



Genetic Improvement of Rice: Activities, Achievements and Aspirations

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SUMMARY

Germplasm collection, maintenance, characterization and promotion for utilization in breeding program in national level have been major activity of the Division for the crop improvement programme. The Division has, so far, developed and released 134 varieties including three hybrids. For ensuring nutritional security, the Institute has recently released, first time in the world, two high-protein (more than 10.0%) rice varieties (CR Dhan 310 and CR Dhan 311). Two climate-smart varieties (CR Dhan 801 and CR Dhan 802), which are tolerant to both submergence and drought and few biotic stresses have also been released, again for the first time in the world, to address the challenges of climate change. Besides, 158 new entries were nominated in different AICRIP trials during 2018. Through commercialisation of these hybrids more than Rs. 1.5 crores has been earned for the Institute. Seed production of different categories are also another major activity of the Division. Every year on an average 1.0 ton of nucleus seed, 50.0 tons of breeder seed and 100.0 tons of truthfully labelled seeds are produced. Besides, identification of genes/QTLs for biotic stresses such as brown planthopper (BPH), sheath blight resistance and high yield enhancing genes, *Gn1a*, *OsSPL14* and *SCM2* were successfully pyramided into the background of MTU 1010, BPT 5204 and Swarna from donors such as Habataki, ST 12 and ST 6 using conventional breeding and genomic tools. The Division also standardized method for somatic embryogenesis in popular *indica* rice varieties which could be utilized in transgenics and CRISPR/Cas9 approach. It is working on to develop super-yielding (more than 10 t ha⁻¹) varieties with tolerance to biotic and abiotic stresses for higher productivity, profitability, climate resilience and sustainability of rice farming.

1. INTRODUCTION

Since inception, the major objective of NRRI remains to carry out basic and applied research on rice in all disciplines so as to improve productivity. The objectives have been broadened from time to time to address the newer challenges with aim to improve the income and livelihood of resource poor



farmers who are depending mainly on rice cultivation under rainfed condition. To meet these objectives, many improved varieties were developed for different ecologies through pure line selection and several breeding lines were developed under *indica-japonica* hybridisation programme. Currently the major activities of the Division are to i) explore and collect the rice genetic resources from different parts of India, their maintenance, characterization and utilization, ii) produce Nucleus, Breeder and TL Seed and their quality control, iii) develop high yielding genotypes with multiple stress tolerance suitable for favourable and unfavourable rainfed lowlands, iv) genetic improvement of rice with novel attributes along with acceptable grain quality and biotic stress tolerance, v) develop Double Haploids (DHs) from elite *indica* rice hybrids and promising recombinants for yield related traits and mapping population, vi) identify and develop parents with good combining ability and develop heterotic hybrids with better grain quality in different duration and genetic backgrounds and vii) develop new generation rice for breaking yield ceiling. The Chapter presents the major activities and achievements of the Division and the aspirations towards higher productivity, profitability, climate resilience and sustainability of rice farming.

2. ACHIEVEMENTS OF THE DIVISION

The rice genetic resources are the basic material for improvement of the crop. The exploration, collection, morpho-agronomic characterization, screening/evaluation, conservation and seed supply were part of the activities of erstwhile 'Botany Division' later named as 'Genetics Division' of the Institute. In 1984, this Division was renamed as 'Plant Breeding and Genetics Division' and a separate 'Genetic Resources Division' was created to look after all such activities on rice germplasm. It, however, was subsequently merged with the newly created 'Division of Crop Improvement' in 2001. Being the leading and premier national research institute, NRRI has been recognized as one of the National Active Germplasm Sites (NAGS).

Heterosis in rice was first documented at NRRI (Barwale, 1993). Studies were made on induced polyploidy and mutagenesis and it was possible to generate tetraploid Sitasail and SR26B genotypes (Misra et al. 1971). Tetraploid and long glumed rice was found to contain more protein than diploid rice with short glumed one.

Pachytene analysis technique unravelled the cause, nature of karyomorphology, evolution and differentiation of species in genus *Oryza*. It was reported that genomes are homeologous and chromosome structural changes are the principal mechanisms of genome differentiation within that series. Total 12 primary trisomics were developed for the first time in the 'Sona' background, which led the path of chromosome engineering studies in rice



(Misra et al. 1985). This primary trisomics have been utilized for associating genes and linkage groups with respective chromosomes. Aneuploid stocks of rice are useful not only for genetic study but also for rice breeding.

Development of monosomic alien addition lines (MAALs) from the wild rice (*Oryza brachyantha* A. Chev. et Roehr.) for introgression of yellow stem borer resistance into cultivated rice was also achieved at NRRI (Mishra et al., 1971) which was later characterized with cross transferrable markers (Aveek et al., 2016). Mutation studies also led to the identification of several novel mutants (Sanchez and Khush 1992; Khush and Librojo 1984; Sanchez and Khush 1997; Nanda and Misra 1975; Misra et al. 1977).

The identification of the source for *Xa21* gene from wild *O. barthii* (Devadath 1983) conferring wide spectrum of resistance to BLB was first carried out at NRRI. This gene shows resistance to all the races of bacterial blight in India (Jachuck and Sampath 1966, Nayak et al. 1978).

2.1. Genetic Resources

The rice genetic resources are the basic material for improvement of the crop. The genus *Oryza* consists of 24 species, of which two are cultivated and rests are found wild in different parts of the world; all of them grow in the tropics only. Khush (1997) in IRRI confirmed that rice cultivation was started at least 7000 years ago and domesticated from its wild ancestor *O. rufipogon*. The perennial wild species was known earlier as *O. perennis* by all rice workers until Bor (1960) identified it as *O. rufipogon*. Sharma and Shastry (1965) assigned a new name (*O. nivara*) to *O. fatua*, which was not a validly published name for the annual wild species. The African wild rice, *Oryza barthii*, which was acquired and maintained at NRRI, was identified as tolerant to bacterial blight with new gene, *Xa21*. Weedy rice appears as hybrid swarms due to introgression of genes between wild and cultivated species in nature. In Asian rice, it is known as *Oryza spontanea* whereas in African context it is said as *O. stapfii*.

Dr. K. Ramiah, the founder Director had brought a set of 2,400 accessions of germplasm from Paddy Breeding Station, Coimbatore to start rice research at NRRI. Subsequently many exploration and collection programmes, introduction and acquisition through exchange activities have helped enrich the Gene Pool. Besides germplasm introduction, the other landmark activity of the Division was the Jeypore Botanical Survey (JBS). This programme was supported by an ICAR scheme and continued for five years (1955-59). It was led by Dr. S. Govindaswami and the mission was the first of its kind, ever organized in the world to collect rice germplasm. The team explored about 27,000 km² and collected 1,745 cultivated and 150 wild rice accessions. A new species was described from this region and was given the name of *Oryza jeyporensis*, which did not exist later as a distinct species.



After establishment of NRRI in 1946, efforts were made to introduce some exotic germplasm from China, Japan, Taiwan and Russia, which were semi tall and nitrogen fertilizer responsive. Some cultivars of Chinese origin like CH 4, CH 45, CH 55, CH 62 and CH 63 proved to be very good donors for better yield and early maturity. Few varieties like CH 27, CH 47, CH 962, CH 971, CH 972 and CH 1039 were found suitable to grow in Kashmir valley. Contrary to the success of the Chinese varieties, the Japanese and Russian germplasm were found unsuitable under Indian condition. The International Rice Commission (IRC) recognized NRRI as a centre for multiplication and maintenance of world genetic stocks of rice mostly the FAO designated germplasm of five countries i.e. India, Indonesia, Japan, Pakistan and USA. In the process many varieties comprising *japonicas*, *indicas*, *bulus* and floating types of south and south-east Asian countries were introduced to the country. Further, NRRI was recognized as the main centre for the inter-racial *japonica-indica* hybridization programme during 1950-1964. In the process many exotic *japonicas* were introduced into India. In early 1960s, Dr. R.H. Richharia introduced 67 varieties from Taiwan, two or three cultures of those were dwarf types, were tested at NRRI and one of them was identified as Taichung Native 1 (TN 1), which later laid the foundation for ushering Green Revolution in the country.

Several joint explorations together with countries like Japan, France and Philippines were undertaken for collection of wild species of rice. Later in 1975, a comprehensive exploration and collection programme was drawn for the whole country especially for the traditional rice growing areas of Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal and Odisha covering 30 districts of 7 states. This programme was popularly known as National Collection from States (NCS) and resulted in collection of 1038 accessions. Increased interest in herbal medicines during last few decades has necessitated collection of rice germplasm with special emphasis on their medicinal value from Bastar region of Chhattisgarh and Palakkad region of Kerala for the famous 'njavara' rice. Recently, FAO recognized Koraput region of Odisha for traditional rice cultivation and Kuttanad region of Kerala for below sea level farming in 2012 and 2013, respectively as Globally Important Agriculture Heritage Systems (GIAHS).

The cold storage system for conservation of rice germplasm was established at NRRI in 1984. After establishment of National Gene Bank at NBPGR, New Delhi, it was decided to deposit all the germplasm of NRRI at NBPGR in 1986. Since then, 35,619 rice accessions have been deposited in the long term storage (LTS) of -18°C and with 3-4% RH. Under the aegis of the Indo-USAID collaborative project, a cold module was gifted to NRRI in 1997. The facility became operative in 1998 with a controlled temperature of $4^{\circ} \pm 2^{\circ}\text{C}$ & 33% \pm 5% RH and was found dependable. The Gene Bank facility thus created was meant for medium term storage (MTS) and the seeds were kept viable for 6-8 years. When accessions in the MTS drops below 50 g or if seed viability falls



below 85%, then the seed is increased (rejuvenated). All the germplasm collections including wild and weedy rice are being characterized for agromorphological traits based on 30 distinctive, uniformity and stability (DUS) characters as per the descriptors which include 19 qualitative and 11 quantitative characters at appropriate stages of plant growth and maturity. Sharing of rice germplasm also remains an important mandate of this Division for utilization in crop improvement programme. Germplasm are supplied to various institutes/organizations through proper signing of Material Transfer Agreement (MTA).

2.2. Cytogenetics of rice and its species

Food and Agriculture Organization (FAO) sponsored *indica* × *japonica* hybridization program was carried out by Dr. S. Sampath and his team during 1952-59. Cytological explanation for the spikelet sterility in *indica* × *japonica* hybrids (Sampath and Krishnaswamy 1948, Sampath and Mohanty 1954, Sampath 1960, 1964) established that it is a complex species. It has been reported that they can be crossed with each other despite their differences (Sampath 1961, Sampath and Seetharaman 1962). Dr. S. Sampath was selected as one among the three members of a Committee appointed on February 4, 1963 during the Symposium on Rice Genetics and Cytogenetics held at International Rice Research Institute to set a standard classification and nomenclature for the species in genus *Oryza* for adoption by all rice research workers worldwide.

2.3. Quality seed production and management

Genetically pure seed is the basic and important input for crop establishment and production. Good quality seed can contribute up to 30% increase in productivity. Therefore, a standing experts committee on seeds was appointed as early as in 1952 by ICAR to formulate a programme to strengthen the seed production and distribution system.

Since beginning NRRI has shouldered the responsibility to carry out the maintenance breeding programme for keeping the integrity and purity of the variety intact by producing Nucleus seed and Breeder seed particularly of these varieties developed by this Institution. It also continued to be one of the voluntary centres of ICAR for breeder seed production of paddy. In last ten years, the institute has produced 598.9 tons of breeder seed of 71 varieties, which were supplied to 15 states of the country, comprising of Government organizations and private companies as per the Government of India allocations. Besides, the institute is also producing TL seeds for distribution to farmers. Participatory seed production programme of the Institute started in 2012 under which 441.2 tons of truthfully labelled (TL) seed of 4 major popular rice varieties of the state was produced in the farmers' field under the supervision of NRRI Scientists and was made available to the farmers.

Presently, 48 rice varieties of the institute are under national seed chain, among them Swarna Sub-1, Pooja, Naveen, Shatabdi, CR Dhan 500, CR Dhan



201 and CR Dhan 303 are highly demanded varieties. The seed demand of high protein rice CR Dhan 310 & CR Dhan 311 (Mukul), high yielding variety CR Dhan 307 (Maudamani) and CR Dhan 800 (Swarna MAS) is picking up at a higher pace.

Along with seed production, seed science research is also a priority area for the institute. All the NRRI released varieties are already characterized for their duration of seed dormancy and viability. The other important seed traits viz., seed vigour and seed dimension are under investigation at molecular level.

With increasing awareness on role of quality seed, the Seed Replacement Rate (SRR) has gradually increased in the country. Like-wise with adaptation of newly developed climate smart and stress tolerant varieties the Variety Replacement Rate (VRR) of the country has also increased considerably in recent years. However, till now, only 30 to 35% of the farmers use their farm-saved seed for crop production either due to lack of awareness or poor accessibility of quality seed. Therefore, the quality seed production and distribution system are to be further strengthened in future to ensure the availability of quality seed for the total farming community.

2.4. Breeding for Biotic stress resistance

Rice suffers from several biotic and abiotic stresses that seriously affect its production. A wide range of pathogens, insects, nematodes and other pests attack the rice plant in different parts of the world. Magnitude and the type of damage caused by pests vary in different regions. Among them, diseases like blast, bacterial blight (BB), sheath blight (ShB) and insects like brown plant hopper (BPH), yellow stem borer (YSB) are of major concern in India as well as many other parts of the world. Despite the availability of several control mechanisms for mitigating pest damage, developing cultivars tolerant to major insect-pests and diseases prevalent in an area is the easiest, most economic and eco-friendly measure available to the farmers. At the same time, the system is highly dynamic in its nature due to continuous co-evolution of genes conferring resistance or susceptibility in hosts and their corresponding gene for virulence in pests. Genes conferring resistance are distributed across primary, secondary and tertiary gene pool of the crop. Judicious use of these genes and genetic resources to minimize losses caused by pests remains an important challenge for rice researchers worldwide.

In India, systematic research efforts to impart host plant resistance in rice has been undergoing since past 70 years. The biotic stress breeding programme at the NRRI has evolved over time depending on the dynamic pest profile of the crop and advances in the technologies available. The institute was established in 1946 in the backdrop of the Bengal famine caused due to *Helminthosporium* brown leaf spot. Hence during the first two decades, the emphasis was given to identify brown spot resistant genotypes for developing



tolerant/resistant varieties. Eventually, breeding for tolerance against blast and yellow stem borer (YSB) was also taken up. With the introduction of high yielding semi-dwarf varieties like TN 1 during early 60's, bacterial blight became a severe threat to rice production. The 1970's and 1980's saw the major focus being directed towards breeding for bacterial blight tolerance. With the outbreak of brown plant hopper in the late 1970's, breeding for BPH tolerance had also taken a centre stage. Sheath blight, though very severe even during 1960's in countries like the Philippines, was not economically important in India until recently. However, consequent upon reports of severe incidences of ShB from different parts of the country in recent years, search for resistance sources has geared up.

2.4.1. Bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance

The *Xa21* gene, imparting resistance to BB, was identified at NRRI in the wild species *Oryza longistaminata*, which was highly effective against all races in South and South-Eastern Asia. The gene was later mapped and cloned at IRRI and is being extensively utilized by breeders across the globe. Varietal improvement programme was also initiated to improve the BLB resistance using popular high yielding varieties as recurrent parents and resistant genotypes viz., Ajaya (*xa5*), IRBB 8 (*xa8*) and IRBB 60 (*xa5*, *xa13* and *Xa21*) as donors through pedigree breeding coupled with artificial screening.

Resistance genes (*xa5*, *xa13* and *Xa21*; either singly or in different combinations) pyramided lines were developed through marker assisted backcross breeding in the genetic background of Swarna and IR64 under the Asian Rice Biotechnology Network (Reddy et al. 1997). Two lines CRMAS 2231-37 (IET 20668) and CRMAS 2231-48 (IET 20669) in the background of IR 64 were found promising for BB endemic areas of Uttarakhand & Andhra Pradesh and Uttarakhand & Haryana, respectively, while one line CRMAS 2232-85 (IET 20672) in the background of Swarna was recommended for the endemic areas of Gujarat and Maharashtra. Pradhan et al. (2015) introgressed three BLB resistance genes (*xa5*, *xa13* and *Xa21*) by marker-assisted backcrossing, in the background of the popular, but highly BLB susceptible deep water variety, Jalmagna. The pyramided lines showed a high level of BLB resistance and a significant yield advantage over Jalmagna. Lines carrying two BB gene combinations (*Xa21+xa13* and *Xa21+xa5*) were also developed in the background of Jalmagna (Pradhan et al. 2016). The pyramided lines showed increased resistance to BB isolates prevalent in the region. The parental line improvement for BB resistance has been successfully undertaken in case of popular rice hybrid of NRRI, Rajalaxmi, by introgressing four resistance genes (*Xa4*, *xa5*, *xa13*, and *Xa21*) through Marker-assisted backcross (MAB) breeding (Dash et al. 2016).

Varietal improvement program at NRRI for BB resistance resulted in the release of Improved Lalat [CRMAS 2621-7-1 (IET 21066)], Improved Tapaswini [CRMAS 2622-7-6 (IET 21070)] and CR Dhan 800 in the genetic background of



popular rice varieties Lalat, Tapaswini and Swarna, respectively. Improved Lalat and Improved Tapaswini carry four genes (*Xa4*, *xa5*, *xa13* and *Xa21*) while CRDhan 800 has three resistance genes *Xa21*, *xa13* and *xa5*. All have been effective for growing in the “bacterial blight” endemic areas of Odisha.

2.4.2. Rice blast (*Magnaporthe oryzae*) resistance

Marker assisted backcross breeding strategy was applied for pyramiding blast resistance genes (*Pi2* and *Pi9*), into Vandana and Kalinga III through the crosses (Kalinga III/C101A51 (*Pi-2(t)*)/Kalinga III/*O. minuta* der. WHD IS 75-127(*Pi-9(t)*) and Vandana/C101A51//Vandana /*O. minuta* der. WHD IS 75-127). Many lines in the background of Vandana and Kalinga III were developed. Among the promising lines, CR 2619-2, CR 2619-5, CR 2619-6, CR 2619-7, CR 2619-8 and CR 2619-9 are in the background of Vandana while CR 2620-1, CR 2620-2, CR 2620-3 and CR 2620-4 are in Kalinga III background. The promising lines were tested in Disease Screening Nursery (DSN) under AICRIP for multi-location trials. The lines are in the pipeline for release as variety.

In an attempts to find out the status of twelve major blast resistance genes (*Pib*, *Piz*, *Piz-t*, *Pik*, *Pik-p*, *Pikm*, *Pik-h*, *Pita/Pita-2*, *Pi2*, *Pi9*, *Pi1* and *Pi5*) and their diversity among eighty released rice varieties of NRRI, it was observed that presence of resistant genes varied from 4 to 12 and the frequencies of the resistance genes ranged from 0 to 100% (Yadav et al. 2017). Among the 80 varieties screened, 19 were resistant, 21 were moderately resistant and 40 were susceptible to the disease.

2.4.3. Sheath blight (*Rhizoctonia solani* Kuhn) tolerance/resistance

Screening experiments conducted at the NRRI using the local strains of the pathogen has shown that international check genotypes for ShB tolerance like Jasmine 85 and Teqing are susceptible to the local strains. Only two genotypes, Tetep and CR 1014, showed consistently moderate resistant for sheath blight. Conventional breeding has been less effective for the development of ShB tolerant genotypes because of the polygenic nature of the trait. In the segregating generations of the crosses made using CR 1014 as the donor for ShB tolerance, selection of superior recombinants has been difficult since ShB tolerance has tight linkage with plant height. A novel ShB QTL on chromosome 1 was identified from an $F_{2,3}$ populations derived from the cross Swarna Sub1 x CR 1014, which need to be fine mapped and its effects in different genetic backgrounds need to be validated.

2.4.4. Brown plant hopper (*Nilaparvata lugens* Stål) resistance

Several landraces showing very high degree of resistance were used for breeding varieties resistant to BPH. The breeding lines CR 3005-77-2 (Samba Mahsuri/Salkathi), CR 3006-8-2 (Pusa 44/Salkathi), CR 3005-230-5 (Samba Mahsuri/Salkathi), CR 2711-76 (Tapaswini/Dhobanumberi) were found to be promising in plant hopper screening trials of AICRIP, 2011 and 2012. Molecular mapping



of resistance genes/QTLs from these two landraces- Salkathi and Dhobanumberi is underway. Two QTLs designated as *qBph4.3* and *qBph4.4* were identified from Salkathi landrace among which *QBph4.3* is novel (Mohanty et al. 2017). Transfer of these two QTLs into two popular susceptible varieties Naveen and Pooja are in progress. Recently, Prahlada et al. (2017) at IRRI identified a single dominant gene, *BPH31* on the long arm of chromosome 3 in CR2711-76.

2.4.5. Yellow stem borer (*Scirpophaga incertulas*) tolerance/resistance in rice

Since gene(s) for resistance to YSB has not been found in the primary gene pool of rice, efforts were made to incorporate alien genes from wild species belonging to the secondary and tertiary gene pool, which are reservoirs of such traits. Wild rice germplasm has been screened against YSB. *O. brachyantha*, *O. officinalis*, *O. ridleyi* and *Porteresia coarctata* were found to be resistant/tolerant against the pest. Subsequently, backcross population of *O. sativa* cv. Savitri / *O. brachyantha* was developed to transfer YSB resistance to the cultivated rice (Behura et al. 2011). The cytogenetic analysis of the chromosomal variants led to the development of monosomic alien addition lines (MAALs). Of the 8 MAALs screened, MAAL 11 was found to be moderately resistant to YSB.

2.5. Breeding for Abiotic stress tolerance

2.5.1. Upland ecosystem

This ecosystem occupies an area of 8 Mha of which 6.2 Mha are in eastern region of the country. Moisture stress is an important limitation for achieving higher yield from this ecology. The main breeding objectives for development of upland rice varieties are early maturity duration (100-105 days) with drought tolerance, weed competitiveness and high yield. Upland varieties should also show biotic stress resistance to blast, brown spot, gundhi bug and termite attack. Many traditional *aus* cultivars from eastern India possess several desirable characters. The traits like early vigour, droopy leaves, deeper roots, moderate tillering and high grain weight are seen in *aus* genotypes. The common upland genotypes used as donor were Brown Gora, Black Gora, N22, C22, Lalnakanda 41, Kalakeri, Salumpkit, Ch 45, Dular, Annada, CR 143-2-2 and IRAT 112. They have provided good recombinants with *indicas*. The varieties developed for this ecosystem by the Institute are Bala, Sattari, Kalinga-III, Neela, Annada, Heera, Kalyani-II, Tara, Vanaprava, Sneha, Phalguni, Satyabhama and Ankit. More focused research on this aspect was initiated by the sub-station (Central Rainfed Upland Rice Research Station; CRURRS) of NRRI at Hazaribag (Jharkhand) where from following varieties have been developed; Vandana, DhalaHeera, Anjali, Sadabahar, Virendra, CR Dhan 40, Sahabhagidhan. Aerobic varieties developed at NRRI are CR Dhan 200 (Pyari), CR Dhan 201, CR Dhan 202, CR Dhan 203 (Sachala), CR Dhan 204, CR Dhan 205, CR Dhan 206, CR Dhan 207 (Srimati) and CR Dhan 209 (Priya) which are suitable for drought prone ecology with assured irrigation.



2.5.2. Lowland ecosystem

Lowland is broadly classified into 3 classes based on duration and depth of water logging. The major class is shallow lowland with water depth of 0-30 cm during growth stages of the crop. This ecology is prone to intermittent flash floods and drought during growth stages. Direct seeding is also a common practice of cultivation in which proper land levelling is rarely achieved. Thus, anaerobic germination trait in the variety is desirable. For this submergence tolerant line, FR13A was selected from landrace Dhalaputia. Other landraces possessing submergence tolerance viz., Khoda, Khadara, Kalaputia etc. were also selected as donor lines. Submergence tolerance has been incorporated in varieties like Pooja, Sarala, Gayatri, Varshadhan and Pratikhya. Currently, the Institute is focussing on development of climate resilient varieties by combining multiple abiotic stress tolerance genes/QTLs into popular varieties. CR Dhan 801 and CR Dhan 802 are two new climate-smart varieties possessing both submergence and drought tolerance in the background of "Swarna". The varieties released by the Institute for the ecosystem are Anamika, Ramakrishna, Samalei, Savitri (Ponmani), Moti, Padmini, Dharitri, CR 1002, Seema, Pooja, Ketikijoha, NuaKalajeera, NuaDhusura, Reeta, NuaChinikamani, Sumit, Swarna-Sub1, CR Dhan 407, CR Dhan 408 (Chakaakhi), CR Dhan 409 (Pradhan Dhan), CR Dhan 800, CR Dhan 801 and CR Dhan 802.

2.5.3. Semi-deep and deep water ecosystem

The semi-deep water ecology consists of low lying areas with water depth up to 75 cm and remain water logged for about a month while in deep water ecosystem it goes up to one-meter water depth and remains submerged for more than a month. By screening thousands of traditional landraces, several tolerant sources of different genetic background have been identified and several rice varieties suitable for deep and semi-deep water ecosystem are developed. The varieties released for water logged ecosystem are Utkalapava, CR 1014, Gayatri, Kalashree, Panidhan, Tulasi, Sarala, Durga, Varshadhan, Haneswari, CR Dhan 501, CR Dhan 502 (Jayantidhan), CR Dhan 503 (Jalamani), CR Dhan 505, CR Dhan 506, CR Dhan 507 (Prasant), CR Dhan 509, CR Dhan 510 and CR Dhan 511.

2.5.4. Ecosystem with salinity and water logging

For high throughput evaluation of rice germplasm and breeding lines for salinity tolerance at seedling and reproductive stages, dug-out cavity structures made of brick-mortar-concrete materials and filled with artificially prepared or natural transported saline soil have been designed and constructed at NRRI which (the prototype of structure and protocol for simulating desired level of salinity) have subsequently been replicated at many research Institutions (Nayak et al. 2017). Novel screening protocol to promote precise screening for salt tolerance at reproductive stage was standardized and validated (Chattopadhyay et al. 2018a). Nine multi-environmental QTLs for salinity



tolerance at reproduction stage were detected using IR64/ AC41585 population for different component traits such as spikelet degeneration, spikelet sterility and K^+ concentration in flag leaf on Chromosome 1, 2, 3, 4 and 11.

Landraces from Sunderbans region of West Bengal were found diverse in respect of salt tolerance. Salt tolerant cultivars from this area such as Kamini, Talmugur, etc. were found to have allelic difference from the widely used *Saltol*-introgression line, FL 478 in the *Saltol* - QTL region (Chattopadhyay et al. 2013a). Raspanjar was found as one of the good performing landraces under salinity stress in coastal Odisha (Patnaik and Sinha, 1972). Unlike Pokkali, Raspanjar was efficient in maintaining higher level of K^+ despite high Na^+ influx in shoot which are found to be located distant from Pokkali in 3-D plot on SSR data. Swarna *Sub1* × Raspanjar and Savitri × SR 26B produced more transgression segregants for tolerance at seedling stage and were found ideal combination (Chattopadhyay et al. 2014). NRRI also identified few unique germplasm for reproductive stage tolerance viz. AC41585, AC39394 (Chattopadhyay et al. 2013b).

2.5.5. Coastal ecosystem

Different breeding methodologies were developed since early 1970s at NRRI to get improved high yielding genotypes with salinity tolerance (De and Sreedharan, 1991) and suitable for the target ecosystem. Lunishree, the first high yielding variety for coastal saline area was developed and released in 1992. This is a gamma irradiated mutant from Nonasail (Sridhar, 1996). CR Dhan 402 (Luna Sampad, IET 19470) and CR Dhan 403 (Luna Suvarna, IET 18697) in 2010 and Luna Barial (CR Dhan 406, IET 19472) in 2012 were released by the Odisha State Sub-Committee on Crop Standards (State Varietal Release Committee) for cultivation during wet season in coastal saline areas of Odisha. Luna Sankhi has been developed by ICAR-NRRI in collaboration with IRRI, Philippines and released in 2012 by SVRC, Odisha for dry season cultivation in coastal saline areas (Chattopadhyay et al. 2018b).

2.5.6. On farm evaluation of climate resilient rice varieties for unfavourable ecology

On-farm trials were conducted in Ersama block of Jagatsingpur district (Odisha) using suitable rice varieties, selected nutrient management practices. They were evaluated and the most promising options were validated in farmers' participatory mode. Among different integrated nutrient management practices, *Sesbania* green manuring (GM) for intermediate lowlands (0-50 cm water depth), *Sesbania* GM + prilled urea (PU) and *Sesbania* GM + *Azolla* for shallow lowlands (0-30 cm water depth) in the wet season, and *Azolla* + PU in the dry season were found to be promising. Substantial yield improvements (91% in wet and 75% in dry season) could be achieved by combining salt-tolerant varieties



with improved nutrient and other crop management practices (Saha et al. 2008). During widespread survey and demonstrations of climate resilient rice varieties in coastal region it was felt that salinity and stagnant flooding in combination affect the rice production in greater extent (Chattopadhyaya et al., 2016). Adoption of rice cultivars tolerant to flooding and salinity gave yield advantage of 0.44 to 2.14 t ha⁻¹ compared to traditional rice cultivars. Luna Suvarna in salinity affected area, Varshadhan in water-logged area and Swarna *Sub 1* and Savitri *Sub 1* in submergence prone areas were found promising in Sunderbans, West Bengal.

2.5.7. High and low temperatures stress tolerance in rice

High temperature stress is prominent in dry season rice cultivation. The Institute has identified many tolerant germplasm lines to high temperature stress (Pradhan et al. 2016). CR3621-6-1-3-1-1, CR3820-2-1-5-1-2, CR3813-4-4-4-2-2, CR3820-4-5-3-1-3, AC 39890, AC 39973, AC 39790, Annapurna, Maudamani (CR Dhan 307) and N22 showed spikelet fertility of >40% under high temperature stress and categorized as highly tolerant while these lines exhibited >80% under normal situation. The strongly associated marker RM547 tagged with fertility under stress and the markers like RM228, RM205, RM247, RM242, INDEL3 and RM314 indirectly controlling temperature stress tolerance were detected through both mixed linear model and general linear model TASSEL analysis (Pradhan et al. 2016). Low temperature stress, on the other hand, limits yield in dry season boro rice and wet season hill and high altitude rice. Many germplasm have been identified as tolerant to cold stress by the Institute (Pradhan et al. 2015c; Pandit et al. 2017). Chilling tolerant varieties can be developed by pyramiding of low temperature stress tolerance QTLs associated with markers like RM1347, RM328, RM152, RM341, RM50, RM2634, RM4112, RM5310, RM7179, RM3701, RM104, RM9, RM1211, RM245, RM3602, RM493, RM1335, RM282, and RM5704 which are significantly associated with chilling stress of 8°C to 4°C for 7–21 days duration. The primers linked to the seedling stage cold tolerance QTLs were also identified namely qCTS9, qCTS-2, qCTS6.1, qSCT2, qSCT11, qSCT1a, qCTS-3.1, qCTS11.1, qCTS12.1, qCTS-1b, and CTB2 (Pandit et al. 2017). CR Dhan 601, CR Boro Dhan 2 and Rajalaxmi have been released for boro cultivation (cold tolerance) while Maudamani (CR Dhan 307) is moderately tolerant to both the stresses.

2.6. Irrigated ecosystem

The quantum leap in productivity was possible mostly by introduction of dwarfing genes in tall varieties. During 1956, Chinese local landrace Ai-zi-zhan and subsequently Dee-geo-woo-gen, Taichung Native 1, and I-geo-tse were used as source of dwarfing gene in breeding to improve tropical landraces. In 1966, the first semi-dwarf, high-yielding modern rice variety, was introduced



for the tropical irrigated lowlands which created history, called green revolution (Khush et al. 2001). The development of IR8 at IRRI increased the yield potential of the irrigated rice varieties substantially in tropics. Subsequently, tropical varieties of enormous yielding capacity, viz., Jaya and Padma in India and Bg. 90-2 in Sri Lanka were developed using the dwarfing genes. In Korea, Tongil-type rice varieties were developed in 1971 from a *japonica/indica* cross (Chung and Heu 1980), which showed 30% yield increment compared to *japonica* varieties. The dwarf plant type was basically attributed to 'sd1' gene in Dee-geo-woo-gen and others was a milestone in breeding high yielding varieties. There was phenomenal modification in plant architecture, viz., dwarf height, more tillering, sturdy stem and erect leaves. The competency further improved due to photo-insensitiveness and fertilizer responsiveness with an enhanced productivity potential. There was continuous effort to improve it further, however, there was less progress for productivity enhancement, rather there was shifting of target towards stability of yield with incorporation of substantial biotic and abiotic stress resistance/tolerance supplemented with quality improvement along with reduction in duration. However, somewhat stagnant yield of semi-dwarf *indica* inbreds have been observed since the release of IR8 (Peng et al. 2008).

In this context, several high yielding rice varieties have been developed for mid early, mid or late duration with seed to seed 125-145 days. These varieties are endowed with characters of dwarfism. However, in this process, several crucial characters viz., dormancy, submergence tolerance, photo and thermo-sensitivity were depleted. Loss of photo-insensitivity was a blessing for intensive agriculture and for improvement of productivity per unit time (Krishnamurthy et al. 1992). Apart from this, synchronous flowering/maturity was another desirable character in this ecology. More than 1200 high yielding varieties (HYVs) have been developed in India. Out of these, 132 varieties have so far been developed by ICAR-NRRI, Cuttack for different ecologies including 52 varieties for irrigated ecology (Pathak et al. 2018).

2.7. Hybrid rice

In India, hybrid technology was adopted in project mode during 1989 when Indian Council of Agricultural Research (ICAR) launched a special goal oriented and time bound project on 'Promotion of Research and Development Efforts on Hybrids in Selected Crops' at 12 network centres. After four years of meticulous research (1989-93), first hybrid rice was released in Andhra Pradesh in 1993 and India became the second country after China to develop and commercialize hybrid rice. So far, 101 rice hybrids (33 from public organizations and 68 from private sectors) have been developed, suitable for different ecologies and duration ranging from 115 to 150 days which covers 3.0 Mha rice area (6.8% of total rice area) in India.



ICAR-NRRI has started breeding hybrid rice during late 1970s and could develop and commercialize three hybrids namely, Ajay, Rajalaxmi and CR Dhan 701 besides several CMS and good combining restorers under diversified genetic back-ground. They are now being utilized for maximizing genetic gain of hybrids in the country.

The primary success of hybrid technology was identified long back at ICAR-NRRI when Sampath and Mohanthy (1954) reported male sterility in reciprocal crosses of *indica/japonica* rice lines. In the beginning, ICAR-NRRI had acquired all the prerequisite materials (CMS lines viz. V 20A, Yar Ai Zhao A, Wu10A, MS 577A, Pankhuri203A, V 41A, Er-JiunanA, respective maintainers, nine other maintainers and 13 restorers) from IRRI (NRRI Annual Report 1981-82). Systematic hybrid rice breeding was initiated in interdisciplinary mode with objectives to develop desirable parental lines viz., cytoplasmic genetic male sterile (CGMS) lines, maintainers and restorers for development of rice hybrids for irrigated and shallow submergence. CR Dhan 701 is the country's first long duration hybrid, substitute for popular variety Swarna. Besides, NRRI has developed several promising CMS lines, which have stable male sterility (WA, Kalinga-I and *O. perennis* etc. cytoplasmic background), maintainers and effective restorers. More than 45 CMS lines in diverse genetic and cytoplasmic background have been developed amongst, Sarasa A, Pusa 33A (WA), Annada A (WA), Kiran A (WA), Deepa A (WA), Manipuri A (WA), Moti A (WA), Krishna A (*O. perennis*), Krishna A (Kalinga I), Mirai (Kalinga I), Padmini A, PS 92A and Sahbhagi Dhan A etc. are prominent. The medium duration CMS, CRMS 31A (WA) and CRMS 32A (Kalinga-I) are utilized more for hybrid development at NRRI and elsewhere in the country. The CRMS 24A and CRMS 40A, developed under the nucleus background of Moti and Padmini are found suitable for late duration hybrid breeding. Moreover, short duration CMS, CRMS 8A, CRMS 51A and CRMS 52A and CRMS 53A having drought tolerance are also being used for development of hybrids for drought prone ecosystem.

The latest release CR Dhan 701 (CRHR32) was found suitable for irrigated-shallow lowland of Bihar, Gujarat and Odisha having MS grain type with an average yield capacity of 7.5 t ha^{-1} . This hybrid shows substantial tolerance to low light intensity, thus having great scope in eastern Indian states where low light limits the potential expression of hybrids/ varieties during wet season. Rajalaxmi (125-130 days) was developed utilizing native CMS line CRMS 32A, released by SVRC 2006/CVRC 2010 for irrigated-shallow lowland of Odisha and *boro* ecosystem of Odisha and Assam as it has seedling stage cold tolerance. Ajay is a medium duration hybrid with long slender grain, released for cultivation in irrigated-shallow lowlands of Odisha. As these hybrids are adaptable for eastern Indian climatic conditions with assured remuneration, 12 private seed agencies over five states have signed MoUs and taken license to commercialize them.



To make this technology more sustainable and amenable to farmers, trait development strategy among the parental lines becomes mandatory. The parents of hybrids Ajay, Rajalaxmi and CR Dhan 701 have been improved for bacterial blight, the most devastating disease of rice (Das et al. 2016). The submergence and salinity are the major abiotic stresses occur frequently in rain-fed shallow lowland area and cause substantial yield loss in rice. Hence, to cope up with the problems, and make hybrid rice more sustainable during these adversities, ICAR-NRRI has successfully stacked submergence and salinity tolerant QTLs in the seed parents of CRMS 31A and CRMS 32A. To enhance the seed producibility, introgression of stigma exertion trait from *O. longistaminata* into CRMS 31A and CRMS 32A, are under progress. To excavate the genetic region responding heterosis in rice, transcriptomic analysis of hybrids Rajalaxmi and Ajay are completed and interpreted. Availability of restorers for WA-CMS lines is very stumpy in nature, only 15% of total rice genotypes are having the ability to restore complete fertility in WA-CMS based hybrid rice (Katara et al. 2017). Hence, good combiner genotypes having partial fertility restorers Mahalaxmi and Gayatri were improved by introgressing fertility restorer gene(s) *Rf3* & *Rf4* through MABB approach. Further, to make clear cut identity and ensure pure seed of parents/hybrids, 12 signature markers that unambiguously distinguish 32 rice hybrids were developed, which can be utilized for DNA fingerprinting and genetic purity testing of hybrids.

Hybrid rice technology is impressive as it enhances farm productivity of 15-25% more than HYVs. Given its yield advantage and economic importance, 101 rice hybrids have been commercialized in the country, which creates a huge seed industry world-wide. Moreover, this venture also has great entrepreneurship opportunity, creates additional employment for 100-105-man days ha⁻¹ in seed production. Hybrids developed in the country are being commercialized under public-private partnership (PPP) mode. Three NRRI bred hybrids are being commercialized with 19 private seed agencies as total 38 MoUs were signed which could generate direct revenue of Rs. 182.00 lakhs (2010-2018) to the Institute.

2.8. Genetic improvement of rice for aroma, nutrition and grain quality

Since inception of the institute, grain quality has remained a focus of researchers, besides yield, dragging the major attention in breeding programs. During 1950s to 60s when *indica-japonica* hybridization program was at its peak, studies were undertaken on traits that could be easily phenotyped, like grain type, grain colour, head rice recovery, milling percentage, etc. Breeding program for fine grain was initiated as early as 1953. Initially, the method of pedigree breeding was chosen for improvement of grain characters of otherwise popular varieties like Ptb10. Later, mutation breeding became the method of choice for altering the grain traits of popular varieties like TN1, Taichung 65



and CR2001. Head rice recovery percentage which has now been accepted as one of the major criteria to decide the release of varieties through AICRIP, was first undertaken as a trait of investigation during 1960s. The nature, texture and type of chalkiness of endosperm were reported to affect the HRR%. These endosperm traits were found to be highly affected by the genetic factors of the variety. Crop harvesting at 30-40 DAF with 20-23% grain moisture content and shade drying to 11-13% moisture, yielded highest hulling recovery by reducing the development of sun cracks in the kernels during field drying. Inheritance studies on complex traits like cooking quality and protein content were undertaken in bi-parental populations and were concluded as to be governed by multiple genes. The role of minor genes in trait expression was also reported. Genetic studies on kernel colour were undertaken in the segregating generations of GEB24 and Ptb10 and linkage of the trait with others were also established. However, breeding initiatives for the complex traits like palatability, sensory traits, cooking quality, value added products, etc. could not become possible due to the difficulty and technical constraints involved in phenotyping of the traits. Traits that could be easily phenotyped, like grain type, aroma, were subjected to improvement keeping yield in the centre.

The existing genetic diversity for short-grained aromatic genotypes in eastern India appealed the plant breeders to enhance the yield potential of the popular landraces. This was initiated with the vision to improve the economic status of farmers by cultivating and preserving them despite the replacement pressure created by high yielding varieties. The initial improvement was through pure line selection of landraces like Dhusara, Chinikamini, Kalajeera yielding $<1.5 \text{ t ha}^{-1}$. The varieties purified from the landraces were renamed as Nuadhusara, Nuachinikamini, Nuakalajeera respectively, which could yield 4.5t/ha, improving the yield potential by three times. Moreover, the varieties fetched higher remuneration in the market due to their qualities. Later came the phase of creation of variability through hybridization. Marker assisted pedigree breeding led to the development of CR Sugandh Dhan-907 by crossing Pusa 44 and Dubaraj, a landrace belonging to Chhattisgarh (Patnaiket al. 2015). Participatory plant breeding was integrated with the molecular breeding for better genetic gain in Kalajeera, Machhakanta and Haladichudi (Roy et al. 2017). Subsequently, aromatic, long grained, non-basmati varieties like Poorna bhog and Geetanjali were developed and released through mutation of basmati genotypes. Among these, Geetanjali variety has been used for establishment of rice value chain in Odisha and Poorna bhog is gaining popularity. Phenotypic descriptors with molecular markers were used to characterize the short grain aromatic landraces of eastern India (Roy et al. 2014 & 2016). A greater insight into the origin and their population structure was derived by integrating the analysis of both nuclear and chloroplast DNA sequence variations (Roy et al. 2016).



Protein in rice has ever been a major concern due to its lowest grain protein content among cereals. Positive correlation of bran and aleurone layers with protein and thiamine content was identified and high protein content genotypes were found to have a cellular patch in spermoderm (NRRI Annual Reports, 1949-50 and 1950-51). Studies on variation in protein content and aleurone layer thickness were undertaken in 450 representative genotypes of *japonica*, *indica* and *javanica* rice. Existence of positive correlation between aleurone layer thickness and percent protein content in kernels was reported earlier. Protein content in rice kernels varies from 6.1% to 10.1% while the aleurone layer thickness varied from 11.2 μ m to 75.0 μ m. Moreover, protein content in *japonica* rice was found to be higher than in white kernelled *indica* types. Inheritance of protein content was studied by the Rice Technology Section established during 1963 and they recorded frequency of occurrence of high protein genotypes among different duration classes of rice varieties in temperate and tropical zones. Due to 4-5% milling, a loss of 11-12% protein as compared to brown rice was recorded during 1966. Studies on inheritance of protein content suggested the role of polygenes in trait expression while appearance of transgressive segregants in F_2 , suggested the possibility to breed pure lines for higher protein content than in parents. Near infrared spectroscopy (NIR) was therefore calibrated and validated for large scale and high throughput phenotyping of GPC (Bagchiet al. 2015). Breeding for high protein rice was initiated involving two landraces from Assam. High protein trait was transferred to high yielding backgrounds of Naveen and Swarna through backcross breeding to release the first high protein variety in Naveen background as CR Dhan 310 (released by CVRC for Odisha, Uttar Pradesh and Madhya Pradesh) with 10.3% GPC in 2016. Another variety CR Dhan 311 has been released by State Variety Release Committee of Odisha in the name of 'Mukul' with GPC of 10.1% and Zn content of 20 ppm. By using a backcross derived mapping population, a consistent QTL (qGPC1.1) over the seasons have also been identified (DARE/ICAR Annual Report 2015-16 and 2016-17 and NRRI Annual Report 2014-15). Quality breeding has come across several phases and has a long way to go with the objective to ensure the nutritional and economic security. With more quality traits coming under non destructive phenotyping, their genetic improvement will further be accelerated.

2.9. Genetic improvement of direct seeded rice

Several studies have reported that grain yield under transplanted system has edge over dry direct seeded rice. On the contrary, no significant differences were observed between the two systems (Mahender et al. 2015). In recent years, ICAR-NRRI has played a significant role in the development of rice varieties for the dry direct aerobic condition with yield potential of 4.0 - 4.5 t/ha. As these aerobic rice varieties require minimum maintenance and save labour they are getting popular across eastern states of West Bengal, Jharkhand, Odisha and Chhattisgarh. On the other hand, developing a hybrid for dry



direct system would be beneficial. As hybrids are heterotic in nature, they yield higher in favourable conditions and marginal lands. Recently, two rice hybrids viz. GK-5022 and KPH 272 were released by private companies for the dry direct aerobic condition with yield potential of 4.0-4.2t/ha.

To surpass the gain of transplanted rice, understanding of direct seeded environment and traits adaptable for that environment need to be addressed. Good seed vigour, high mesocotyl length with anaerobic germination is very much essential to break the earth crust to have uniform emergence. However, anaerobic germination will address the problem of water stagnation in the improperly levelled field. Rice has the fibrous root system and developed for the transplanted condition. Over several decades, the below-ground portion for rice plant has been neglected. Attaining vigorous seedling growth and improving root parameters are necessary to improve nutrient acquisition and to compete against the weeds under direct seeded condition. Increase in nodal root in the early phase of the cropping period and deep root with more branching will facilitate the nutrient uptake under limited moisture. Erect leaves, thin and strong culm will have proper assimilation of photosynthates. That would address the problem of improving water productivity and helps in more spikelet fertility and high grain yield. ICAR-NRRI in collaboration with International Rice Research Institute, Philippines, has developed a comprehensive breeding program to develop new generation rice for direct seeded condition by introgressing the above discussed QTLs/ genes (Table 1).

Table 1. Donors, QTLs, genes, trait information used to improve dry direct seeded rice.

Donors	QTLs/Gene	Trait	Reference
IR93312-30-101-20-13-66-6	qAG _{9,1'} , qAG _{9,2}	Anaerobic germination	Angaji <i>et al.</i> (2010)
IR94226-B-177-B	qNR _{5,1'} , qEVV _{9,1'} , qRHD _{1,1}	High nutrient uptake (N,P, Fe, Zn), Early vigor, nodal root,	Sandhu <i>et al.</i> (2015)
WAB 880-1-27-9-2	qNR _{5,1}	High nutrient uptake (N,P, Fe, Zn)	Sandhu <i>et al.</i> (2015)
IR94225-B-82-B	qGYDS _{1,1'} , qGYDS _{6,1'} , qGYDS _{9,1} , qGYDS _{10,1}	Grain yield DS	Sandhu <i>et al.</i> (2015)
IR91648-B-289-B	qLDG _{3,1'} , qLDG _{4,1}	Lodging resistance	Dixit <i>et al.</i> (2015)
IR91648-B-336-B	qCS1.1	Lodging resistance	Yadav <i>et al.</i> (2017)
IR91648-B-13-B	qEUE3.1	Early uniform emergence	Singh <i>et al.</i> (2017)
IR91648-B-32-B-B	qEUE11.1	Early uniform emergence	Dixit <i>et al.</i> (2015)



Thus, the stacking of multiple QTLs/gene in single background for nutrient use efficiency, lodging resistance, seedling vigor, anaerobic germination, yield related QTLs could assist in development of dry direct seeded rice varieties with greater productivity and would drastically reduce the input cost and water which ultimately leads to greater economic returns to the farmers.

2.10. Biotechnological applications in rice improvement

2.10.1. Doubled haploids

The ICAR-NRRI, Cuttack initiated the work on doubled haploid technique in 1997 to overcome the constraints associated with the rice hybrids such as (1) high seed cost (2) high sensitivity to unpredictable environmental condition and (3) asynchronous flowering. Since doubled haploid approach can effectively address the problems associated with hybrid rice through production of high yielding doubled haploids with uniform grain quality, the basic studies on *indica* rice anther culture assumes great significance.

Considerable progress has been made as evidenced by release of two DHs as new varieties named Satyakrishna and Phalguni in 2008 and 2010, respectively. Satyakrishna (CRDhan 10) has been developed from hybrid PHB-71 and is best cultivated in shallow irrigated low lands. Its maturity duration is 135 days. Satyakrishna is semi dwarf (105 cm) and possesses long slender grains with yield potential of 4.5-5 tha^{-1} in *kharif* and 5-6 tha^{-1} in *rabi*. Phalguni was obtained from the popular hybrid KRH-2 and is cultivated in bunded upland and irrigated land. It is 105 cm tall and matures in 117 days. It also possesses long slender grains with resistance to leaf folder and leaf blast and moderate resistance to sheath rot, rice tungro virus, gall midge, brown spot and sheath blight. In 2013, attempt was made to standardize androgenic protocols in two *indica* rice hybrids i.e. CRHR32 (an elite long duration rice hybrid of NRRI, Cuttack) and BS6444G (a popular rice hybrid of Bayer Seed Pvt. Ltd.) for generation of doubled haploids. Further, anther culture was used for development of mapping population from Savitri (a high yielding *indica* rice variety) \times Pokkali (a salt tolerant *indica* rice genotype) for identification of salt tolerant QTL/gene. Besides, two crosses of B \times B and R \times R were taken for generation of DHs using anther culture for parental line improvement to be used in development of rice hybrid. However, the production of albino shoots (60-100%) in all the cultures proved to be detrimental for optimization of androgenic response. Therefore, NRRI attempted to develop a protocol for suppression of albinism, which is also a frustrating feature. This led to standardization of 100% albino free shoot regeneration method in *indica* rice; patent and filed 1355/KOL/2015 entitled "Method for albino free shoot regeneration in rice through anther culture".

Subsequently, the improved protocol could generate 150, 200, 117, 73 and 30 DHs from CRHR32, BS6444G, Savitri \times Pokkali, B \times B and R \times R respectively and surprisingly no haploids were observed in BS6444G, which showed 100%



spontaneous doubling. After proper assessment of ploidy status of the regenerants, 20 and 13 DH lines developed from CRHR32 and BS6444G respectively, were selected based on the morpho-agronomic characters and advanced for replicated trial for 5 consecutive seasons (*kharif* 2014-2018). Further, 6 promising DH lines derived each from CRHR32 and BS6444G showed promising grain yield and quality. Six promising DH lines developed from CRHR32 and BS6444G were nominated for AICRP trial.

All the 6 DH lines of BS6444G showing promising yield (BS6444G) were found to be aromatic confirmed by PCR and sequencing of *badh2*. A combination of BLB resistance genes (*Xa21+* *Xa4+* *Xa7+* *xa5*) were also confirmed in 2 DH lines developed from BS6444G through molecular markers and bioassay. Besides, four DHs each derived from CRHR32 and BS6444G showed superior grain quality as compared to other DHs. Moreover, 4 DH lines derived from rice hybrid, CRHR32, were identified containing high protein (11.59-12.11%). Furthermore, isocytorestorers lines were developed through test cross of the 13 DHs (BS6444G) carrying positive Rf4 genes with the CMS, with an average of 500-600 grains per panicle.

A systematic study was carried out to find out the salinity tolerant lines during germination in different salinity levels for which 117 DHs derived from F₁s of Savitri (popular HYV) and Pokkali (salinity tolerant) were screened. This resulted in the identification of 4 candidate genes such as LOC_Os01g09550 (no apical meristem protein), LOC_Os01g09560 (mitochondrial processing peptidase subunit alpha), LOC_Os12g06560 (putative protein) and LOC_Os12g06570 (cyclic nucleotide-gated ion channel) using BSA. Subsequently, an efficient androgenic protocol was developed for another popular quality *indica* rice hybrid, 27P63 (M/s Pioneer, Hyderabad) and generated 345 green plants. After proper examination of ploidy status with further confirmation of 325 DHs using molecular markers, field evaluation was made for identification of superior lines using morpho-agronomic characters from which 92 DHs were selected for further evaluation out of which 25 DHs were found superior in terms of grain yield and quality.

About 15 years efforts of NRRI have overcome some of the problems that have enabled high frequency generation of doubled haploids from a number of commercial and experimental hybrids. Now, this expertise attracts the researchers from public sector institutions and private companies engaged in rice breeding to undertake training in this area. It is high time that country should take full advantage of this technology to apply at commercial scale.

2.10.2. Transgenics

Biotechnology research at the NRRI was initiated in the year 1999-2000 with an objective to build suitable capabilities for rice tissue and protoplast culture. Work was initiated on use of RFLP/RAPD and other DNA markers for genome analysis for rice improvement and genetic analysis of gall-midge resistance. A



working molecular plant-pathology laboratory was also set up with the objective of marker-assisted selection for pathogen resistance.

During 2000-2001, a chymotrypsin inhibitor gene construct (pSKB: pMasJ35S-potato chymotrypsin inhibitor-nospolyA) was mobilized into an *Agrobacterium tumefaciens* strain LBA4404. *Agrobacterium* transformation of *indica* rice Pusa Basmati 1 was performed using 25 days-old-calli and resistant calli were placed for regeneration. Of the 38 putative regenerants produced, 34 plants were positive for the gus both through histochemical and PCR assays. In the year 2001-02, further attempts were made for genetic transformation of *indica* rice lines, Pusa Basmati 1 and Swarna. Three-week-old callus derived from the mature seeds used for *Agrobacterium tumefaciens* (LBA 4404) mediated transformation plasmid construct having potato chymotrypsin gene cloned under 35S dual promoter and a nos terminator. A total 65 putative transgenics were obtained from Pusa Basmati 1 and 11 putative transgenics from Swarna. Molecular analysis confirmed the presence of *GUS* (reporter gene) and *hpt* (marker gene) genes. Work was also reported on standardization of callusing and regeneration protocols in Savitri, Naveen and Tapaswani for genetic transformation with *CryIAcandXa21*, and chitinase genes through biolistic approach. Satisfactory callusing (51 to 86 %) was reported on MS medium with 2,4-D (2.0 mg/1) and kinetin (0.5 mg/1). For obtaining required level of regeneration, MS medium with BAP (1.0 mg/1), kinetin (1.0 mg/1) and NAA (0.25 mg/1), MS medium with BAP (2.0 mg/1) and NAA (0.25 mg/1) and MS medium with kinetin (2.0 mg/1) and NAA (0.25 mg/1) were examined.

During 2002-03, transformation of Swarna with a potato trypsin inhibitor gene was accomplished. Southern analysis confirmed the presence of trypsin inhibitor gene in the putative transgenic rice lines. Later in 2004-05, transgenic lines expressing a *pin ii* gene were developed in the genetic background of PusaBasmati-1 and Swarna and these lines were subjected to the insect bioassays against YSB and leaf folder, and two transgenic lines from both PusaBasmati-1 and Swarna showed higher levels of resistance to both the insects. In the year 2007-08, putative transgenics containing *Cry 1AB* gene were developed using both biolistic and *Agrobacterium*-mediated approaches for conferring resistance against yellow stem borer (YSB). A *Trichoderma* derived chitinase gene conferring resistance against rice sheath blight and *DREB1A* gene for conferring tolerance against drought were also used for rice transformation. The plants were grown to maturity and no morphological abnormalities were observed. In the year 2007-08, work was initiated to develop transgenic rice line over-expressing ferritin cDNA. Binary vector carrying ferritin cDNA was mobilized into *Agrobacterium tumefaciens* strain EHA105.

In another attempt to develop transgenic Swarna line with *Cry1Aabc* gene for YSB resistance, 150 calli survived after selection. In another experiment, highly embryogenic calli derived from Gayatri and Swarna were co-cultivated with Agro strain LBA 4404 having pDREB1A and 120 and 150 calli, respectively, of the genotypes are on pre-regeneration media.



The high yielding variety Naveen was later used for transformation of NADP-Malate dehydrogenase gene using embryogenic callus. The regenerated shoots arising from calli on regeneration media were transferred to rooting media and rooted plantlets were transferred to soil for further analysis which is underway.

Transgenics were also developed with phosphomannose isomerase (*pmi*) gene in the genetic background of an elite *indica* rice cultivar, Pusa Basmati-1 and with zinc transporter gene into *indica* rice cultivars PB-1 and BPT 5204. A Swarna line with *Pennisetum glaucum* eukaryotic translation initiation factor 4A (*PG-eif4A*) was developed for increased salinity tolerance at germination and seedling stage. Enhanced activity of antioxidant enzymes (SOD and APX) was observed in PG-eif4A over expressed transgenic lines of Swarna. Rice variety Naveen was also transformed with NADP-Malic Enzyme gene and higher transformation efficiency was achieved. The GUS expression of the transformants confirmed the existence of the desired gene in the callus and the putative transgenic plant developed was used for further analysis.

3. IMPACTS OF THE DIVISION

The hybrids developed from this Division have been licensed and are being commercialised through various Seed companies (Table 2) and the farmers are getting benefitted.

Besides the hybrid technology, one patent entitled “Protocol standardized for albino free shoot regeneration in *indica* rice to hasten breeding cycle” has been filed in Indian Patent Office vide File No. 1355/KOL/2015.NRRI has imparted training to the researchers of several institutes on the doubled haploid technique.

4. ASPIRATIONS

Germplasm is always a basic material for any crop improvement programs for sustainable agriculture. A road map depicting collection sites need to be prepared so that areas which are not covered in the map will be explored. Wild and primitive populations are the reserves of cryptic variability and hence, their capacity for adaptive response is high. Such genetic variation is as important as prevalent varietal diversity for genetic conservation. It is, therefore, important to collect and conserve them. It has been estimated that even 5% of rice germplasm conserved in different gene banks have not been utilized. Our research should be oriented towards developing a core collection which represents the diversity of entire collection and removes duplicate accessions that would enhance the use of germplasm by identifying diverse source of parents and also would ease in evaluating the germplasm against biotic and abiotic stresses. Identifying trait-specific, genetically diverse parents i.e., salt tolerance, cold tolerance, drought tolerance, early/late heading, low chilling,

Table 2: Varieties and technologies commercialized during 2009-2018.

Sl. No.	Variety/hybrid/technology	Institute/company
1	Ajay (CRHR 7)-Hybrid	Nodai Seed India Pvt. Ltd., Gurgaon
2	Rajalaxmi (CRHR 5)-Hybrid	Nodai Seed India Pvt. Ltd., Gurgaon
3	Ajay (CRHR 7)-Hybrid	Annapurna Seeds, Kolkata
4	Rajalaxmi (CRHR 5)-Hybrid	Annapurna Seeds, Kolkata
5	Ajay (CRHR 7)-Hybrid	Vicky Agrisciences Pvt. Ltd., Hyderabad
6	Rajalaxmi (CRHR 5)-Hybrid	Vicky Agrisciences Pvt. Ltd., Hyderabad
7	Ajay (CRHR 7)-Hybrid	Signet Crop Sciences, Pvt. Ltd., New Delhi
8	Rajalaxmi (CRHR 5)-Hybrid	Signet Crop Sciences, Pvt. Ltd., New Delhi
9	Parental lines of Ajay- Hybrid	IFSSA, Hyderabad
10	Parental lines of Rajalaxmi- Hybrid	IFSSA, Hyderabad
11	Ajay (CRHR-7)-Hybrid	Sansar Agropol Pvt. Ltd. Bhubaneswar
12	Rajalaxmi (CRHR-5) Hybrid	Sansar Agropol Pvt. Ltd. Bhubaneswar
13	Rajalaxmi-(CRHR 5)-Hybrid	Nath Biogene(I) Ltd., Aurangabad, Maharashtra
14	CR Dhan 701-(CRHR-32) -Hybrid	NathBiogene (I) Ltd., Aurangabad, Maharashtra
15	Ajay (CRHR-7)-Hybrid	PAN Seeds Pvt. Ltd., Kolkata
16	Rajalaxmi-(CRHR 5)-Hybrid	PAN Seeds Pvt. Ltd., Kolkata
17	Ajay (CRHR-7)-Hybrid	Vikkys Agrisciences Pvt. Ltd., Hyderabad
18	Rajalaxmi-(CRHR 5)-Hybrid	Delta Agrigenetics Pvt. Ltd., Hyderabad
19	CR Dhan 701-(CRHR-32) -Hybrid	Delta Agrigenetics Pvt. Ltd., Hyderabad
20	Ajay (CRHR-7)-Hybrid	Balaji Agri Biotech Pvt. Ltd. Paikmal, Odisha
21	Rajalaxmi-(CRHR 5)-Hybrid	Bharat Nursery Pvt. Ltd. Kolkata, West Bengal
22	Ajay (CRHR-7)-Hybrid	SaiShradha Agronomics and Husbandry Pvt. Ltd. Puri, Odisha

Contd...



Sl. No.	Variety/hybrid/technology	Institute/company
23	CR Dhan 701-(CRHR-32) -Hybrid	Sri Sai Swarupa Seeds Pvt. Ltd., Warangal, Andhra Pradesh
24	Rajalaxmi-(CRHR 5)-Hybrid	Vibha Agrotech Ltd., Hyderabad
25	CR Dhan 701-(CRHR-32) -Hybrid	Vibha Agrotech Ltd., Hyderabad
26	Rajalaxmi-(CRHR 5)-Hybrid	Sansar Agropol Pvt. Ltd., Bhubaneswar
27	Ajay (CRHR-7)-Hybrid	Sansar Agropol Pvt. Ltd., Bhubaneswar
28	CR Dhan 701-(CRHR-32) -Hybrid	GV Seeds, Hyderabad
29	Rajalaxmi-(CRHR 5)-Hybrid	GV Seeds, Hyderabad
30	CR Dhan 701-(CRHR-32) -Hybrid	Bioseed research India
31	CR Dhan 701-(CRHR-32) -Hybrid	Delta Agri Genetics Pvt. Ltd., Hyderabad
32	Rajalaxmi-(CRHR 5)-Hybrid	BharatiyaBeej Nigam Ltd.
33	CR Dhan 701-(CRHR-32) -Hybrid	Nath bio-genes PVT. LTD
34	Rajalaxmi-(CRHR 5)-Hybrid	Nath bio-genes PVT. LTD
35	Rajalaxmi-(CRHR 5)-Hybrid	PAN Seeds PVT. LTD.
36	Rajalaxmi-(CRHR 5)-Hybrid	Bharat Nursery Pvt Ltd., Kolkatta
37	Rajalaxmi-(CRHR 5)-Hybrid	Mahalaxmi Seeds Corporation, Begusarai, Bihar
38	CR Dhan 701-(CRHR-32) -Hybrid	Jay Shankar Agro Inputs Pvt. Ltd. Delhi

tolerance/resistance to particular pests/diseases, adaptability to water logged habitats, tillering capacity, root system, leafiness, etc., apart from quality characteristics are the primary need of the plant breeder for trait enhancement. A rice seed file depicting photograph of individual germplasm may be prepared for identification of germplasm and avoiding misrepresentation of germplasm. Morphological and molecular characterization of a core/ minicore and trait specific subsets would further enhance the usefulness of the germplasm accessions. Work in this line has been initiated at this Institute (Jambhulkar et al. 2017).

Under changing climatic and agriculture scenario, rice hybrid is likely to face stiff competition to sustain in future. Despite having great potential to enhance production and productivity, it has not been adopted on large scale as was expected. This is due to several constraints like lack of acceptability of hybrids in some regions such as southern India, due to region specific choice grain quality requirement. Moderate (15-20%) yield advantage in hybrids is not economically very attractive and there is a need to increase the magnitude of heterosis further. Lower market price offered for the hybrid rice produce by millers/traders is acting as a deterrent for many farmers to take up hybrid rice



cultivation. Higher seed cost is another restraint for large scale adoption and hence, there is a need to enhance the seed yield in hybrid rice seed production plots in order to reduce seed cost. Efforts for creating awareness for technology transfer were inadequate in initial stages. Involvement of public sector seed corporations in large-scale seed production has been less than expected. Hybrids for aerobic/upland, *boro* season and long duration for shallow lowland conditions are to be developed. Most of the constraints mentioned above are being addressed with right earnestness through the on-going research projects and transfer of technology efforts.

5. CONCLUSIONS

Though rice germplasm are collected either through explorations or exchange programmes and conserved in the gene bank but information on their trait characteristics are not complete. Germplasm without characterization and evaluation data are meaningless as per as their utilization aspect in crop improvement programme is concerned. Therefore, systematic characterization, evaluation and documentation of important traits against each germplasm are required to be done for better utilization by the breeders. The database on agro-morphological characters of all the germplasm conserved in gene banks need to be prepared. Also a National/central rice data base be prepared in collaboration with the research centres working in collaboration with NBPGR. Research work should be oriented towards developing a core collection for better management and utilization of the germplasm. Human resource development by imparting training to persons engaged in PGR activities is required for proper utilisation and conservation of germplasm.

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