.

Table 1. Performance of exotic rice hybrids.

Hybrids	Yield (t ha^{-1})	Heterosis (%)	Days to heading	Height (cm)	Tillers per plant (no.)	Phenotypic acceptability	Cooking quality
IR76715H (IR68897A/ RP2087-194-1-2-2R)	11.8	142	120	118	23.0	3	<KS282
IR76712H (IR68897A/ IR68427-8-3-3-2R)	11.3	133	103	106	15.6	3	<KS282
IR79118H (IR73328A/ IR69702-78-3-3R)	10.8	123	96	112	16.2	3	<KS282
IR68284H (IR58025A/ IR34686-179-1-2-1R)	10.6	118	104	110	15.0	3	<KS282
IR75587H (IR68899A/ IR62162-184-3-1-3-2R)	9.7	100	101	104	14.0	3	<KS282
IR80641H (IR 75596A/ IR73014-59-2-2-2R)	8.8	80	109	113	23.2	5	<KS282
IR64616H (IR68829A/ IR29723-143-3-2-1R)	8.2	68	103	97	22.0	3	<KS282
IR73410H (IR69626A /IR46R)	7.4	51	102	117	20.0	1	<KS282
IR75583H (IR68897A/ IR62653-8-3-3R)	7.3	50	119	116	18.0	3	<KS282
IR68284 H (IR58025A/ IR34686-179-1-2-1R)	7.2	48	104	110	15.0	3	<KS282
KS282 (local check)	4.9	–					Good

Evaluation of locally developed rice hybrids

Among two-line hybrids, LH-72, LH-76, and LH-64 yielded 8.1, 7.9, and 7.4 t ha^{-1}, respectively, exhibiting 138%, 132%, and 117% heterosis over Super Basmati (Table 2). LH-26, LH-27, LH-28, LH-35, LH-54, LH-58, and LH-76 had more cooked grain length than check variety Super Basmati. Moreover, LH-35 had the longest cooked grain length (13.8 mm) among all the hybrids tested, followed by LH-58 (13.7 mm). LH-72, LH-76, LH-64, and LH-39 are potential two-line rice hybrids with better quality and agronomic characters than the approved check variety. Among the three-line hybrids, LH-1, LH-18, LH-19, and LH-20 had more than 58% heterosis over that of the check variety. All of them, except for LH-20, have extra long grains. Among the hybrids tested, LH-1, LH-18, and LH-19 show the most potential. Seeds of these hybrids are being multiplied on a large scale. Furthermore, we conclude that hybrids have more potential than approved commercial varieties.

Table 2. Agronomic and quality traits of promising rice hybrids.

Hybrid no.	Hybrid	Plant height (cm)	Days to maturity	Tillers	Grains per panicle	Yield (t ha^{-1})	Yield heterosis (%)	Grain length (mm)	Cooked grain length (mm)
Two-line hybrids									
LH26	IR75589-31-27-8-33/Shaheen Basmati	156.2	110	24.4	64.4	4.6	+35	6.59	13.5
LH27	IR73834-21-26-15-25-4/KSK436	125.2	93	14.2	73.2	4.3	+26	6.19	12.6
LH28	IR73827-23-26-15-7/Shaheen Basmati	140.0	113	18.0	117.8	4.8	+41	6.48	13.3
LH34	IR75589-31-27-8-33/Super Basmati	134.9	112	36.0	109.4	4.2	+23	7.51	12.2
LH35	IR73834-21-26-15-25-4/33608	140.6	100	24.4	110.6	4.2	+23	6.85	13.8
LH39	IR73827-23-26-15-7/KSK202	121.4	103	27.8	79.8	6.5	+91	6.65	11.8
LH54	IR75589-31-27-8-33/Basmati 385	145.0	127	14.6	79.2	6.2	+82	6.59	13.3
LH58	IR75589-31-27-8-33/4029B	127.8	98	23.0	131.4	5.9	+73	6.33	13.7
LH64	IR73827-23-26-15-7/Basmati 385	145.6	110	18.8	154.0	7.4	+117	6.43	12.3
LH72	IR76753-41-6-34-13/KSK133	124.0	90	29.0	97.4	8.1	+138	6.98	11.7
LH76	IR73834-21-26-15-25-4/IR506	105.0	95	31.0	98.8	7.9	+132	7.54	13.5
Three-line hybrids									
LH1	IR58025A/Basmati 385R	121	105	18	131	5.61	65	7.15	13.8
LH18	IR75596A/IR73014-59-2-2-2R	115	94	18	189	5.60	65	7.26	12.2
LH19	IR68897A/IR69714-28-1-2-6-2R	119	99	18	153	5.64	66	7.09	11.9
LH20	IR70369A/IR72998-93-3-3-2R	110	90	15	171	5.39	58	6.93	10.6
LH21	IR79128A/IR72906-24-1-3-1R	117	97	15	167	4.82	42	7.53	11.1
LH22	IR58025A/IR63870-123-2-2-2-2R	103	81	18	104	4.95	43	6.91	12.0
	Basmati 320/10486 (check)	120.4	111	25.8	113.6	3.4	–	7.31	12.5

Commercialization of rice hybrids

Progress and scope of hybrid rice

In spite of advances in rice production, rice yield remains lower than in many other countries. The stagnant yield is attributed to a lack of high-yielding varieties, a decline in land resources, and water scarcity. The adoption of hybrid rice technology is one of the options to achieve 20–25% higher yield than pureline varieties, particularly in Sindh, Punjab, and Balochistan. Hybrid rice can be planted on an area of about 1 million ha, which is about 40% of the total rice area. Initially, about 7 t of hybrid seed was imported in 2002; this increased to about 2,500 t in 2008. Consequently, hybrid rice area increased steadily from 400 ha in 2002 to about 63,000 in 2007, covering about 8.3% of the area for nonbasmati varieties. The area under hybrid rice is projected to reach 350,000 ha, with an expected production of 3.4 million t by 2012.

Factors contributing to the success of hybrid rice

The income of rice farmers has doubled as the yield of hybrid rice doubled. The early-maturing hybrid rice crop ensured the timely sowing of rabi crops. Due to a shorter maturity period, hybrid rice can be planted late in the season. Hybrid rice also requires less irrigation than do traditional rice varieties. Moreover, it can be successfully grown in stressed areas such as saline-, drought-, and waterlogging-prone environments.

Challenges to the adoption of hybrid rice

Hybrid rice technology has not been popular among farmers in spite of the 20–25% higher yield over existing rice varieties. This is due to the inconsistent performance of hybrids in farmers' fields, lack of understanding of agronomic management, poor grain quality, high cost of hybrid rice seed, and use of farmers' own seed. To popularize hybrid rice in Pakistan, there is a need to develop better quality rice hybrids, along with the dissemination of effective production technology to farmers. This may be achieved by establishing effective mechanisms for research, hybrid seed production, and technology transfer. Once adopted, hybrid rice technology will help increase rice production and feed Pakistan's ever-increasing population.

Approved rice hybrids

The rice hybrids mentioned in Table 3 (except for GNY 50 and GNY 53) have been recommended by the Variety Evaluation Committee of the Pakistan Agricultural Research Council, Islamabad, for importation in 2008. GNY 50 and GNY 53 were recommended in 2003. The agronomic and quality characteristics of the recommended hybrids are given in Tables 3 and 4. Among these recommended hybrids, Emkay-H401 is the only locally developed hybrid.

Table 3. Agronomic characteristics of approved rice hybrids.

Designation	Plant height (cm)	Days to maturity	Tillers plant^{-1}	Grains panicle^{-1}	Spikelet fertility (%)	1,000-grain wt. (g)	Paddy yield (t ha^{-1})
GNY 50	100	100	11	150	82	26.5	6.0
GNY 53	97	96	10	143	86	25.4	5.4
Guard 402	120	100	17	148	80	26.0	6.0
Guard 403	105	90	18	135	86	27.0	6.5
Arize 403	120	100	12	162	80	25.0	5.9
Dagha-1	135	85	9	185	82	26.5	7.2
Emkay-H401	120	100	14	112	83	25.9	5.7

Table 4. Quality characteristics of approved rice hybrids.

Designation	Brown rice (%)	Total milling recovery (%)	Head rice (%)	Grain length (mm)	Grain width (mm)	Grain thickness (mm)	Cooked grain length (mm)	Bursting (%)
GNY 50	79	70	54	6.60	1.90	1.65	10.9	18
GNY 53	79	71	54	6.54	1.87	1.56	10.5	20
Guard 402	78	71	55	6.90	2.0	1.65	10.9	16
Guard 403	78	69	46	6.95	1.95	1.65	12.2	12
Arize 403	81	71	56	6.49	1.94	1.53	11.2	25
Dagha-1	79	71	56.5	6.66	1.98	1.72	11.6	35
Emkay-H401	80	69	34.5	6.77	1.97	1.58	11.5	27

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Authors' addresses: Muhammad Akhter, Muhammad Afzal Zahid, Syed Sultan Ali, Mushtaq Ahamd, Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan; Muhammad Ramzan, Rice Program, Pakistan Agricultural Research Council, Kala Shah Kaku, Lahore, Pakistan. Email: makhter64@yahoo.com.

Status of and prospects for hybrid rice commercialization in the Philippines

Madonna C. Casimero and Niña Gracel B. Dimaano

Hybrid rice production was launched in the Philippines as a component of the *Gintong Ani* Program in 1997. Five years later, the Hybrid Rice Commercialization Program (HRCP) was launched by President Gloria Macapagal-Arroyo as a strategy to increase rice productivity and profitability for Filipino farmers. The HRCP aims to promote the widespread use of hybrid seeds in the country to enhance farmers' productivity and income, and to generate employment in the rural areas. It has seven major components: hybrid rice commercial production (F_1 cultivation), hybrid seed production, technology demonstrations, training, an information campaign, marketing assistance, and research and development. The HRCP has continuously been monitored and analyzed for its profitability and productivity impacts among its major stakeholders. From only 5,472 ha in the 2001 wet season (WS), hybrid rice production increased to 225,371 ha in the 2006 dry season (DS). Production increased significantly as well, from 29,233 tons in the 2001 WS to 1,309,502 tons in the 2006 DS. The actual farm yield average is at 5.8 t ha^{-1}. Likewise, the number of farmer beneficiaries per season rose from 3,847 in the 2001 WS to 132,747 in the 2006 DS. In the recently launched rice self-sufficiency plan, hybrid rice is targeted to be planted on about 1.14 million hectares, of which 700,000 hectares will be planted for the dry season, and these are expected to produce 6.8 million tons of rice in 2009-10. The R&D activities in upstream research on hybrid parental lines, integrated crop management, and on-farm adaptive research are done continuously to develop suitable hybrids and the corresponding crop management strategies to attain optimum yields. Technology promotion is also done in conjunction with training of trainers and farmers to attain long-term national capacity development in hybrid rice.

Hybrid rice technology has proven its potential as a key strategy for increasing national rice production and achieving food security and rice self-sufficiency. Much needs to be done, though, as we face more challenges ahead. Accordingly, there is a vital need to sustain and further enhance the productivity gains posted by the HRCP; refocus the HRCP in high-impact areas; continue funding support for R&D, technology promotion, and training; revisit the seed subsidy policy; strengthen the National Seed Quality Control Services; adopt a private sector–led approach in hybrid rice industry development; form strategic alliances; and continue and expand socioeconomic monitoring and evaluation of the HRCP.

The Philippine population is steadily growing, at 2.3% per year, while the total land area for rice production has been decreasing by about 10,000 ha per year because of industrialization and urbanization. Moreover, production from the high-yielding varieties recommended to farmers has not increased significantly since the early 1970s. By 2025, the country will be needing 40–50% more rice and this increased production must come from less land using less water (Redoña and Gaspar 2001). To meet this food challenge, the Philippine government has recognized the potential of hybrid rice technology to increase national rice production and attain rice self-sufficiency and food security. Consequently, hybrid rice production was launched by the government as a component of the *Gintong Ani* Program in 1997. This program elevated hybrid rice technology to the national agricultural forefront. Through this program, hybrid rice research and promotion activities were intensified with the cooperation of local government units (LGUs) and the private sector. In 1999, commercialization efforts for hybrid rice production started through various promotion activities and initial seed subsidy programs. In 2002, these activities were further strengthened through the Hybrid Rice Commercialization Program (HRCP).

The Hybrid Rice Commercialization Program in the Philippines

Inspired by the success story of the People's Republic of China, a Hybrid Rice Commercialization Program (HRCP) was launched by President Gloria Macapagal Arroyo under the Million Jobs Program on 4 March 2002. With the use of hybrid rice technology, it is assumed that the country's average rice production would increase by at least 15%. Under EO No. 76, the Chief Executive designated the Philippine Rice Research Institute (PhilRice) as the lead agency in implementing the program.

The major objectives of the HRCP were to reduce the importation of rice and attain self-sufficiency of the crop, increase rice production with less land and improve farm yields, and create employment opportunities in rural areas. Accordingly, the program's specific objectives were to effectively disseminate information on hybrid rice technology, including the benefits derived therefrom; provide farmers with support services by delivering technical training, credit assistance, and high-quality agricultural inputs; and monitor the effectiveness of the program by tracking national yields, jobs, and incomes.

The HRCP is rooted in seven major components: hybrid rice commercial production (F_1 cultivation), hybrid seed production, technology demonstrations, training, an information campaign, marketing assistance, and research and development. From the total budget of PhP428,685,000, the budget allocation to F_1 cultivation included seed procurement (80%), seed production (6%), program management (7%), R&D

activities (4%) and technology demonstration, training, an information campaign, and facility construction (3%) (Gaspar et al 2007).

Implementation of the HRCP

PhilRice, together with the Department of Agriculture Regional Field Units and LGUs, embarked on implementing the HRCP with the full support of the Philippine government. Strategies were mapped out to ensure success in the implementation despite hybrid rice being a new technology in the country.

Program empowerment. To effectively implement the HRCP, a total of 459,250 extension workers, R&D implementers, seed inspectors, agricultural technicians, and farmers attended technical briefings on hybrid rice seed technology. Many plant breeders, R&D implementers, and other extension workers from PhilRice, the Department of Agriculture (DA), and Agricultural Training Institute (ATI) were also trained for the promotion of the technology (Gaspar et al 2007).

Information dissemination. Information dissemination for the HRCP was accomplished through various campaign materials such as billboards, bulletins, newsletters, pamphlets, and other printed materials. TV advertisements, radio plugs and jingles, instructional and promotional videos, and other broadcast media releases were also used to ensure nationwide awareness and appreciation of hybrid rice. For easier understanding by the target clienteles, various information materials were translated into the target area's dialect. Hybrid rice is not like conventional inbred rice. The development of hybrids as well as seed production activities have many new terms and concepts that have not been encountered by nontechnical staff and the consuming public. Concepts and technical terms were simplified during the development of the information materials for easier grasp, understanding, and adoption of the recommended technologies on hybrid rice.

R&D efforts. Research strategies focused on the use of a multidisciplinary approach in solving various problems and the use of biotechnology techniques to expedite hybrid varietal improvement. The collaboration of PhilRice with the International Rice Research Institute (IRRI), China, and other international hybrid rice R&D institutions was strengthened with the launching of the HRCP. The national seed production network (SeedNet) and the private sector for hybrid rice seed production were tapped to produce the required volume of parentals and F_1s for planting. Research and training activities and a public awareness campaign of the technology, particularly in the target areas, were intensified to ensure that we developed the proper technologies for the hybrids and that these reached the farmers in time. Private-sector participation, including NGOs, in seed production, distribution, and marketing, as well as with LGUs and other government agencies in technology promotion and commercialization, was also enlisted. These steps facilitated the sourcing out of external funds both locally and internationally. In addition, the program strongly supported the development of experimental and seed production farms; the construction of greenhouses, growth chambers, and biotechnology and grain quality laboratories; and the improvement of drum seeders, mechanical dryers, power tillers, and other specialized farm equipment to suit the characteristics of hybrid rice (Gaspar et al 2007).

HRCP status

Government resources were poured into promoting hybrid rice technology to ensure that the goals of the program were met. Thus, there has been a concern about the worthiness of government investment in the program. Accordingly, a midterm assessment of the status and performance of the HRCP (2002 WS to 2004 DS) was conducted by STRIVE Foundation and PhilRice to determine its national impact and effectiveness. The PhilRice Socioeconomics Division (PhilRice-SED) studied and presented the extent of hybrid rice adoption in the country from the 2004 DS to 2006 DS. These midterm assessments were both conducted in the five major hybrid rice-producing provinces in the Philippines: Nueva Ecija, Isabela, Davao del Sur, Davao del Norte, and Iloilo.

Overall program impact assessment

National rice production. Results of the program assessments showed that the total area planted to hybrid rice increased from 5,472 ha in the 2001 wet season (WS) to 225,371 ha in the 2006 dry season (DS). The area harvested in hybrid rice grew from 5,371 ha in the 2001 WS to 221,952 ha in the 2006 DS. Production increased significantly as well from 29,233 tons in the 2001 WS to 709,778 tons in the 2004 WS. In 2004, hybrid rice had contributed 10% to the total production from irrigated areas. Production volume further increased in the 2005 DS to 1,157,750 tons and in the 2007 DS to 1,309,502 tons. Likewise, the number of farmer beneficiaries per season rose from 3,847 in the 2001 WS to 132,747 in the 2006 DS. Up to the 2007 DS, actual farm yield averaged 5.8 t ha^{-1} (Table 1).

Job creation. Labor requirements for hybrid rice production are much higher than for inbred rice. Activities that require higher labor include seed production, seedling management, transplanting, harvesting, and threshing operations. Results of the four-season analysis of STRIVE and PhilRice indicated that the HRCP generated a total of 85,266 jobs during the entire period. Likewise, the program had benefited a total of 247,887 hybrid rice farmers and 1,857 seed growers.

Diffusion of other technologies. Hybrid seed also proved to be an efficient tool for the promotion of other new technologies. The HRCP had brought about indirect technology diffusion by inducing synergies between hybrid and inbred rice farmers in terms of lower seed use per hectare, patterns of fertilizer usage, soil analysis, integrated nutrient and pest management, and synchronous farming, among others. Specifically, the technologies introduced along with the use of hybrid seeds included the use of 20 kg seed ha^{-1}, sparse seeding rates, 1–2 per hill planting rates, and the use of the minus-one-element technique (MOET) and the leaf color chart (Redoña et al 2004).

Financial and economic impact. Results of the midterm analysis showed that the program's benefits far exceeded its costs. From the 2002 WS to 2004 DS, the financial and economic benefit-cost ratios of hybrid rice production were estimated at 1.56 and 1.13, respectively. In terms of U.S. dollar savings from rice imports, the program had earned $23.25 million (STRIVE-PhilRice 2007).

Table 1. Area planted, area harvested, production, yield, and number of farmer-beneficiaries of commercial hybrid rice by season, 2001 WS to 2007 WS.

Season[a]	Area planted (ha)	Area harvested (ha)	Production (tons)	Yield (t ha^{-1})	No. of farmer beneficiaries
2001 WS	5,472	5,371	29,233	5.34	3,847
2002 DS	7,078	6,825	46,747	6.60	4,890
2002 WS	21,301	21,089	121,917	5.72	14,949
2003 DS	25,523	25,247	152,715	5.98	17,704
2003 WS	54,538	52,111	308,844	5.66	38,137
2004 DS	77,982	77,481	463,359	5.94	54,781
2004 WS	130,903	126,513	709,778	5.42	100,695
2005 DS	189,606	187,444	1,157,750	6.11	145,851
2005 WS	184,601	165,385	965,612	5.23	130,092
2006 DS	225,371	221,952	1,309,502	5.90	132,747
2007 DS	256,548	256,548	2,435,404	9.49	–
2007 WS	85,456	85,456	504,319	5.90	–

[a]The wet season is defined as May to October while the dry season runs from November to April planting.
Source: GMA Rice Program.

Hybrid rice adopters. Results of the PhilRice-SED study showed that, among the 508 farmers, 30% (152) have tried planting hybrid rice at least once in the last two years. Among the provinces, Nueva Ecija has the highest percentage of adoption at 42%, followed by Isabela at 38%. Iloilo has the lowest adopters at 16% (Table 2) since hybrid farmers in this area have not encountered a significant yield and profit difference compared with farmers who planted inbred varieties (Manalili et al 2008).

Of the 152 adopters, 63% have planted hybrid rice for only one season, 17% have tried it for two seasons, and 20% have planted it for three to five consecutive seasons (Fig. 1). One-fifth of the farmers who planted hybrid rice are full adopters. This indicates a positive sign for hybrid rice production given that inbred varieties have already been in the market for almost four decades (Manalili et al 2008).

Hybrid rice varieties. Since the launching of the HRCP, demand for hybrid seeds by farmers has steadily increased. Four public hybrids, Magat, Mestizo 1, Mestizo 2, and Mestizo 3, and four proprietary hybrids, including two from multinationals Monsanto and Bayer Crop Science (*Magilas* and *Bigante,* respectively), were released commercially in 2004 (Table 3). One hybrid, NSIC Rc136H (SL 8H) from local seed company SL Agritech, was also released in the same year. Rizalina 28, developed by HyRice Corporation, had temporary accreditation for commercial planting by farmers. The involvement of private seed corporations in promoting their rice hybrids without

Table 2. Percentage of hybrid rice adopters by province, 2004 DS to 2006 DS.

Province	Adopters (%)	Nonadopters (%)
Nueva Ecija	42	58
Isabela	38	62
Iloilo	16	84
Davao del Sur	28	72
Davao del Norte	27	73
All	30	70

Source: PhilRice Socioeconomics Division survey.

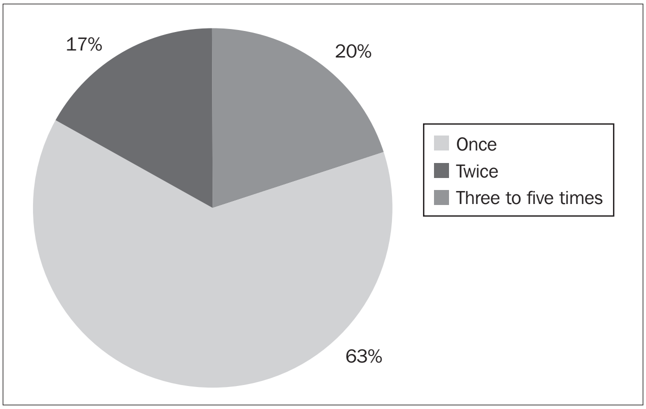

Fig. 1. Number of times adopters planted hybrid rice. Source: PhilRice Socioeconomics Division survey, 2006.

support from the public sector has greatly enhanced the development and adoption of hybrid rice technology in the country (Redoña et al 2004).

In 2007, four more privately bred hybrids had been commercially released—NSIC Rc162H (Bio 401), NSIC Rc164H (Rizalina 28), NSIC Rc166H (PSD 3), and NSIC Rc168H (BCS 064) (Table 4). Another hybrid, Arize H 64 developed by Bayer Crop Science, is being evaluated in the National Cooperative Test for Hybrids. Arize H 64 is said to be an improved version of the existing Arize variety and it has a shorter maturity period and longer slender grains, and is slightly aromatic. Another unnamed hybrid, which is resistant to the dreaded bacterial leaf blight (BLB) disease, will be introduced by Bayer soon.

Results of the PhilRice-SED study showed that, across all seasons, 45% of hybrid rice adopters identified NSIC Rc 132H, popularly known as SL 8H, as the most popular hybrid rice variety. With a 69% adoption rate, SL 8H was the most popular in

Table 3. Commercially released hybrid rice varieties in the Philippines, 1994-2007.

Variety	Line designation	Breeding institution	Year	Average yield (t ha^{-1})	Maximum yield (t ha^{-1})
PSB Rc26H (Magat)	IR64616H	IRRI	1994	5.6	7.6
PSB Rc72H (Mestizo)	IR68284H	IRRI	1997	5.4	9.9
PSB Rc76H (Panay)	CRH 05	Monsanto	1998	4.8	7.9
NSIC Rc114H (Mestizo 2)	IR75207H	IRRI	2002	5.8	8.7
NSIC Rc116H (Mestizo 3)	IR75217H	IRRI	2002	5.8	8.6
NSIC Rc124H (Mestizo 4)	Bigante	Bayer	2004	5.7	9.1
NSIC Rc126H (Mestizo 5)	MRH 005	Monsanto	2004	6.2	9.5
NSIC Rc132H (Mestizo 6)	SL 8	SL Agritech	2004	5.9	8.7
NSIC Rc163H (Mestizo 7)	IR78386H	IRRI	2006	6.7	10.6
NSIC Rc162H (Mestizo 8)	BIO 401	Bioseed	2007	5.8	7.7
NSIC Rc164H (Mestizo 8)	Rizalina 28	HyRice	2007	6.4	8.5
NSIC Rc166H (Mestizo 10)	PSD 3	Syngenta	2007	6.5	10.6
NSIC Rc168H (Mestizo 11)	BCS 064	Bayer	2007	6.5	10.5

Source: Philippine Seed Board (PSB), National Seed Industry Council, and PhilRice.

Table 4. Hybrid rice varieties commonly planted by farmers, 2004 DS to 2006 DS.

Variety	2004 DS	2004 WS	2005 DS	2005 WS	2006 DS	All seasons
	(%)					
SL 8H	26	29	35	36	69	45
Mestizo 1	45	40	43	44	18	34
Bigante	18	10	12	7	7	10
Mestizo 3	8	10	7	13	3	7
Rizalina	3	10	0	0	0	2
Bioseed	0	0	0	0	2	1

Source: PhilRice Socioeconomics Division survey.

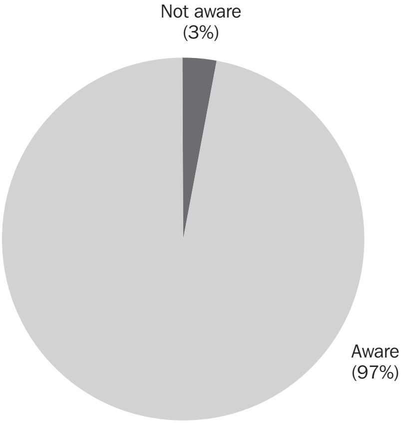

Fig. 2. Percentage of farmers aware and not aware of hybrid rice. Source: PhilRice Socioeconomics Division survey, 2006.

the 2006 DS in the major rice-growing provinces of Nueva Ecija, Isabela, and Davao del Norte. SL 8H started gaining popularity among farmers in the 2005 DS and it became the most accessible and available variety since it was promoted by the DA and a private company in the 2006 DS.

Mestizo 1 or PSB Rc 72H, a public-bred variety, which is aromatic and soft and has an eating quality that is comparable with that of inbred rice variety IR64, ranked second most popular at 34% (Table 4). Mestizo 1 was most popular during the 2004 DS to 2005 WS among farmers in Isabela, Davao del Sur, and Davao del Norte. Bigante ranked third, planted by 10% of hybrid rice adopters, followed by Mestizo 3, Rizalina, and Bioseed (Manalili et al 2008).

Awareness and perception of hybrid rice technology by nonadopters. Out of the 356 farmer respondents who did not try hybrid rice, 97% claimed that they were already aware of the technology (Fig. 2). However, they did not adopt it because of constraints such as high costs of seeds and labor, particularly for pulling of seedlings and transplanting activities. In this regard, 9% of the farmers interviewed perceived that hybrid rice cultivation was more laborious than inbred cultivation. Other farmers (8%) claimed that they lacked technical know-how on the production practices of hybrid rice. Its susceptibility to pests and diseases and unsuitability during the WS were also reasons why these farmers were hesitant to use the technology (Manalili et al 2008).

Adoption level without a seed subsidy. Provision of a seed subsidy has been a key for the promotion and initial adoption of the HRCP in the country. This strategy indeed has given the HRCP its preliminary success. However, a gradual phasing-out of the seed subsidy is to be done by the government, assuming that even without the seed subsidy farmers will still get a reasonable return on their additional investment in hybrid rice.

Table 5. Percentage of farmers who will plant and who will not plant hybrid rice without seed subsidy.

Province	Will plant (%)	Will not plant (%)
Nueva Ecija	81	19
Isabela	62	38
Iloilo	75	25
Davao del Sur	68	32
Davao del Norte	15	85
All	61	39

Source: PhilRice Socioeconomics Division survey (2006).

Accordingly, PhilRice-SED determined the reaction of farmers to the removal of the seed subsidy. Results of the survey showed that, among the 152 hybrid rice adopters, 61% intended to continue planting hybrid rice even if the seed subsidy were removed. Across provinces, Nueva Ecija had the highest percentage of farmers (81%) that intended to continue planting hybrid rice in the absence of a seed subsidy, followed by Iloilo (75%), Davao del Sur (68%), Isabela (62%), and Davao del Norte (15%) (Table 5).

The yield advantage over inbred rice was the main reason 83% of the farmers intended to continue planting hybrid rice (Table 6). Early on-farm experiments showed that hybrid rice has a yield advantage of at least 15% over inbred varieties. From 2001 to 2004, the yield advantage of hybrid rice over inbred rice in the program areas was estimated at 27% during the WS and 39% during the DS (Manalili et al 2008). The STRIVE and PhilRice midterm impact evaluation of hybrid rice technology showed that hybrid rice had an average yield advantage of 11%. Additionally, more hybrid rice users obtained a higher yield of more than 5 t ha^{-1} than their inbred counterparts. The study also revealed that hybrid rice production would still give superior net profits to inbred rice even if the seed subsidy were removed.

Another reason for planting hybrid rice even without a seed subsidy is the higher output price (Table 6). Hybrid rice has a price advantage over inbred rice of about 30 centavos per kg, signifying that hybrid rice has better quality or at least the same eating quality as inbred rice. Though the production cost per hectare was higher in hybrid rice due to the higher cost of seed, fertilizer, pesticide, and labor, the profit from hybrid rice farming was still significantly higher than that of inbred rice during the DS (Manalili et al 2008). With the higher yield and output price for hybrid rice, there is a corresponding high gross income for Filipino farmers.

With the higher income and profit, farmers considered themselves as better providers and they claimed that they had purchased more possessions such as household

Table 6. Major reasons for continuing and discontinuing to plant hybrid rice without the seed subsidy.

Reasons	Percentage
Reasons for continuing	
Higher yield	83
Higher output price	4
Suitable for dry season	3
Higher income/profitability	3
Good eating quality	1
High milling recovery	2
If yield performance remains high	4
Reasons for discontinuing	
High cost of seeds/lack of capital	49
Susceptible to pests and diseases	19
Broken rice/low milling recovery	10
High fertilizer requirement	9
Complexity of the technology	5
Farm area is too small	2
Poor germination	2
Same yield as inbreds	2
Low price/marketing problem	2

Source: PhilRice Socioeconomics Division survey (2006).

appliances after continuous planting of hybrid rice. Respondents also indicated improvement in their confidence as rice farmers upon adopting the technology (STRIVE-PhilRice 2007).

Other reasons for continually adopting hybrid rice include its suitability during the dry season, good eating quality, and high milling recovery. Conversely, the high cost of seed is the major reason for dropping the technology once the seed subsidy is removed. Other reasons for discontinuing were its susceptibility to pests and diseases, broken rice and low milling recovery, low selling price of some hybrid rice varieties, high fertilizer requirement, complexity of the technology, poor germination, farm area that is too small, and the same yield as inbred rice (Table 6).

Table 7. Target area for certified hybrids by region, irrigated ecosystem, 2009-10.

Region	Target hybrid area (ha)	
	2009	2010
I	53,947	62,185
II	116,150	134,570
III	91,249	109,841
IV-A	12,327	16,266
IV-B	18,057	23,118
V	21,719	27,454
VI	19,223	24,667
VII	9,306	11,456
VIII	31,965	39,915
IX	24,868	29,409
X	12,503	15,219
XI	25,677	30,963
XII	26,227	32,575
XIII	28,195	31,895
CAR	17,935	19,540
ARMM	7,902	9,734
Total per year	517,250	618,807
Total	1,136,057	

Source: Philippine Rice Self-Sufficiency Plan, 2009-10.

Prospects for the HRCP

Since the launching of the HRCP, the potential of hybrid rice technology as a key strategy for increasing Philippine rice production and achieving food security and self-sufficiency has been analyzed. Thus, in the recently launched rice self-sufficiency plan, hybrid rice is targeted to be planted on about 1.14 million hectares (Table 7), on which 700,000 hectares will be planted for the dry season, and expected to produce 6.8 million tons of rice in 2009 to 2010.

Undeniably, HRCP assessments and studies showed promising results; however, many challenges are still at hand. Foremost, there is a need to sustain and further enhance the productivity gains brought about by the HRCP over the past years. Consequently, funding support for the program must be prioritized, as well as a further strengthening of linkages and coordination among the government, PhilRice, and other major stakeholders of the program. Moreover, there is a need to identify and characterize the most suitable areas for cultivation, given the agro-climatic variances across the regions, level of coordination, technical services, and infrastructure development at the LGU level. The targeting should focus on areas where hybrid rice production has demonstrated clear superiority over inbreds.

In ensuring national capacity development in hybrid rice, upstream research on hybrid parental lines and on-farm adaptive research on hybrid commercial production through technology promotion and training must be continued in the medium to long term (STRIVE-PhilRice 2007). A challenge is also posed for strengthening the institutional capacity of the National Seed Quality Control System (NSQCS) to ensure quality standards in hybrid rice seed production.

Private sector–led hybrid rice commercialization is envisioned by 2010. The private sector must play a greater role in the commercialization of the technology while the government makes the policy environment conducive for the private sector to do its business (Third National Workshop on Hybrid Rice 2005). However, the combined efforts of both the public and private sector will be fundamental in guaranteeing more rapid growth in the development and adoption of hybrid rice technology in the country.

With the limited resources of the government, a gradual phasing out of the seed subsidy should be implemented. Government funds could then be re-allocated to farm-to-market roads, irrigation facilities, extension activities, information dissemination, and rice research, among other things that represent more tangible projects. However, in the provinces where adoption is still quite low, a seed subsidy should still be implemented to encourage farmers to try the technology and evaluate its performance. This recommendation is a result of the PhilRice-SED assessment, which revealed that more than half of the farmer adopters in the five surveyed provinces indicated that they would still adopt the technology even without the seed subsidy. Besides, farmers' net income is still higher for hybrid rice than for inbreds even without this financial incentive due to a higher yield advantage and output price.

Filipino farmers preferred rice varieties that possess high yield, a high output price, good eating quality, long grain quality, and high milling recovery. These characteristics should be considered by plant breeders in a future breeding program of hybrid rice. There is also a need to address the major constraints to hybrid rice adoption in the country. These constraints include not only the high costs of seeds but also the high cost of labor for pulling and transplanting activities. Consequently, research on direct-seeded hybrid rice, particularly on the use of a drum seeder, should be continued and intensified to lessen the production costs of farmers, thereby increasing their net income (Manalili et al 2008).

Lastly, there is a need to continue and expand the socioeconomic monitoring and evaluation of the HRCP to continually monitor and analyze the profitability and productivity impact of the HRCP among its major stakeholders—seed growers and F_1 commercial rice farmers. Constant monitoring will also elucidate the long-term impact of the HRCP on national rice production, job generation, financial and economic conditions, rice self-sufficiency plans, and poverty alleviation.

Conclusions

Results from the assessment of HRCP status in the Philippines are promising. It has been confirmed that hybrid rice has superior yield potential, resulting in high profitability for seed production and the generation of rural employment in some major provinces. In addition, hybrid rice promotion has created sequential adoption of other component technologies in rice production such as lower seed use per hectare, patterns of fertilizer usage, soil analysis, integrated nutrient and pest management, and synchronous farming. On the national scale, government investments in hybrid rice commercialization have outweighed its cost. Moreover, the estimated dollar savings from rice importation resulting from hybrid rice commercialization amount to $23.25 million.

For the claim that hybrid rice technology is a key strategy for increasing national rice production and achieving food security and rice self-sufficiency, much still has to be proven. A lot of challenges still have to be faced. Accordingly, there is a vital need to sustain and further enhance the productivity gains posted by the HRCP; refocus the HRCP in high-impact areas; continue funding support for R&D, technology promotion, and training; revisit the seed subsidy policy; strengthen the National Seed Quality Control Services; adopt the private sector–led approach in hybrid rice industry development; form strategic alliances; and continue and expand socioeconomic monitoring and evaluation of the HRCP.

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Authors' address: Philippine Rice Research Institute, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines.

Hybrid rice research and development in Sri Lanka

S.W. Abeysekera, S.N. Jayawardena, D.M.N. Dissanayake, S. Bandara,
W.K. Jayasinghe, and D.M.N.D. Dasanayake

With a population growth rate of 1.2% per year, overall rice demand in 2010 and 2020 in Sri Lanka would be 3.46 and 3.82 million tons. Current problems facing the rice sector in Sri Lanka are the low yield of marginal lands despite the high adoption of semidwarf high-yielding varieties. Sri Lanka's future national food security goals can be achieved only by increasing paddy production. However, Sri Lanka has reached a stage at which further expansion in rice area is not possible. Therefore, finding alternative ways to increase the productivity of the unit area of land and to narrow the yield gap between researchers' plots and farmers' fields is essential. The Rice Research and Development Institute (RRDI) has identified hybrid rice technology as one of the best options to overcome low yield and meet the future demand for rice in the country.

In 1996, a collaborative hybrid rice research and development (R & D) program began with support from an IRRI/ADB project. Four CMS lines have been developed by the RRDI, of which Bg CMS 2A and Bg CMS 3A have small round (Samba) grain type with 100% sterility and around 23% outcrossing rate (OCR%). Bg CMS 1A has been used to develop the first Sri Lankan rice hybrid, Bg 407H. Bg CMS 4A has shown 100% sterility with a high outcrossing rate (35%). In 2005, RRDI released its first rice hybrid variety, Bg 407H, for commercial cultivation with cultural management practices. Farmers now grow Bg 407H on about 200 ha. Its F_1 seed production is done on a government research farm. The average F_1 seed yield is 0.75–1.0 t ha^{-1}. Seed production techniques still need further improvement to attain higher yield. Recently, RRDI has developed 22 promising high-yielding hybrid combinations using RRDI- and IRRI-developed CMS and restorer lines. Of these, seven combinations have shown high standard heterosis with medium age and resistance to major pests and diseases, with superior grain quality. Small-scale F_1 seed production is being done at RRDI.

One of the barriers to the large-scale adoption of hybrid rice technology in the country is the high labor requirement for transplanting. Because of the high production cost, more than 95% of the total rice crop is grown using

broadcasting. This practice hinders the wide adoption of hybrid rice technology in Sri Lanka. One of the establishment methods introduced, the seedling broadcasting method (parachute method), is now widely practiced. In 2007, FAO granted a Technical Collaboration Program for "strengthening national capacity for hybrid rice development and use for food security and poverty alleviation." It is expected that this assistance will support the government of Sri Lanka in its drive to assure sustainable food security.

Rice is the staple food of 19 million people in Sri Lanka. Significant gains have been achieved in national rice production during the last two decades. National rice yield has reached 4.1 t ha^{-1}. However, national paddy production is sufficient to meet only 90% of the nation's requirement. Projected annual rough rice consumption by 2010 is 3.46 million tons. The majority of the rice farmers in the country have small holdings (0.2 ha) and are resource-poor. The main problems now facing the rice sector in Sri Lanka are low yield, a marginal rate of yield increase, the high cost of production, and the abandoning of marginal rice lands despite the high adoption of semidwarf high-yielding varieties (Abeysiriwardena and Sandanayake 2000). Therefore, it has been proposed that average yield be elevated from 4.1 t ha^{-1} to 5.1 t ha^{-1} immediately. Increasing productivity cannot be sustained, however, without extensive research efforts, mainly in the area of varietal improvement. Hybrid rice is one of the potential areas for further improving Sri Lankan rice varieties and, hence, hybrid rice research has been given national priority (Abeysekara 2000). The hybrid rice research program in Sri Lanka began in 1994 at the Rice Research and Development Institute (RRDI) of the Department of Agriculture Sri Lanka (DOASL). From 1996 onward, the Hybrid Rice Research and Development (R&D) Program gained technical and financial assistance from the International Rice Research Institute and Asian Development Bank (IRRI/ADB) and helped in developing adaptable hybrid rice technology in Sri Lanka (Abeysekara et al 2000).The second phase of this project terminated in 2004, leaving the recipient country to bear the total cost of continuing the program. With assistance from the IRRI/ADB project, RRDI released its first hybrid rice variety, Bg 407H, in December 2005 and F_1 seed production is in progress.

In October 2007, the Food and Agriculture Organization (FAO) granted a Technical Collaboration Program (TCP) for "strengthening national capacity for hybrid rice development and use for food security and poverty alleviation." Improving parental lines to enhance hybrid rice breeding efficiency, developing high-yielding hybrids, and optimizing technological packages for hybrid rice cultivation and hybrid rice seed production, training, and awareness are the major areas covered under this program. The current status of hybrid rice R&D in Sri Lanka is presented in this paper.

Improvement of parental lines

Promising CMS, maintainer, and restorer lines

The three-line system has been identified as the most feasible system for the development of hybrid rice in Sri Lanka. In hybrid rice breeding, elite lines and released varieties from varietal improvement programs are now used as parental lines. Besides narrow genetic diversity, the frequency of the occurrence of restorers (R) and maintainers (B) among these lines can range from only 0 to 25% and 0 to 10%. The remaining parental lines (70–80%) cannot be directly used in a hybrid breeding program. Within the identified restorers and maintainers, frequency of combining ability, resistance to biotic and abiotic stresses, and good grain quality are still lower. Therefore, an improvement program for parental lines was undertaken at RRDI using cross-breeding methods for further improvement. Many desirable segregates possessing good restorability, better grain quality, stigma exsertion, and favorable outcrossing lines were selected. The potential restorers identified are being purified and multiplied for producing new hybrids.

Work on developing new CMS lines is actively done at RRDI. Some 10 to 15 promising maintainers are at various stages of conversion in the backcross nursery at RRDI, along with IRRI CMS lines.

IRRI- and RRDI-developed CMS lines were tested for stability, sterility, and other desirable traits. Four CMS lines developed by RRDI and seven IRRI CMS lines have been identified as adaptable and stable under local conditions (Table 1). The outcrossing rate of the IRRI CMS lines ranged from 30.2% to 43.0% while showing 100% pollen sterility. Four CMS lines, Bg CMS 1A, Bg CMS 2A, Bg CMS 3A, and Bg CMS 4A, possess desirable characteristics such as high outcrossing rate (23−34%) and higher adaptability. Bg CMS 1A was already used to developed the first hybrid rice variety, Bg 407H.

Bg CMS 2A and Bg CMS 3A are small round-grain (Samba-type) varieties that could be used to develop Samba-type rice hybrids in the future. Bg CMS 4A has a high outcrossing rate (34%) and promising desirable characters. It could be used to develop new hybrid combinations with high heterosis.

Evaluation of hybrids

During the past two years, RRDI was able to develop 22 promising high-yielding hybrid varieties using RRDI- and IRRI-developed CMS and restorer lines. Of these, seven combinations showed high standard heterosis (1.6−34.4%) with medium age (105–110 d), resistance to major pests and diseases, and superior grain quality.

Of the tested new experimental hybrids, seven, Bg CMS 4A/At 95-10-4, Bg CMS 1A/BN 6986-108-2R, IR69616/IR69702-32-1-3, IR80156A/IR68077-37-2-3R, IR77289A/HRSP 948, IR76768A/IR73012-2-2-2, and Bg CMS 4A/At 95-10-4, showed higher yield heterosis (21–34%) over presently recommended hybrid variety Bg 407H. Bg CMS 4A/At 95-10-4 showed yield heterosis above 34% (Table 2) and other desirable characteristics such as resistance to major pests and diseases.

Table 1. Performance of IRRI and Sri Lankan CMS lines at RRDI during the minor seasons, 2007.

CMS line	Panicle length	Spikelets panicle^{-1}	Duration of floral opening (min)	Stigma exsertion (%)	Pollen sterility (%)	Outcrossing rate (%)
IR69616A	21.0	180.0	325	22.4	100	30
IR68275A	24.2	196.2	290	14.3	100	7
IR68897A	25.5	162.6	270	12.8	98	14
IR58025A	19.2	180.0	265	12.9	100	37
IR77289A	23.4	170.0	255	20.3	100	32
IR80156A	24.2	165.3	270	22.1	100	28
IR77298A	21.2	182.6	272	18.3	100	30
Bg CMS 1A	26.3	170.2	280	70.0	100	30
Bg CMS 2A	25.2	185.3	325	71.0	100	23
Bg CMS 3A	25.8	195.2	350	75.0	100	24
Bg CMS 4A	26.8	175.5	270	76.0	100	34

Table 2. Performance of selected hybrids in preliminary yield trials at RRDI, wet season, 2006-07.

Hybrid	Plant height (cm)	Number of tillers plant^{-1}	Maturity (days)	Yield (t ha^{-1})	Standard heterosis (%)
IR69616A/BR1356-13-2-IR	105	18	105	6.0	–1.6
Bg CMS 1A/IR55838-32-2-3-2-2R	100	22	107	7.1	16.4
IR68275A/Bg 95-454	98	18	110	5.9	–3.3
IR68897A/Bg 95-276	90	17	103	6.2	1.6
IR58025A/P 2087-194-12-2R	97	16	102	5.8	–4.9
Bg CMS 2A/Bg 95388	98	17	104	6.3	3.3
IR77289A/HRSP 923	100	18	102	6.1	0.0
Bg CMS 2A/IR33380-7-2-1-3	100	21	100	7.4	21.3
Bg CMS 1A/BN 6986-108-2R	102	20	103	7.5	23.0
Bg CMS 1A/Bg 98-445	100	17	102	6.2	1.6
Bg CMS 4A/IR62030-97-3-2-2	98	18	104	6.3	3.3
IR69616/IR69702-32-1-3	102	22	105	8.1	32.8
IR80156A/IR68077-37-2-3R	98	20	100	7.9	29.5
IR77289A/HRSP 948	96	23	102	7.8	27.9
IR76768A/IR73012-2-2-2	98	21	105	7.5	23.0
Bg CMS 4A/At 95-10-4	99	24	102	8.2	34.4
Bg CMS 4A/R 49735-4-13-1	100	17	105	6.0	–1.6
Bg 407H (std.)	115	23	108	6.1	–

Released hybrids

In 2005, RRDI released its first rice hybrid variety, Bg 407H, for commercial cultivation with cultural management practices. Bg 407H matured in 115 to 120 days and had resistance to the major pests (brown planthopper, rice gall midge) and diseases (blast and bacterial leaf blight) found in the country. It also performs well in soils with iron toxicity in the low-country wet zone of Sri Lanka. It possesses excellent grain quality to fulfill consumers' and farmers' preferences. Around 200 ha of Bg 407H are now grown by farmers in high-potential areas under major irrigation schemes. The yield potential of this variety is 12 t ha^{-1}.

Seed production

The success of hybrid rice technology mainly depends on the yield advantage of the hybrid and the cost of seeds. Seed cost depends on seed yield and operational and input costs such as labor and GA_3. Seed yield mainly depends on outcrossing rate, which is highly influenced by environmental factors. One of the most difficult parts in hybrid rice technology is seed production. Currently, large-scale seed production (5 ha) has started at one government research station at Girandurukotte in the intermediate zone of Sri Lanka. The average seed yield is around 0.75 t ha^{-1}. Synchronization of flowering was found to be the most difficult part in seed production. Past results also showed that the dry/minor season (March-September) is favorable for seed production.

Agronomic studies on hybrid rice cultivation and seed production

To realize the full potential of hybrid rice or any other crop variety, the adoption of appropriate management practices is important. We have already developed agronomic management practices that include nursery management, establishment, and nutrient management. Research on areas such as flowering synchronization, row arrangements, etc., needs further improvement.

Effect of different establishment methods on the yield of hybrid rice variety Bg 407H

The high cost of seed is one of the problems in hybrid rice cultivation. Hence, methods for lowering seed cost are important. Seedling broadcasting and transplanting are two alternative methods to lower the seed paddy requirement. However, their production cost is still higher than that of direct seeding. In this study, we compared direct seeding with varying seed rates with other establishment methods (Table 3).

The highest yield (4.82 t ha^{-1}) was recorded from seedling broadcasting, followed by transplanting (4.66 t ha^{-1}). Line sowing (4.54 t ha^{-1}), row sowing (4.46 t ha^{-1}), and direct seeding at 25 kg ha^{-1} seed rate (4.57 t ha^{-1}) gave similar yield. Direct seeding at 100 kg ha^{-1} recorded the lowest yield (3.85 t ha^{-1}). Increasing seed rate decreased grain yield significantly. The highest yield (4.57 t ha^{-1}) occurred with 25 kg ha^{-1} and the lowest yield (3.85 kg ha^{-1}) with 100 kg ha^{-1} (the recommended seed rate for inbred rice). The same trend of yield in direct-seeding treatments was observed in the last season also. Grain-filling percentage and 1,000-grain weight were not affected by different establishment methods.

Table 3. Effect of different seed rates on grain yield and yield components of Bg 407H, wet season, 2007.

Treatment	Panicles m^{-2}	Yield (t ha^{-1})	Filled grains $panicle^{-1}$	Unfilled grains $panicle^{-1}$	Total grains $panicle^{-1}$	Filling (%)	1,000-grain weight (g)
Direct seeding (25 kg ha^{-1})	305	4.57	115	6	141	81.6	28.58
Direct seeding (50 kg ha^{-1})	339	4.33	110	25	135	81.4	28.73
Direct seeding (75 kg ha^{-1})	382	4.13	104	23	127	81.7	28.10
Direct seeding (100 kg ha^{-1})	342	3.85	95	24	119	80.1	28.65
Line sowing (15 × 20 cm)	370	4.54	105	30	135	77.9	28.38
Seedling broadcasting	344	4.82	113	20	133	85.0	28.60
Row seeding	347	4.46	91	20	111	82.3	27.95
Transplanting (20 × 15 cm)	326	4.66	117	30	147	79.5	27.93

This result suggests the possibility of adopting a lower seed rate (25 to 50 kg ha^{-1}) for direct seeding of Bg 407H. This seed rate is similar to that recommended for transplanting hybrid rice.

Seedling broadcasting/parachute method for crop establishment

Because of the high cost of rice production, more than 90% of the farmers in Sri Lanka practice direct seeding for rice crop establishment. But, to minimize seed cost (amount), hybrid rice has to be transplanted. This is one of the biggest problems for promoting hybrid rice in Sri Lanka as it requires additional labor for transplanting. The seedling broadcasting/parachute method for rice crop establishment has been introduced and recommended by the DOA as an alternative establishment method for manual transplanting to cut down on labor cost and seed cost. Studies showed that the labor requirement for transplanting and seed requirement could be reduced by around 50% and 25%, respectively, by adopting the seedling broadcasting method (Jayawardena and Abeysekara 2006).

Nursery seeding density and transplanting distance

Results show that nursery seeding density around 20 to 30 g m^{-2} and transplanting at 20 × 20-cm spacing were good for higher yields in hybrid rice (Jayawardena et al 2004).

Effect of different mulching materials on the quality of seedlings raised in a parachute nursery

A study on the improvement of nursery management practices in the parachute method found that covering the nursery with black polythene and transparent polythene had a more adverse effect on seed germination than covering it with newspapers, banana leaves, and Polysacks. Woven *cajans* were also good for covering nurseries.

Effect of row ratio and CMS density on F_1 seed yield

Low yield of CMS and F_1 seed is one of the major problems in hybrid rice seed production. Currently, we adopt the IRRI recommendation for CMS and F_1 seed production in which 3 rows of R/B lines and 10 rows of CMS lines are planted alternately (IRRI *Seed production manual*). However, we believe that the width of the CMS lines is too much. Therefore, studies were conducted to find ways to increase F_1 yields by changing the row ratios. Results revealed that row ratio is more important than CMS density. The lower the row ratio, the better the seed yield (4–6 for A lines:3 for R lines).

Row arrangement and early application of nitrogen fertilizer for F_1 seed yield

Currently, we adopt the IRRI recommendation for CMS and F_1 seed production in which 3 rows of R/B lines and 10 rows of CMS lines are planted alternately along with an early application of nitrogen fertilizer. However, low seed yield obtained in F_1 and CMS is still a problem. Therefore, we studied the effect of row arrangement of CMS and B lines with an early application of a high dose of N fertilizer on CMS seed yield. Results showed that leaving a border at the center of CMS lines instead of having a border between B and CMS lines gave around 16% more yield than the IRRI recommendation (Table 4). Early application of N fertilizer did not show much advantage over current practices (T1), except for T4, in which three split applications of N gave the lowest CMS seed yield.

A study on the leaf counting method to determine exact flowering dates

Flowering synchronization is one of the most difficult events in successful seed production. Therefore, knowledge of exact dates of flowering of parental lines is quite important. We now adopt visual observation of panicle initiation and flowering, which are highly influenced by climatic conditions. Therefore, flowering adjustment is difficult. Recently, we began a study of the possibilities to adopt the leaf counting method instead of visual observation as in China and some other countries.

Training and awareness

National and international linkage is very useful for developing human resources and improving infrastructure. Therefore, RRDI maintains a good relationship with international organizations such as IRRI and the International Hybrid Rice Research and Development Centre (INHRRDC) in Hunan Province of China. The government of China plays a vital role in human resource development. During the past few years,

Table 4. Effect of row arrangement and N fertilizer application on CMS yield (wet season, 2007).

N fertilizer management	CMS seed yield (kg ha^{-1})			
	Border between CMS lines (new method)	Border between B and CMS lines (IRRI method)	Average yield	Yield increase over check variety (%)
T1: 5%, 25%, 30%, and 40% N at 0, 2.5, and 7 WAT[a] (current practice)	506	586	546	15.8
T2: 20%, 20%, 20%, and 40% N at 0, 1, 3, and 7 WAT	524	604	544	15.2
T3: 40%, 20%, 20%, and 20% at 0, 2, 4, and 7 WAT	514	596	554	15.9
T4: 30, 30, and 40 at 0, 3, and 7 WAT	290	330	310	13.7
Average	458	529	488	15.5

[a] WAT = weeks after transplanting. Total N = 100 kg ha^{-1}.

the Chinese government has generously supported the training of many mid-level DOA officials on hybrid rice technology.

FAO/TCP

FAO granted a TCP for two years starting in 2007 on "strengthening national capacity for hybrid rice development and use for food security and poverty alleviation." During the project period, this work is expected to increase people's awareness of hybrid rice technology and the development of hybrid rice with high heterosis, and improve the skills of the officers involved in hybrid rice.

Constraints identified and suggested remedies for the successful development of a hybrid rice R&D program

The constraints identified for the successful development of a hybrid rice R&D program are summarized below:

1. Because of the cost of production, a majority of the farmers practice direct seeding. The high cost of hybrid rice seed prevents the direct seeding of hybrid rice. Therefore, one must find alternative cost-effective establishment methods for saving seed.
2. Difficulty in finding isolated large-scale fields for paddy seed production.
3. The high cost of GA_3 used for hybrid rice seed production. GA_3 accounts for 15–20% of the total cost. Therefore, finding alternative chemicals/other means for GA_3 is essential.

4. Limited yield heterosis in developed hybrids. The low genetic diversity used for hybrid rice development may be one of the problems. Hence, widening the genetic diversity of the materials used for hybrid rice development is essential.
5. Poor and inconsistent F_1 seed yield. We need to develop highly stable and highly adoptable hybrids using widely adaptable parental lines.

Future outlook

To overcome these constraints, the hybrid rice R&D program has been reoriented to

- Develop hybrids with acceptable grain quality to meet the specific requirements of consumers.
- Enhance the magnitude of heterosis to 20% and above by developing two-line and intersubspecific hybrids.
- Develop hybrids resistant to the major pests and diseases of target areas.
- Enhance seed yields beyond 1.5 t ha^{-1} to bring down F_1 seed cost.
- Promote seedling broadcasting/parachute method as an alternative establishment method for manual transplanting to cut down labor and seed costs.

In addition, technology dissemination has to be intensified through the conduct of a large number of on-farm demonstrations and training programs to increase awareness about the benefits of hybrid rice among farmers and consumers. Policy intervention by the government to increase support, aggressive efforts to popularize hybrid rice technology, and the assured procurement of hybrid rice seeds (F_1) at a subsidized price are needed at this stage. It is envisaged that, if the above constraints are overcome effectively, hybrid rice is likely to be cultivated on around 10,000 ha in high-potential areas in the future to help sustain food security in Sri Lanka.

Conclusions

During the past few years, the hybrid rice R&D program in Sri Lanka has shown remarkable progress under numerous constraints. Many activities exist to achieve the goals. The current activities of the hybrid rice R&D program in the country could have good success with the collaboration of IRRI and China's INHRRDC and with financial support from the FAO TCP.

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Authors' address: Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Sri Lanka; email: rice@sltnet.lk, rrdihybrid@yahoo.com.

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Research on and development of hybrid rice in Vietnam

Nguyen Tri Hoan

To develop new adaptable thermosensitive genic male sterile (TGMS) lines, a set of short-duration adapted varieties and available maintainer lines (Kim 23B, IR58025B, BoB, and II-32B) were crossed with available TGMS lines Peiai 64S, TQ 125S, 7S, and CN26S. The TGMS lines have been developed through pedigree selection from single crosses, from backcross generations, or through anther culture of F_1 plants of crosses between TGMS lines and inbred cultivars.

Several TGMS lines with stable male sterility, short duration, and high stigma exsertion have been developed. Known maintainer lines Kim 23B, IR58025B, II-32B, and BoB were converted into TGMS lines. These newly developed TGMS showed stability for male sterility. The two-line hybrid approach used these TGMS lines as female parents and these gave higher yield than the three-line hybrids. Additionally, several restorer lines (male parents) as well as TGMS lines having the *Wc* gene have been developed. These lines have been crossed with indica and japonica varieties to produce super hybrids (indica × japonica hybrids). Several indica-japonica hybrids have been developed and super high yields of some were recorded in experimental fields. Of the new TGMS lines developed in Vietnam, 103S , T1S-96, AMS30s, and P5s were exploited commercially. These lines were used as female parents of released two-line hybrid varieties such as VL20, TH 3-3, TH3-4, HC1, TH5-1, HYT102, and HYT103. For diversification of CMS, wild rice or CMS lines were crossed with identified maintainer lines. New CMS lines such as OMS1-2, AMS 71A, AMS72A, and AMS73A were developed. Several CMS lines were developed by continuously backcrossing existing CMS lines with newly developed maintainer lines. In 2001-05, 12 CMS lines and 10 TGMS lines were crossed with good male parents. Of the 8,130 test crosses made, 434 good crosses were identified. Some 134 promising hybrids were selected for primary and regional yield trials. Several hybrids—HYT83, HYT92, HYT100 (three-line hybrids), Vl20, TH3-3, Th3-4, HC1TH5-1, HYT102, and HYT103 (two-line hybrids)—have already been developed and released for commercial production in Vietnam. For quality, hybrid rice developed in Vietnam has better eating quality than existing hybrids. An F_1 seed production package for each released hybrid was developed by the different research institutes and was used by seed companies or cooperatives involved with the F_1 seed production program of MARD. The average yield of F_1 seeds in Vietnam was 2 t ha^{-1}. A total of 1,500–2,000 ha are used for hybrid seed production every year, producing 3,000–4,000 tons of hybrid rice. However, hybrid rice seed area has decreased in recent years. For commercial rice production, hybrid rice has been planted on about 600,000–650,000 ha per year in Vietnam, with a yield average of 6.0–6.5 t ha^{-1}.

Keywords: CMS, TGMS, hybrids, maintainer lines

The area for rice cultivation in Vietnam has decreased rapidly in recent years—from 4.5 million ha to around 4.0 million ha annually. In general, two rice crops are cultivated in the northern provinces, while two to three crops can be grown in the southern provinces.

In 2007, Vietnam produced around 35.9 million t of paddy from the 7.2 million ha devoted to rice. The average yield of rice in Vietnam was 4.98 t ha^{-1} (Statistical Yearbook of Vietnam 2007).

For the food security of 86 million people in the country and to maintain exports of 4–5 million t of milled rice to the world market, Vietnam plans to keep 4 million ha of land for rice cultivation and produce about 40 million t of paddy every year. To reach this rice production target, the development and use of hybrid rice has been considered as a readily available approach.

Recent achievements in hybrid rice research

Developing new tropical TGMS lines in Vietnam

To create new tropical TGMS lines adapted to tropical conditions, adapted cultivars were crossed to available TGMS lines and new TGMS lines were selected in segregating generations. The new TGMS lines have stable pollen sterility under critical temperatures of 23–24 °C, and are uniform in phenotype with good combining ability and floral characteristics. These were selected and used for making new hybrid rice combinations. The new TGMS lines created by the different research institutions are presented in Table 1.

Among the newly developed TGMS lines, T1S-96, 103s, AM30s, and P5s were used as female parents of the released two-line hybrids (VL20, TH3-3, TH3-4, HC1, TH5-1, HYT103, HYT102, and VL24). The other TGMS lines (P5S, AMS29S, AMS30S TGMS1, and TGMS20) were used as female parents of promising two-line hybrids such as HYT106, HYT107, LHD5, and LHD6.

Developing new TGMS lines based on genotype of parental lines (maintainer lines and restorer lines of adapted three-line hybrids)

To overcome the instability of several CMS lines such as II-32A, IR58025A, and Jin 23A, some CMS maintainer lines such as II-32B, IR58025B, Jin23B, BoB, Zhenshan

Table 1. Available TGMS lines developed and used in Vietnam, 2001-05.

Line	Source	Institution	Critical temperature (°C) (fully sterile)	Critical temperature (°C) (fertile)	Level of use
T1s-96, 103s	T1S/Peiai64	HAU	25.5	22–23	Commercial
P5s	T1S/Peiai64	HAU	25 and daylength of 12 h 16 min	≤24	Commercial
P47S	Selected from Peiai64S	HAU	25	≤24	For breeding
AMS27S(7)	TGMS/inbred	VASI	25	23.5	For breeding
AMS 28S	TGMS/inbred	VASI	25	23.5	For breeding
AMS 29S	Selected from segregating material	VASI	24.5	23	Promising variety
AMS 30S	Selected from segregating material	VASI	24.5	23	Commercial
AMS26S (CL64S)	Selected from Peiai 64S	VASI	24.5	23	For breeding
AMS31S	CL64S/VN292-2	VASI	25.5	≤23	For breeding, promising variety
AMS33S	CL64S/BM9820-11	VASI	25.5	≤23	For breeding
TGMS1	Cross	FCRI	25	≤24	For breeding LDH5, LDH6
TGMS20	Pollen culture				
TGMS6/Xi23	FCRI	25	≤24	For breeding promising LDH4	
D101S	VN1/DT12	AGI	>24	≤24	For breeding
D102S	VN1/DT12	AGI	>24	≤24	For breeding
D103S	VN1/DT12	AGI	>24	≤24	For breeding
TGMS18-2	H84/CR203	AGI	>24	<24	For breeding
TG1	103S/Peiai64S	Vanlam			For breeding
TG22	TGMS2/R15	Vanlam			For breeding

Table 2. Pollen sterility of newly developed TGMS lines observed in the summer season.

TGMS line	Original cross	Pollen sterility (%)	Type of pollen sterility	Average temperature (°C)
Bos	TG125S/BoB	100	WA	27.6–30.8
255-1	TG125S/25B	100	No pollen	26.2–30.8
25S-2	TG125S/25B	99–100	WA	26.2–30.8
II32S	7S/II32B	100	WA	26.2–30.8
Kim S	7S/Kim23B	100	WA	26.2–30.8
II32S	CN26S/II32B	98–100	WA	26.2–30.8

97B, and restorer lines such as Fuhui838, 827R, and Gui 99 were converted to TGMS lines. These parental lines are characterized by short duration, short plant height, good combining ability, and adaptability to local conditions. These parental lines were used in crosses with four TGMS lines—Peiai64S, 7S, CN26S, and TG125S—and successively backcrossed to a recurrent male parent up to the BC_2F_1 and BC_3F_1 generations before selecting pure TGMS lines. The work was done in the spring of 2002. A total of 735 TGMS lines were isolated and screened under low natural temperature (9–15 °C) for 1 week.

The results of the study involving 426 TGMS lines showed good cold tolerance and these lines were further screened to develop new TGMS lines. The first set of TGMS lines based on genotypes of maintainer lines was selected in the F_8 of the BC_2 generation. These newly developed TGMS have genotypes as well as phenotypes similar to corresponding CMS B lines (IR58025A, BoA, Kim 23A, and II-32A). For convenience, they were named as 25s, Bos, Kim23s, and II32s, respectively.The pollen and growth characters of these TGMS are presented in Tables 2 and 3.

These new TGMS lines were selected and used to cross with good restorers of the original three-line hybrids as well as with new, high-yielding inbred lines. In the summer of 2005, several highly heterotic hybrids were identified among 86 hybrids.

To show the advantage of the newly developed TGMS lines based on available CMS lines, the corresponding restorer lines RTQ5, R100, and PM3 of the existing three-line hybrids HYT83, HYT100, and HYT92 were crossed with 25s, a new TGMS line similar to IR58025A. IR58025A is the female parent of HYT83, HYT100, and HYT92. Results showed that the yield components and yields of the two-line and three-line hybrids with the same male parent have similar phenotype; however, the two-line hybrids had more spikelets per panicle and lower percentage of sterility than the three-line hybrids (Table 4).

The results of the test crosses confirmed the success of new TGMS line development using maintainer lines from the three-line hybrid system. This opens up opportunities to replace three-line hybrids with two-line hybrids to overcome several

Table 3. Characteristics of newly developed TGMS lines derived from existing CMS lines.

TGMS line	Duration from seeding to flowering (days)	Plant height (cm)	Panicles hill^{-1} (no.)	Spikelets panicle^{-1} (no.)	Stigma exsertion	Color of stigma
25S-2	87	65.3	7.4	194.0	Good	White
25S-2	87	73.7	7.0	227.7	Good	Black
II32S	85	77.0	8.0	173.7	Very good	Black
BoS	72	67.7	8.5	156.0	Very good	Black
Kim23S	72	63.3	6.6	197.3	Very good	Black

Table 4. Yield components of newly developed two-line hybrids compared with those of original three-line hybrids.

Type of hybrid	Panicles hill^{-1} (no.)	Spikelets panicle^{-1} (no.)	Percentage of sterile seed (%)	1,000-grain weight (g)	Weight of five plants (g)	Yield (t ha^{-1})
HYT100-2 lines	7.2	219.3	8.4	28.6	159.0	10.4
HYT100-3 lines	6.0	170.0	21.0	28.0	101.0	6.7
HYT83-2 lines	6.4	239.3	11.3	22.2	122.0	8.1
HYT83-3 lines	6.6	185.3	19.4	23.3	99.3	6.6
HYT92-2 lines	7.4	244.3	9.6	24.8	160.0	10.6
HYT92-3 lines	6.0	206.0	18.4	24.6	110.0	7.3

limitations—e.g., instability of pollen sterility in II-32A and medium stigma exsertion of IR58025A. In addition, new TGMS lines based on B lines require no restorer genes in their male parents. Therefore, there are more chances of breeding high heterosis into two-line hybrids than with existing three-line hybrids.

Development of parental lines having the *Wc* gene

The conventional high-yielding varieties are adapted to Vietnamese conditions so varieties such as Xi23, Q5, Chiem77, R242, and BM9855 were used as male parents in crosses involving donors having the *Wc* gene—Peiai 64S, N22, Palawan, Dular, Calotoc, Lambayeque 1, and Moroberekan. Single crosses were made and selection for parental lines followed the steps: (1) select fertile plants in segregating generations of male parents having the *Wc* gene; (2) select sterile plants in segregating generations of single crosses or in backcrossing generations. Sterile plants at the F_6BC_1 generation were used and test-crossed with an indica/japonica check to identify female parents having the *Wc* gene in the spring and summer of 2005. Eight uniform TGMS lines having 100% pollen sterility were selected. The selected TGMS lines have good stigma

Table 5. Yields obtained from promising super hybrid rice (indica/japonica), spring 2008, Ankhanh, Hoai Duc, Hatay, Vietnam.

Cross	Plant height (cm)	Growth duration (d)	Yield (t ha^{-1})	Type of cross
D64S/RV126	102	97	11.44	Indica/japonica
D66-1/R838	103	105	10.31	Indica/indica
D59-1/R838	100	99	10.06	Indica/indica
D60-3/RV126	98	91	9.93	Indica/japonica
D59-1/R725	104	101	9.88	Indica/indica
D59-4/RV126	104	97	9.72	Indica/japonica
D59-4/RV114	105	105	9.54	Indica/japonica
D52-5/RV114	106	101	9.41	Indica/japonica
AMS30S/R8	105	97	9.62	Indica/indica
D59-1/R253	105	105	9.00	Indica/indica
33S/R1	104	95	8.87	Indica/indica
31S/R36P	106	98	8.88	Indica/indica
HYT83	105	98	8.22	Indica/indica
Er you 838	106	97	7.42	Indica/indica

exsertion, short duration, and good phenotype acceptability. TGMS lines with the *Wc* gene were produced and were named D59, D60, and D28.

From 80 test crosses, several promising hybrids were identified. The yields of these hybrids are presented in Table 5.

Developing TGMS lines through anther culture of F_1 from TGMS lines/inbred varieties

Anther culture was used to develop TGMS lines from crosses between TGMS lines and inbred varieties (including maintainer lines of the three-line system). Anthers of F_1 generations of 18 hybrids were used in anther culture. TGMS lines with stable pollen sterility were isolated. Six TGMS with good growth characteristics—short duration, 100% pollen sterility, and good stigma exsertion—were selected for use in the breeding program. With anther culture, new TGMS lines can be developed within 2 years compared with 4–5 years using pedigree selection.

Developing new maintainer lines for new CMS lines

To develop new CMS lines, 17 good maintainer lines with short duration, short stature, good adaptability to Vietnamese conditions, tolerance of diseases, and good combining ability were selected from a test-cross nursery. Completely male sterile plants were selected for backcrossing to their corresponding CMS lines.

The backcrossing populations were in different states. From 12 crosses, three completely sterile CMS lines were developed—OM1-2A, AMS 71A, and AMS 72A. These new CMS lines have been used for test crosses in the hybrid rice breeding program. The remaining backcross generations from BC_4F_1 to BC_7F_1 showed 60–96.5% of the population with complete pollen male sterility. The percentage of completely pollen sterile plants increased gradually in later backcrossing generations.

To diversify and improve maintainer lines for use in three-line hybrid rice breeding, seven available maintainer lines were crossed in pairs, with the objective of developing new maintainer lines with short duration, good phenotype, good tillering, high stigma exsertion, and better quality of CMS lines. The plants of the F_1, F_2, and F_3 generations of six crosses involved eight maintainer lines as parents, and they were selected following the bulk method. In the F_4 generation, 20–25 individual plants were selected for backcrossing in pairs with the corresponding individual plants of each CMS line.

From the F_5 generation, 132 promising lines were selected and 38 lines were crossed in pairs again with the corresponding CMS line. Twenty-four lines were found to be good maintainers for pollen sterility of CMS (WA cytoplasm). Backcrossing was continued to convert these promising maintainer lines into new CMS lines. Several new CMS lines have uniform and complete pollen sterility.

Results of developing new hybrid rice combinations for commercial hybrid rice production

Twelve stable male sterile CMS lines, 10 TGMS lines, and 2,000 male parents (inbred lines and varieties) were used in test crosses to develop two-line and three-line hybrids for Vietnam. A total of 8,130 test crosses were made during 2001-05 and 434 crosses were selected for re-test-crossing at the Hybrid Rice Research Center. Seed production of 340 selected hybrids was done using the IRRI-developed isolation-free method. A total of 481 rice hybrids were evaluated in observation yield trials and 134 promising hybrids were selected for primary yield trials. About 15–18 promising hybrids were selected each year for testing in the National Hybrid Rice Yield Trials (NHYT), conducted under different ecological conditions in 6–9 locations.

Promising two-line hybrids identified in the National Multilocation Yield Trial and the National Testing Program of MARD are presented in Table 6.

For quality aspects, studies on percentages of milling rice, head rice, length and width of grain, amylose content, etc., showed that hybrid rice is similar to the inbred check. However, the amylose content of hybrid rice is in a lower range (18–20%). For eating quality, hybrid rice lines HYT100 and HYT92 were evaluated to have very good quality with good aroma. HYT83 and TH3-3 likewise have good eating quality.

The high-yielding hybrids with wide adaptability, good quality, and tolerance of diseases and insects were selected and released for commercial rice production. The released hybrids were HYT83, HYT92, and HYT100 (three-line hybrids) and VL20, TH3-3, TH3-4, HC1, HYT102, and HYT103 (two-line hybrids). Several promising two-line hybrids such as VL1, HYT106, HYT107, TH3-5, LHD5, and LHD6 and

Table 6. Average yield of some promising combinations in the National Hybrid Rice Yield Trials in 6–9 locations.

Season	Good nominated combinations	Average yield (kg ha^{-1})	Remarks	Type of hybrid
Spring 2003	HYT100	6,837	Very good quality	Three-line
	HYT83	6,752	Good quality	Three-line
	HYT92	6,651	Very good quality	Three-line
	TH3- 3	5,591	High seed yield	Two-line
	Eryou 838 (check)	6,501	Chinese hybrid	Three-line
Spring 2004	HYT83	7,708	Good quality	Three-line
	Dyou 527	7,700	Chinese hybrid	Three-line
	HYT100	7,445	Very good quality	Three-line
	CV1 (Peiai64S/9311)	7,481	Chinese hybrid	Two-line
	HYT92	7,120	Good quality	Three-line
	HYT88	7,251	–	Three-line
	Eryou 838 (check)	6,985	Chinese hybrid	Three-line
Spring 2005	Dyou527	7,330	Chinese hybrid	Three-line
	HYT102	7,077	Short duration	Two-line
	HYT100	6,757	Short duration	Three-line
	Eryou 838 (check)	6,347	Chinese hybrid	Three-line

three-line hybrid HYT105 have been identified for inclusion in farmers' field demonstrations (Hoan 2003,Tram 2005).

The advantages of two-line hybrids developed in Vietnam are short duration and suitability for late spring and early/very early summer rice cropping. Most of the existing three-line hybrids are not adapted for growing in early summer. In addition, seeds of two-line hybrids are easily produced, obtaining 2.5–3.0 t ha^{-1}, compared with only 1.5–2.0 t ha^{-1} for the three-line hybrids (HYT83, HYT100, HYT92). The rice hybrids used for commercial production are shown in Table 7.

Studies on disease resistance

Twenty-seven parental lines of two- and three-line hybrids were screened against 10 races of *Xanthomonas oryzae* pv. *oryzae* isolated from IRRI. A reaction of parental lines to the disease was reported (Lang et al 2003). The data showed that II-32B was resistant to 10 races; Peiai 64 and Son thanh were resistant to nine races. Four parents (IR78595A, IR75601A, II-32A, and IR68885B) had resistance to eight races. The parents with resistance to seven races were BoA, IR73328A, and Buc Khoi 838. It is important to note that only 12 among 27 varieties showed resistance to race 3. Studies have shown that races 2 and 3 are the most prevalent in the northern provinces of Vietnam.

Table 7. Hybrid rice varieties released for commercial production in Vietnam.

Hybrid	Season	Source	Yield (t ha^{-1})	Year of release
Boyou 903 (three-line)	Summer	China	6–8	1996
Bo you 253 (three-line)	Summer	China	6–8	2004
Shan you 63 (three-line)	Spring	China	7–9	1992
Er you 838 (three-line)	Spring	China	7–8.5	2000
Er you 63 (three-line)	Spring	China	7–8 .5	2000
Dyou 527 (three-line))	Spring	China	7–9	2001
CNR36 (three-line)	Spring	China	7–9	2006
Van quang 14 (two-line)	Summer	China	6–8	2006
VL 20 (two-line)	Summer, spring	Vietnam	6–8	2002
HC1 (two-line)		Vietnam	6–8	2005
VL24 (two-line)	Summer	Vietnam	6–7	2006
TH3-3 (two-line)	Summer, spring	Vietnam	6–8	2003
TH3-4 (two-line)		Vietnam	6–8	2005
HYT102 (two-line)	Summer, spring	Vietnam	7–9	2007
HYT103 (two-line)	Summer, spring	Vietnam	7–9	2007
TH5-1 (two-line)	Spring	Vietnam	7–8	2006
HYT83 (three-line)	Summer, spring	Vietnam	7–9	2003
HYT92 (three-line)	Summer, spring	Vietnam	6.5–8	2005
HYT100 (three-line)	Spring	Vietnam	7–9	2005
B-Te1 (three-line)	Summer	India	6–9	2007
Thuc Hung 6 (three-line)	Spring, summer	China	7–9	2007

We used the PCR technique to find a linkage with *xa13*, *xa5*, and *Xa21*. No correspondence to genes *xa5* and *Xa21* was found among the 27 parental lines. However, using marker RG 136, with resistant check IRBB13 and susceptible check IR24, we found that three varieties, Son Thanh Re, IR68897B, and IR75601B, had a 1,500-bp resistance band of IRBB13.

The standard lines with known resistance genes from IRRI were screened for 11 races of the bacteria. The data showed that four lines—IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB7 (*Xa7*), and IRBB21 (*Xa21*)—have good resistance to 6–9 races of the bacteria that cause bacterial leaf blight. However, *xa5*, *Xa7*, and *Xa21* are very important genes that must be incorporated into the parental lines of rice hybrids in Vietnam.

The 66 F_1 plants from test crosses between IRBB4, IRBB5, IRBB7, IRBB11, IRBB13, IRBB21, Nep, Trum bong, and several male parents were screened against 11 races of bacterial leaf blight. The results showed that only eight hybrids were resistant to 8–11 races of the disease. Among the eight hybrids, two involved IRBB4 having a dominant gene (*Xa4*). One hybrid involved IRBB 21 (*Xa21*). The remaining five hybrids involved recessive genes *xa7*, *xa11*, and *xa13*. The study suggests that CMS or TGMS lines need to acquire *Xa4*, *Xa7*, *xa11*, *xa13*, and *Xa21* to develop hybrid rice with resistance to bacterial leaf blight.

The best restorer lines (such as Ce64, Gui99, RTQ5, R242, 827R, Minghui 63, and R253) have been used as female parents in crosses with IRBB4, IRBB5, IRBB7, and IRBB21. The F_1s were successively backcrossed with a corresponding restorer. New restorer lines with bacterial leaf blight resistance genes *Xa4*, *xa5*, *Xa7*, and *Xa21* have been developed for use in hybrid rice breeding in Vietnam. Restorer lines with *Xa21* have been used as pollen parental lines of VL24 (two-line hybrid) and Nanyou 2 (three-line hybrid). These hybrids showed moderate resistance to bacterial leaf blight in northern Vietnam. Work is focusing on transferring 2–3 genes (*Xa4*, *xa5*, *Xa7*, and *Xa21*) into newly developed parental lines for breeding resistance to bacterial leaf blight–resistant hybrid rice in Vietnam.

Hybrid rice seed production

To spread seed production technology in Vietnam, an in-country training program was organized to teach seed technology to seed growers through the help of different organizations such as the local extension department, the Hybrid Rice Research Center, and the ADB/IRRI project.

Because of training activities on seed production, the area for producing hybrid seeds increased from 267 ha in 1996 to 620 ha in 2001. Yield and production of hybrid seeds also increased from 1.75 to 2.3 t ha^{-1} and from 467.5 t to 1,426 t in the same period, respectively. In 2001, about 1,400 ha of rice land was used for hybrid seed production. In some areas, more than 2 t ha^{-1} seed yield could be obtained, but unfavorable climate conditions in 2001 reduced yield to 1.7 t ha^{-1}. From 2002 to 2007, Vietnam used 1,500–2,000 ha for production; average yield was 2,000 kg.

F_1 seed production in Vietnam decreased in recent years for the following reasons:

- Subsidy from the government declined (\$250 ha^{-1}) (about 10% of seed production cost per ha) while the cost of fertilizers, chemicals, and labor increased.
- Crop damage occurred brought about by climate change, leading to less benefits for seed growers.
- The cooperative system involved in F_1 seed production made it difficult to sell and distribute F_1 seeds as goods in commercial markets.
- A new approach for hybrid seed production was implemented in Vietnam.
- Private or joint-venture foreign seed companies purchased breeder rights and exclusive rights to produce and sell hybrid rice seeds. The rights to hybrids VL20, TH3-3, TH3-4, and HYT103 have been bought by four seed companies (three Vietnamese and one Chinese).

- The Ministry of Agriculture and Rural Development set a policy to support every seed company to carry out seed production on areas of more than 100 ha. The support includes a subsidy of US\$250 ha^{-1}, and support funding to develop irrigation systems, maintain dryers, and conduct training.

Commercial hybrid rice production

Hybrid rice was planted on about 100 ha in Vietnam in 1991. The area covered by hybrid rice increased substantially to 200,000 ha in 1998, to 480,000 ha in 2001, and to 600,000 ha in 2003. Hybrid rice cultivation is now spreading to the south-central coast, central highlands, and Cuu Long Delta in addition to the 31 northern provinces. In general, yield of hybrid rice is 6.3–6.5 t ha^{-1}, higher than the national average of about 5.0 t ha^{-1}.

Issues identified for future research and development of the technology

- Lack of a strong local seed production system that involves both the public and private sector.
- Unsuitable conditions for hybrid rice seed production in the north, where hybrid rice is mostly cultivated to date, due to erratic climate and late harvesting time. There is a need to develop alternative seed production sites.
- Except for the Hybrid Rice Research Center, other R&D institutes are not well equipped to take the responsibility for nucleus and breeder seed production of locally developed or imported hybrids.
- Reluctance of small farmers to produce hybrid rice seeds locally because of greater risk; very high financial requirement; lack of proper warehouses, space, and cold storage for unsold seed; unavailability of pure CMS lines; and farmer preference for imported seeds.
- Lack of hybrid rice combinations with good grain quality, tolerance of pests and diseases, and short duration (105–115 days) that can meet the requirements of the various agroecological zones of the country.

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Notes

Author's address: Field Crops Research Institute, Vietnam Academy of Agricultural Sciences.

Yield advantage of hybrid rice over conventional and Clearfield® long-grain rice in the southern United States

Xueyan Sha, Steve D. Linscombe, S. Brooks Blanche, and Donald E. Groth

Hybrid rice has expanded rapidly in the U.S. since its first commercialization in 2000 by RiceTec Inc. We will compare the performance of hybrid rice with that of leading inbred cultivars in grain yield, milling recovery, disease resistance, lodging susceptibility, and other agronomic characteristics under U.S. conditions.

Hybrid rice technology and production have expanded rapidly in the southern United States since its first commercialization by RiceTec Inc. in 2000. The majority of the hybrid rice cultivars employ Clearfield® (CL) technology that offers the first-ever selective control of noxious weedy red rice. Since there are limited public reports on the yield advantage of hybrid rice over inbred cultivars under southern U.S. conditions, field research was conducted at five Louisiana locations during 2006 and 2007 to evaluate the grain yield, milling quality, maturity, plant height, reactions to diseases, and lodging susceptibility of the latest hybrids, along with predominant conventional long-grain (LG) and CL inbred cultivars. Our results indicated that hybrid rice had a 15.9% to 39.2% grain yield advantage over inbred cultivars. The average yield of commercial hybrids was 9.97 and 9.87 t ha^{-1} compared with 7.99 and 7.09 t ha^{-1} for CL cultivars and 8.60 and 7.50 t ha^{-1} for LG cultivars, respectively. However, head rice recovery of hybrids was 1.4% to 7.0% lower than that of inbred cultivars. Hybrid rice had similar maturity but was 16 to 20 cm taller than inbred cultivars and more susceptible to lodging. Nevertheless, hybrid rice had higher resistance to both sheath blight and bacterial panicle blight.

Background

Hybrid rice and Clearfield® rice are two technological breakthroughs that have revolutionized southern U.S. rice production since early 2000. Through collaboration with the China National Hybrid Rice Research and Development Center (Hunan, China), RiceTec Inc. (Alvin, Texas) commercialized the first three-line rice hybrid (XL6) for the southern U.S. in 2000, which has since been replaced with two-line hybrids (Walton 2003). On the other hand, imidazolinone-resistant CL rice was invented at the Louisiana State University AgCenter Rice Research Station, Crowley, Louisiana, USA, by chemical mutation combined with conventional breeding procedures (Croughan 2003). Combined with NewPath herbicide (*Imazethapyr*), CL rice allows selective control of red rice in a commercial rice field for the first time ever. In 2003, the first CL hybrid, CLXL8, was commercialized by RiceTec (Walton 2003). Both hybrids and CL rice have been rapidly adopted by rice growers throughout the southern rice-growing states since 2002. It was estimated that, in 2007, LG, CL, CL hybrid, and conventional hybrid rice were planted on 60%, 12%, 11%, and 7% of the southern U.S. rice area, respectively (Saichuk J, personal communication, 2008; Buehring NW, personal communication, 2008; Wilson CE, personal communication, 2008).

The yield advantage of hybrid rice over inbred cultivars has been well documented in China, the Philippines, and other Asian countries, where rice is produced on small farms and involves extensive labor (Virmani 2005, Yang et al 2007). However, limited public information is available regarding the yield advantage of hybrids over inbred cultivars in the southern United States (Ottis and Talbert 2005, Sha and Linscombe 2007, Walker et al 2008). Hybrids developed in the United States by RiceTec, Inc. are indica/japonica hybrids because their pollen parents are derived from U.S. long-grain germplasm, which taxonomically belongs to the tropical japonica (Ni et al 2002). More than five hybrids were being marketed by RiceTec, Inc. during 2006-07 (Anonymous 2008). Nevertheless, all previous yield comparison studies conducted in the U.S. involved only a single hybrid and a few inbred cultivars. Thus, this study was carried out to compare hybrid rice with leading inbred cultivars (CL and LG cultivars) across years and locations for grain yield, milling yield (head and total milled rice), plant height, lodging susceptibility, maturity, and disease reactions to sheath blight and bacterial panicle blight.

Materials and methods

Four hybrids were tested in 2006 and five in 2007 (Fig. 1). All hybrids were long-grain and those with a "CL" prefix are CL hybrids. In both 2006 and 2007, three CL inbred cultivars and four LG cultivars were also tested. All the hybrids and inbred cultivars were widely grown and/or recently released, which represented the highest yield potential available (Saichuk J, personal communication). Field experiments were carried out at three southwest Louisiana locations and two northeast Louisiana locations in both 2006 and 2007, resulting in a total of 10 year × location environments. A completely randomized block design was applied with three blocks for each test location. Seeding rates were 112 kg ha^{-1} for inbred cultivars and 36 kg ha^{-1} for hybrids. Test plots with seven rows 4.8 m long and 0.19 m apart were drill-seeded. The rice was fertilized at 154 kg N ha^{-1} by broadcasting on the dry soil surface preflood. Neither fungicides nor plant growth regulators were applied. Data collection was conducted by following the Standard Evaluation System (IRRI 2002). Sheath blight and bacterial panicle blight severity were rated at the Lake Arthur location under natural infections. This location has served as a test site for more than 20 years and consistently has high disease pressure. Milling tests were conducted by following the procedure described by Sha et al (2007). All tests were harvested by a plot combine, and grain yield was estimated based on 120 g kg^{-1} moisture content. The Mixed Model procedure of SAS version 9.0 was used in the data analysis (SAS Institute, Cary, NC). Because of the different genotypes being tested, separate analyses were carried out for 2006 and 2007 data, as well as for individual cultivar/hybrid and three cultivar types (hybrid, CL cultivar, and LG cultivar). Cultivar, cultivar type, and location were treated as fixed effects, while replication and any interaction involving replication were treated as random effects. All fixed effects and their interactions were tested by Type III statistics, and a significance level of 0.05 was used for mean separation.

Results and discussion

In both 2006 and 2007, hybrid rice cultivars yielded significantly ($P<0.01$) higher than leading commercial CL and LG rice cultivars (Fig. 1). The average grain yield of hybrids was 9.97 t ha^{-1} compared with 7.99 and 8.60 t ha^{-1} for CL and LG cultivars, respectively, which was equivalent to a 24.8% and 15.9% yield advantages. The yield gaps broadened in 2007, when hybrids produced 39.2% and 30.7% higher grain yields than CL and LG cultivars, respectively (Table 1). In 2006, XL723 produced the highest yield of 11.06 t ha^{-1} among all hybrids and inbred cultivars, which was 1.79 t ha^{-1} more than the highest-yielding inbred cultivar, Wells. CL hybrid CLXL730 yielded slightly lower than XL723 but was about 1.97 t ha^{-1} higher than the highest CL cultivar, CL151. In 2007, CLXL730 had the highest yield of 10.08 t ha^{-1} among all hybrids and inbred cultivars and had a 2.52 t ha^{-1} advantage over the highest-yielding CL cultivar, CL151 (Fig. 1).

Hybrids in general had lower head rice yields than inbred cultivars, but the difference was significant ($P=0.0573$) only in 2006 (Table 1). The average head rice yield of hybrids in 2006 across locations and genotypes was 45 g kg^{-1} (7.0%) and 26 g kg^{-1} (4.2%) less than CL and LG cultivars, respectively. However, the head rice yield difference between hybrids and inbred cultivars was much smaller in 2007, when hybrids produced 34 g kg^{-1} (5.5%) and 8 g kg^{-1} (1.4%) less head rice than CL and LG cultivars (Table 1). In 2006, the best milling hybrid (XL723) produced 26 g kg^{-1} less head rice than the best milling LG cultivar, Cheniere (Fig. 1). CLXL730 had an average head rice yield of 604 g kg^{-1}, which was 51 g kg^{-1} less than the best milling CL cultivar, CL161. In 2007, CL131 had the highest head rice yield of 632 g kg^{-1} among all genotypes, which was 33 g kg^{-1} higher than the best milling hybrid, XL723, and 38 g kg^{-1} better than the best milling CL hybrid, CLXL729 (Fig. 1). No significant differences in total milled rice yield were detected between different cultivar types (Table 1).

Differences in maturity measured by days to 50% heading between hybrids and inbred cultivars were negligible (Table 1). On average, hybrids headed 0.9 day earlier than CL cultivars but 1.5 days later than LG cultivars in 2006. However, in 2007, hybrids were 0.5 and 2 days earlier than LG and CL cultivars, respectively. Nevertheless, hybrids were significantly ($P<0.001$) taller than inbred cultivars (Table 1). The average plant heights of hybrids were 110 and 117 cm in 2006 and 2007, respectively, which were 20 and 20 cm taller than CL cultivars and 16 and 19 cm taller than LG

Table 1. Grain yield, head rice recovery, total milled rice, days to 50% heading, plant height, sheath blight reaction, bacterial panicle blight reaction, and lodging incidence of hybrid, Clearfield® long-grain, and conventional long-grain cultivar types in Louisiana, USA, 2006-07.

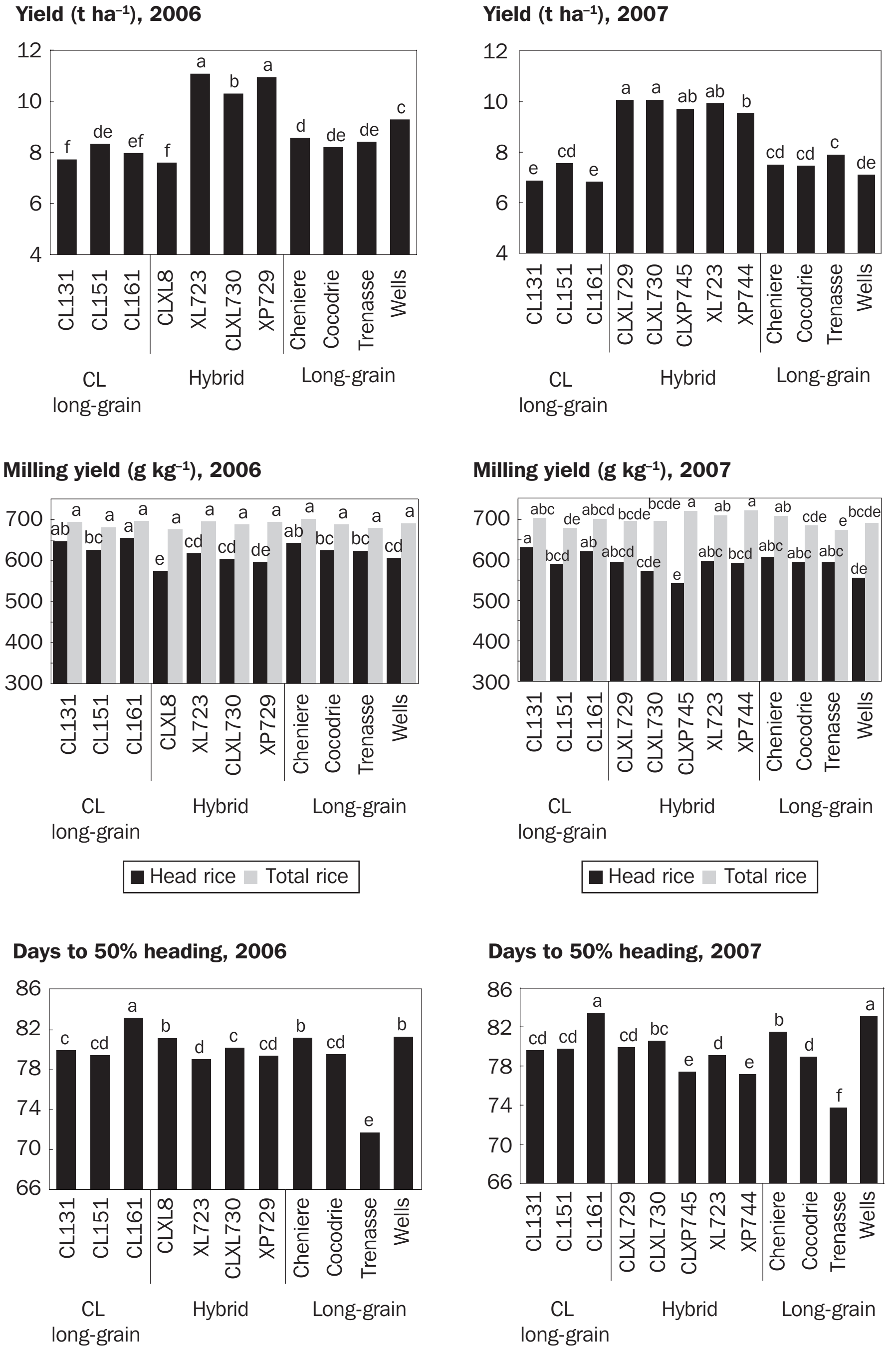
Cultivar type	Yield (t ha^{-1})	Head rice (g kg^{-1})	Total rice (g kg^{-1})	Days to 50% heading	Plant height (cm)	Lodging incidence (%)	ShB[a]	BPB[a]
2006								
Hybrid	9.97 a[b]	598 b	688 a	80.0 a	110 a	26 a	5.8 b	na[c]
CL	7.99 b	643 a	691 a	80.9 a	90 c	0 b	7.1 a	na
LG	8.60 b	624 ab	690 a	78.5 b	94 b	2 b	6.2 b	na
2007								
Hybrid	9.87 a	580 a	710 a	78.8 b	117 a	na	6.0 b	1.8 b
CL	7.09 c	614 a	694 a	80.9 a	95 b	na	7.8 a	5.6 a
LG	7.50 b	588 a	690 a	79.3 ab	98 b	na	7.4 a	5.0 a

[a] 0–9 rating, 0 = no disease and 9 = maximum disease. [b] Means followed by the same letter are not significantly different at $P \leq 0.05$. [c] na = not analyzed.

cultivars, respectively. Lodging is the most important constraint to achieving high grain yield in a high-input and highly mechanized rice production system (Islam et al 2006, Walton 2003). Significant lodging occurred in 2006 but not in 2007, even though rice plants were much taller in 2007 (Table 1). Averaged across genotypes and locations, hybrids showed a 26% lodging incidence compared with 0 and 2% for CL and LG cultivars, respectively (Table 1).

Sheath blight was more severe in 2007 than in 2006 (Table 1). The average sheath blight ratings of hybrids were 5.8 and 6.0 compared with 7.1 and 7.8 of CL cultivars and 6.2 and 7.4 of LG cultivars for 2006 and 2007, respectively. Significant bacterial panicle blight incidence was observed in 2007 (Table 1). Again, hybrids were significantly less susceptible than inbred cultivars. The average rating of hybrids was 1.8 compared with 5.6 and 5.0 of CL and LG cultivars, respectively. All five hybrids had significantly lower bacterial panicle blight ratings than any inbred cultivars (Fig. 1).

A 15.9% to 24.8% yield advantage of hybrid rice found in 2006 was similar to those reported in China and other countries. However, in 2007, a yield advantage of hybrid rice over inbred cultivars was much higher than that in 2006. Since there was no fungicide being used in this research, such an elevated yield advantage might be partially attributed to severe sheath blight and bacterial panicle blight infections that substantially reduced the grain yields of inbred cultivars. Groth and Bond (2007) reported that, under artificially inoculated conditions, yield loss to sheath blight increased from 4% in the moderately susceptible cultivar Francis to 21% in the very susceptible cultivar Cocodrie. Replacement of the older hybrid CLXL8 with newer hybrids, such



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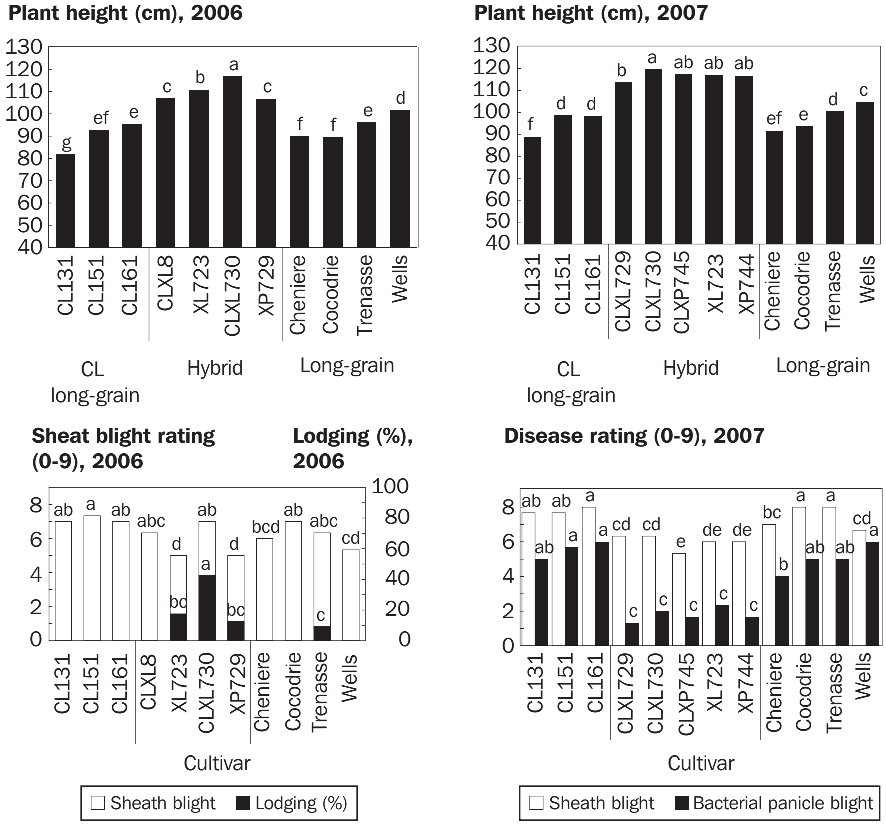

Fig. 1. Yield, maturity, plant height, milling yields, disease reactions, and lodging tolerance of individual hybrids as compared with leading commercial Clearfield® (CL) and conventional long-grain (LG) rice cultivars in Louisiana, USA, 2006-07.

as CLXP745 and XP744, in 2007 might have also contributed to the increased yield advantage. Likewise, the diminished head rice reduction between hybrids and inbred cultivars in 2007 can also be explained by differential reactions to heavier sheath blight and bacterial panicle blight pressures among three cultivar types.

The tall stature of hybrids was part of the hybrid vigor and considered to be associated with high yield potential through increased biomass production (Yamauchi 1994). However, it will remain a challenge to find a balance between further increasing yield potential and reducing plant height to improve lodging resistance. Hybrid rice was significantly less susceptible to sheath blight and much more resistant to bacterial panicle blight. It appears that heterosis exists in RiceTec indica/japonica hybrids for resistance to sheath blight and bacterial panicle blight. This is in contrast to the results from earlier research on indica/indica hybrids carried out in China (Mew et al 1988). Further studies are needed to identify the mechanisms of such heterosis.

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Notes

Authors' address: Rice Research Station, Louisiana State University AgCenter, Rayne, LA 70578, USA.

CIRAD's hybrid rice breeding strategy for Latin America

James Taillebois, Joanna Dossmann, Cécile Grenier, Péricles de Carvalho Ferreira Neves, Roger Taboada Paniagua, and Juana Viruez Justiniano

In Latin America, CIRAD and its partners are undertaking several research programs for hybrid rice development and marketing. These programs are conducted in Colombia and neighboring countries with the private company El Aceituno, in Brazil with the Embrapa state enterprise, and in Bolivia with the private organization ASPAR and an international agricultural research institute (CIAT). The selected hybrids are bred for irrigated systems and, in the case of Brazil and Bolivia, for rainfed systems. The hybrids, breeding schemes, and seed production systems are developed for highly mechanized agriculture in which labor availability is a limiting factor. The breeding scheme is first based on early evaluation for seed production ability. As parental lines are selected through early tests for combining ability, the breeding program is totally independent of available varieties and conventional breeding programs. Parental lines are selected from populations produced by the recurrent selection breeding process. Nuclear genetic sterility is used as a tool for population recombination as well as for combining ability tests and selection for outcrossing ability. Seed production techniques, based on WA and Gam cytoplasm exploitation, are developed for mechanized production systems. In Brazil, seed production for irrigated hybrid rice is developed in rainfed conditions. In Bolivia, because of the available ressources for the breeding program, the objective is the creation of three-way-cross hybrid varieties. Currently, in Brazil and Colombia, several hybrids have gone through the process of registration.

In Latin America, CIRAD and its partners are undertaking several research programs for hybrid rice development and marketing. In Colombia, CIRAD and the private company El Acetiuno are working jointly to develop hybrid varieties for Colombia, Ecuador, Venezuela, and Central America. For all these countries, hybrids are mainly bred for irrigated conditions. In Brazil, CIRAD and Embrapa develop hybrids for the different Brazilian rice agroecologies (temperate and tropical irrigated rice and upland rice). Finally, in Bolivia, CIRAD, in partnership with CIAT and ASPAR, a rice producers' association, is developing hybrids for upland conditions. For this last collaboration, the goal is not the creation of common single-cross hybrids but three-way-cross hybrids. Three-way-cross hybrids result from the crossing of an inbred A line with an R population coming from a single heterozygous plant.

Several constraints concerning the use of hybrid rice are characteristic of Latin America: the high mechanization, direct seeding, and less labor available, particularly for seed production.

In this context, CIRAD and its partners have been developing, in the past few years, specific breeding and seed production technologies to allow hybrid rice technologies to be adapted to Latin American agricultural conditions.

The value of a hybrid parental line is not its value as an inbred but its value when combined with another line. For this reason, all the hybrid breeding programs led by CIRAD are completely independent and autonomous from conventional inbred selection programs. The parental lines are specifically created to breed hybrids: they are bred, from various heterozygous germplasm sources, by pedigree selection for combining ability. For seed production of both single- and three-way-cross hybrids, we are using sporophytic genic cytoplasmic male sterility (three-line hybrids). Two cytoplasmic sources are used: WA (wild-abortive) and Gam. Our breeding plans are structured through two steps: first, the creation of parental lines; second, the creation of hybrids from these lines.

The selection of the parental lines is based on the exploitation of populations led through a recurrent selection process allowing the accumulation of genetic progress. A recessive male sterility gene, segregating within the populations, facilitates the control of the recurrent selection schemes. From these populations, parental lines are continuously extracted to be used for creating new hybrid combinations. Two populations are reciprocally used: one, the R population, provides the restorer parental lines; the other, the B population, provides the A/B female parental lines.

The R population is improved according to the best available A lines. Some 100 to 200 S_0 plants from the R population are tested for combining ability with one of the best female lines. Because S_0 seeds were harvested on male sterile plants, all fertile S_0 plants used are heterozygous for the male sterile recessive gene. Two different strategies can be used to test combining ability:

- S_0 plants are test-crossed with an A plant on chimney-like isolation plots and, in the next season, testcross progenies are evaluated in a yield trial. Because of the few available seeds (200 to 400 testcross seeds), no repetitions are possible for evaluating yield.
- At blooming time, 4 to 6 male sterile plants from each S_1 line are transferred to a B line plot for outcrossing. Testcross progenies are then evaluated in multilocation replicated trials.

With the first strategy, a recurrent selection cycle is achieved in two seasons: for the second strategy, four seasons are necessary. Nevertheless, yield evaluation is far more precise with strategy two. Strategy one is necessary when maintainer genes are still segregating in the population.

Remnant S_1 seeds corresponding to the best testcrosses are used in two different ways: (1) they are bulked and interbred to initiate a new cycle of selection; (2) they are submitted to a pedigree selection for combining ability to create new R lines.

The bulk seeds of the elite S_1 lines are planted in an isolated field. Segregating male sterile plants are naturally outcrossed and bulk harvested. Seeds harvested on male plants form the next population, which is ready for a new selection cycle. With this outcrossing process, the most allogamous plants are favored. According to off-season facilities and the testcross strategy, a recurrent selection cycle lasts from 1 to 4 years.

Hundreds of new R lines are bred every year from the selected S_1 lines. Breeding is achieved through a process of pedigree selection for combining ability on S_2 lines. Some two-thirds of the S_2 lines are segregating for male sterility and can thus be tested for combining ability through the same process with which S_1 lines were tested for combining ability. To breed three-way-cross hybrids, S_1 lines are used directly as parental restorer populations.

The recurrent selection process of B populations is far more complex. From a B population, 100 to 200 S_0 fertile plants are tested for the following three characters: outcrossing ability, maintainer ability, and combining ability. The ability to maintain sterility is obtained simply by crossing the S_0 plants with an A line. Outcrossing ability is evaluated by productivity of the male sterile plants of each S_1 line in transplant plots. Combining ability is evaluated by crossing some male sterile plants of each S_1 line with the best available R lines. Through a selection index combining the three characters, the best S_1 lines are selected. As for the restorer population, the selected S_1 lines are interbred for a new recurrent selection cycle and, in parallel, through combining ability pedigree selection, used for the creation of new A/B lines. One recurrent cycle for the B population requires 2 to 3 years according to the off-season facilities. Pedigree selection and CMS cytoplasm transfer are a long process and only a few new A/B lines are bred every year.

Thus, every year, thanks to the recurrent selection process, 100 to 150 new R lines and some A lines are bred. From this material and some of the best A and R lines of previous cycles, 1,000 to 2,000 hybrid combinations are tested annually for seed production ability. Small plots (25 cm^2) made up of several A lines and one R line are sown in a single totally mechanized operation. Each plot is isolated from its neighbors by a vegetable barrier made up of sorghum in upland conditions or with a tall and late-maturity rice variety in irrigated conditions. Using one single sowing date for both parental lines is not, considering equal resources, a limiting factor; quite to the contrary, when area is not a problem, as is common in Latin America, it is possible to test a greater number of hybrids. From this trial, the hybrids having the best seed production ability are evaluated for their agronomic value (yield and grain quality). The quantities of harvested seeds (100 to 300 g) allow a precise evaluation with multilocation replicated trials. In parallel to this agronomic evaluation, the seed production ability of these hybrids is evaluated once again on increasingly large plots. This process of joint selection of seed production ability and agronomic potential, repeated in several cycles, makes it possible to progressively identify the best hybrids. Once the process is installed, new hybrids can be regularly proposed for marketing.

If seed production is a drawback for a large diffusion of hybrids in Asia, this is even more so in Latin America, where direct seeding is commonly practiced. Several techniques have been developed by CIRAD and its partners to facilitate the production of hybrid seeds under the agro-economic conditions of Latin America.

- With direct mechanized seeding, sowing densities are often higher than 100 kg ha^{-1}, sometimes at about 200 kg ha^{-1}, which is completely incompatible with the possible cost of the hybrid seeds. To obtain hybrids able to express their yield potential with very low seeding density, all yield trials, throughout the selection process, are direct seeded with low seed density (25 kg ha^{-1}). The agronomists of El Aceituno, in Colombia, showed, on important surfaces, that our hybrids could be sown, with the seeder equipment commonly available, with low densities of up to 20 kg ha^{-1}: what is important is to obtain good seed distribution and good weed control. The use of seed treatments and coating techniques will probably facilitate the use of low seeding densities: a seeding density of 10 kg ha^{-1} seems possible in mechanized direct seeding. The integration of total herbicide resistance, such as Clearfield® technology developed by BASF, would be an important element to facilitate the use of these ultra-low seeding densities.
- To facilitate seed production using direct mechanized seeding, all our hybrids are selected so that a single sowing date for both parents allows good seed production. The practice of several sowing dates to obtain synchronized flowering of both parents is very risky in direct seeding: weather conditions will not always allow the entry of the machines for a second or third sowing date. In our breeding plans, all hybrid combinations are tested for seed production ability, practicing a single sowing date for both parental lines. These decisions indirectly reduced breeding costs and allowed us to test a larger number of hybrids.
- For the selection of parental lines, and more especially for A lines, outcrossing ability is one of the most important selection criteria.
- Finally, when possible, as in Brazil or Bolivia, hybrid seed production, even for irrigated hybrid rice varieties, is carried out in upland conditions, where mechanization is easier and production costs are lower.

Thanks to the exploitation of all these strategies, CIRAD and its partners currently have several R and B populations undergoing recurrent selection. Numerous R lines bred from recurrent selection and more than 50 A lines are available. Currently, hundreds of hybrids are tested and, in Colombia and Brazil, several hybrids are in the registration process.

Notes

Authors' addresses: James Taillebois, CIRAD, UR6, F-34378, Montpellier, France, james.taillebois@CIRAD.fr; Joanna Dossmann, El Aceituno, Ibagué, Colombia, Joanna_dossmann@telesat.com.co; Cécile Grenier, CIRAD, UR6, F-34378, Montpellier, France, Cecile.grenier@CIRAD.fr; Péricles de Carvalho Ferreira Neves, Embrapa-Arroz e Feijão, Goiânia, Brazil, pericles@cnpaf.com.bo; Roger Taboada Paniagua, CIAT/ASPAR, Santa Cruz, Bolivia, rogertaboada@cotas.com.bo; Juana Viruez Justiniano, CIAT/ASPAR, Santa Cruz, Bolivia, juanitavj@hotmail.com.