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Comparative analysis of effectiveness of wide compatibility (WC) trait between improved maintainer line having WC and without WC gene in rice

RAHUL PRIYADARSHI[abc1*], HARI PRASAD A.S.[b2], AKHILESH KUMAR SINGH[c3], ULAGANATHAN K.[a4] and VINAY SHENOY[c5]

[a] *Deptt. of Genetics, Osmania University, Tarnaka, Hyderabad - 500 007 (Telangana)*

[b] *ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad - 500 030 (Telangana)*

[c] *Barwale Foundation Research Centre, Himayathnagar, Hyderabad - 500 029 (Telangana)*

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ABSTRACT

Hybrid rice technology is one of the potential options for increasing rice yield. The present study was carried out to know the effectiveness of wide compatibility trait. The compatibility as measured by percentage spikelet fertility in F_1s revealed it to vary between the IR58025WCB having wide compatibility (*WC*) gene and IR58025B. The F_1s were also analyzed for pollen fertility with I_2-KI solution. In crossing of IR58025WCB with *indica*, *japonica* and *tropical japonica* testers, group mean spikelet fertility 83.62%, 77.75% and 69.16% respectively; and in comparison to crossing of recurrent parent IR58025B with *indica*, *japonica* and *tropical japonica* testers, group mean spikelet fertility 81.80%, 43.90% and 59.94% respectively. The anticipated increase in group mean percentage overcome with *indica*, *japonica* and *tropical japonica* testers 2.50%, 13.93% and 36.99% for pollen fertility and 2.22%, 77.11% and 15.38% for spikelet fertility respectively. Such kind of approach helps in the combination of genetic background of the two dissimilar sub-species. The effectiveness of wide compatibility trait in improved parental line would be used for exploitation *indica* and *japonica* in hybrid rice breeding programme.

Key words : *Hybrid sterility, Inter-subspecific crosses, Maintainer line, Rice, Wide compatibility trait.*

INTRODUCTION

Rice (*Oryza sativa*) is a staple food for almost two thirds of the world population plays a key role in Indian economy. To feed an increasing population of India needs to produce at least 125 million tonnes of rice by year of 2020. Hybrids possess a yield advantage of 15–20% yield over the best commercial rice varieties (Virmani, 1996; Normile, 1999) and at least 72 public and private bred hybrids are available for commercial cultivation in India. Exploitation of inter-subspecific heterosis in rice is recognized as one of the innovative approaches to further enhance genetic yield potential. A major difficulty encountered in the development of such intersubspecific hybrid is the complete or partial hybrid sterility (HS) frequently observed in most *indica/japonica* crosses (Kato *et al*., 1928). The magnitude of heterosis in Asian cultivated rice (*Oryza sativa* L.) is reported to be in the order of *indica/japonica* > *indica / javanica* > *japonica / javanica* > *indica/indica* > *japonica / japonica* (Yuan, 1994; Zhang *et al.,* 1993). The inter sub specific hybrids (*indica/japonica*) possess approximately 30% higher yield potential compared to inter-varietal hybrid (Cao and Zhan, 2014).

The wide compatibility is a neutral allele (*n*), which overcome hybrid sterility between *indica* and *japonica* and useful for better heterosis. The *indica* allele *i* and *japonica* allele *j* show hybrid sterility when come together in crosses. So far, all homozygotes (*i/i, j/j*, and *n/n*) and heterozygotes with *n* allele (*j/n* and *i/n*) remain fertile. These results made Prof. Ikehashi and his group to conclude that the neutral allele *n* or *WC gene* was capable of overcoming the complete or semi-sterility in *indica/japonica* hybrids. The *wide compatibility gene* has been incorporated into several *indica* and *japonica* varieties in Japan, China and at IRRI for development of higher yielding varieties and hybrids, through conventional breeding (Araki *et al.,* 1990; Ikehashi, 1991). The objective to comparative study of effectiveness of wide compatibility trait between improved line having *WC* gene and original parent without *WC* gene through estimation of the magnitude of pollen and spikelet fertility.

[1] Ph.D. Scholar Presently - IIRR, Hyderabad *(rhl.priyadarshi@gmail.com)
[2] Principal Scientist (IIRR), [4] Professor (CPMB),
[3] Breeder (Presently - Rasi Seeds Pvt. Ltd., Hyderabad, Telangana)
[5] Head Biotechnology (Presently Kaveri Seed Co. Ltd., Secunderabad)

MATERIALS AND METHODS

IR58025B is an elite maintainer line of WA cytoplasm of rice derived from the cross between female IR48483A/8*PUSA 167-120-3-2 and male PUSA167-120-3-2 having long slender grain and mild aroma. It is a maintainer line of IR58025A. The study was conducted at the research farm of the Barwale Foundation (BF), Maharajpet, Hyderabad, India during *kharif* 2014 to *rabi* 2014-15. IR58025WCB having *WC* gene and IR58025B were crossed independently with five *Indica* testers (APO, IR36, IR72, IR 64 and Shan Huang Zhan 2), five *Japonica* testers (Kinmaze, Nipponbare, Tainung 67, CT9993 and M 201) and five *Tropical Japonica* testers (Moroberekan, IR68552-55-3-2, Azucena, Banten and Calotoc) to produce 20-25 F_1 seeds per cross during *kharif* 2014. The pollen parent and the respective F_1 was planted side by side followed by another set of pollen parent and F_1 during *rabi* 2014-15. About 25 day old F_1 seedlings from each of the crosses were planted adopting a spacing of 15 x 15 cm between pollen parent and F_1 and 30 x 15 cm between F_1 and pollen parent of another set. Recommended package of cultivation practices was followed. Care was taken to plant single seedling per hill.

Pollen was anticipated as percentage pollen staining, based on panicles of main tillers of five individual plants of each F_1. For pollen fertility, one or two panicles per plant were sampled at the time of flowering, unopen six florets per panicles were taken randomly and fixed in 70% (v/v) ethanol. One anther per floret was collected, and the six anthers from the same panicle were mixed and spread on a microscopic slide and crushed using 10-20 µl 1% Iodine-Potassium Iodide solution (Rosamma and Vijayakumar, 2005); the slides were examined under microscope by using 40x magnification. Approx 300 pollen grains per sample, were scored for pollen fertility. The pollen with deeply stained and round was considered as fertile and the pollen with pale stain and shriveled was considered as sterile. Staining of pollen depends on the relative starch contents. Pollen fertility (%) of plants was calculated as the ratio of the number of stained grains to the total number of counted pollen grains.

$$\text{Spikelet fertility (SF) \%} = \frac{\text{Number of filled spikelet in the panicles}}{\text{Total number of spikelet in the panicle}} \times 100$$

Based on percentage spikelet fertility of testcross F_1 plants were classified as above 65% (normal fertile), intermediate fertile (40-65%) and less than 40% (semi-sterile) (Vijay Kumar and Virmani, 1992).

RESULTS AND DISCUSSION

In crossing of IR58025WCB with *indica*, *japonica* and *tropical japonica* pollen fertility ranged from 73.33% (IR 64) to 88.33% (IR72), 60.00% (Tainung 67) to 88.33% (M 201) and 36.67% (Banten) to 81.67% (Moroberekan) respectively; and in comparison to IR58025B with *indica*, *japonica* and *tropical japonica* testers, pollen fertility ranged from 75.00% (Shan Huang Zhan 2) to 85.00% (IR72), 35.00 (Kinmaze) to 93.33% (M 201) and 25.00% (Banten) to 68.33% (Calotoc) respectively. It was observed that estimates of group mean pollen fertility in IR58025WCB with *indica*, *japonica* and *tropical japonica* testers 82.00%, 76.33%, and 61.00% respectively; and in comparison to crossing of recurrent parent IR58025B with *indica*, *japonica* and *tropical japonica* testers, group mean pollen fertility 80.00%, 67.00% and 44.53% respectively. In crossing of IR58025WCB with *indica*, *japonica* and *tropical japonica* standard deviation in percentage pollen fertility ranged from 5.00 (APO) to 10.41(IR36), 0.00 (CT9993) to 11.55 (Kinmaze) and 5.00 (IR68552-55-3-2) to 14.43 (Azucena) respectively; and in comparison to IR58025B with *indica*, *japonica* and *tropical japonica* testers, standard deviation in pollen fertility ranged from 5.00 (IR 64, Shan Huang Zhan 2) to 10.00 (IR72), 2.89% (Nipponbare, Tainung 67, M 201) to 10.41 (CT9993) and 5.00 (Banten) to 30.55 (Calotoc) respectively. Details of pollen fertility was mentioned in **Table 1**.

In crossing of IR58025WCB with *indica*, *japonica* and *tropical japonica* testers, spikelet fertility ranged from 78.29% (APO) to 88.48% (IR72), 67.63% (Kinmaze) to 86.13% (Nipponbare) and 67.71% (Azucena) to 72.70% (Calotoc)

Table 1. Comparative study of F_1 percentage pollen fertility (% PF±SD) of crosses involving IR58025B and IR58025WCB with *Indica, Japonica* and *Tropical Japonica* testers

	Mean pollen fertility with					
Genotypes	*Indica testers*	% PF	*Japonica testers*	% PF	*Tropical Japonica testers*	% PF
IR58025B	APO	78.33±7.64	Kinmaze	35.00±5.00	Moroberekan	38.33±15.28
	IR36	81.67±7.64	Nipponbare	81.67±2.89	IR68552-55-3-2	40.00±8.66
	IR72	85.00±10.00	Tainung 67	61.67±2.89	Azucena	51.00±21.63
	IR64	80.00±5.00	CT9993	63.33±10.41	Banten	25.00±5.00
	Shan Huang Zhan 2	75.00±5.00	M 201	93.33±2.89	Calotoc	68.33±30.55
	Group mean	80.00	Group mean	67.00	Group mean	44.53
IR58025WCB	APO	85.00±5.00	Kinmaze	73.33±11.55	Moroberekan	81.67±7.64
	IR36	86.67±10.41	Nipponbare	85.00±10.00	IR68552-55-3-2	55.00±5.00
	IR72	88.33±5.77	Tainung 67	60.00±5.00	Azucena	58.33±14.43
	IR64	73.33±7.64	CT9993	75.00±0.00	Banten	36.67±11.55
	Shan Huang Zhan 2	76.67±7.64	M 201	88.33±5.77	Calotoc	73.33±12.58
	Group mean	82.00	Group mean	76.33	Group mean	61.00

respectively; and in comparison to IR58025B with *indica*, *japonica* and *tropical japonica* testers, spikelet fertility ranged from 75.39% (APO) to 86.02% (IR72), 28.71 (Kinmaze) to 62.22% (Nipponbare) and 50.98% (Banten) to 69.70% (Calotoc) respectively. Representative F_1 panicles showing effectiveness of wide compatibility trait was presented in **Fig. 1**.

In crossing of IR58025WCB with *indica*, *japonica* and *tropical japonica* testers, group mean spikelet fertility 83.62%, 77.75% and 69.16% respectively; and in comparison to crossing of recurrent parent IR58025B with *indica*, *japonica* and *tropical japonica* testers, group mean spikelet fertility 81.80%, 43.90% and 59.94% respectively. In crossing of IR58025WCB with *indica*, *japonica* and *tropical japonica* testers, standard deviation in spikelet fertility ranged from 0.13 (IR36) to 6.16 (IR64), 1.53 (Kinmaze) to 9.17 (CT9993) and 2.27 (IR68552-55-3-2) to 6.63 (Calotoc) respectively; and in comparison to IR58025B with *indica*, *japonica* and *tropical japonica* testers, standard deviation in spikelet fertility ranged from 0.93 (Shan Huang Zhan 2) to 2.91 (IR72), 0.49 (CT9993) to 6.13 (Kinmaze) & 0.34 (Banten) to 2.02 (Moroberekan) respectively. Details of spikelet fertility was presented in **Table 2**.

It was found that more standard deviation in percentage pollen fertility, compare to percentage spikelet fertility in F_1s. The estimated group mean pollen percentage overcome with *indica*, *japonica* and *tropical japonica* testers were 2.50%, 13.93% and 36.99% respectively; and for spikelet fertility percentage overcome 2.22%, 77.11% and 15.38% respectively. The hybrid fertility analysis showed a considerable variation in F_1s with improved maintainer line IR58025WCB having *WC* gene and IR58025B without *WC* gene with *japonica* and *tropical japonica* testers but almost similar with *indica* testers. The cause of differential expression in different backgrounds may be epistasis or non-allelic interactions. It was observed different degree of expression of *WC* genes and suggested the set of modifier gene(s) and epistasis (Kumar and Chakraborty, 2000). It was emphasized the role of additional genes modifying hybrid fertility in the presence of *WC* gene in one way or another (Kinoshita, 1995), whereas, it was proposed other loci with

A B C

Fig. 1. Spikelet fertility in F_1 of

A. IR58025B/IR36 and IR58025WCB/IR36;
B. IR58025B/Tainung67 and IR58025WCB/Tainung67;
C. IR58025B/IR68552-55-3-2 & IR58025WCB/IR68552-55-3-2 respectively

minor effect in addition to *S5* in improving hybrid fertility (Ikehashi and Wan, 1996). An *indica* variety when crossed with a set of *japonica* varieties would give hybrids of relatively uniform fertility as against variable fertility in crosses of a *japonica* with a set of *indica* varieties. *Indica* varieties are therefore considered to be the determiners of fertility behaviour in *indica*/*japonica* hybrids. This is possibly due to the fact that *indica* group as a whole is more diverse than *japonica* (Richharia *et al.,* 1962). High spikelet fertility of F_1 hybrids depends on the presence of *WC* gene either in male sterile or restorer line (Tao and Zhou, 1997). Wide compatibility varities (WCVs), when used as male parents exhibited positive effect in respect of number of spikelets/panicle and percentage spikelet fertility in their hybrids with *indica* and *japonica* testers (Vijaya Kumar *et al.* 1999). The hybrids between WCV on one hand and either *Indicas* or *Japonicas* on the other show normal pollen fertility. It was first used as *indica* (IR 36 and IR 50) *testers* and as *japonica* (Nipponbare and Akihikari) testers. A wide compatibility variety (WCV) should produce F_1 hybrid with over 90% pollen fertility and over 75-80% spikelet fertility when crossed with these testers (Ikehashi and Araki, 1984). It has been subsequently observed that *S5*^{n} did not neutralize hybrid semi-sterility in all combinations suggesting the possibility of involvement of more neutral genes. More than 50 loci across the rice genome have been shown to affect hybrid sterility. Of these

Table 2. Comparative study of F_1 percentage spikelet fertility (% SF±SD) of crosses involving IR58025B and IR58025WCB with *Indica, Japonica* and *Tropical Japonica* testers

	Mean pollen fertility with					
Genotypes	*Indica testers*	% PF	*Japonica testers*	% PF	*Tropical Japonica testers*	% PF
IR58025B	APO	75.39±1.54	Kinmaze	28.71±6.13	Moroberekan	57.81±2.02
	IR36	82.96±2.71	Nipponbare	62.22±0.84	IR68552-55-3-2	68.49±1.29
	IR72	86.02±2.91	Tainung 67	43.47±1.10	Azucena	52.71±0.65
	IR64	82.58±1.02	CT9993	38.96±0.49	Banten	50.98±0.34
	Shan Huang Zhan 2	82.04±0.93	M 201	46.12±2.05	Calotoc	69.70±1.00
	Group mean	81.80	Group mean	43.90	Group mean	59.94
IR58025WCB	APO	78.29±1.64	Kinmaze	67.63±1.53	Moroberekan	67.96±4.58
	IR36	83.94±0.13	Nipponbare	86.13±3.40	IR68552-55-3-2	68.49±2.27
	IR72	88.48±2.13	Tainung 67	78.38±1.80	Azucena	67.71±3.12
	IR64	81.30±6.16	CT9993	83.57±9.17	Banten	68.96±2.96
	Shan Huang Zhan 2	86.10±3.65	M 201	73.02±1.98	Calotoc	72.70±6.33
	Group mean	83.62	Group mean	77.75	Group mean	69.16

loci, approximately 20 loci control female gamete abortion, more than 30 are involved in male gamete abortion, and over 10 affect spikelet sterility (Ouyang *et al*., 2009). *Indica-Japonica* hybrids showed a pollen sterility of a about 45-55% on the basis of stain ability. It was reported that low germinability of morphologically normal pollen (<10%) could be a factor for sterility in *indica* x *japonica* hybrids (Liu *et al*., 1992). Spikelet fertility ranging from as low as 2.7% to as high as 88.4% in the inter-subspecific crosses indicated sterility neutralizing effect of *WC* genes to depend as well as on the genetic background of the parents involved (Reddy *et al*., 2000). Testcrosses with IR 64446-7-3-2-2 and IR 65598-112-2 showed spikelet fertility to be either similar to or higher than that of the parents and tester lines, suggesting these two lines to possess the *WC* genes. Both the lines are semi dwarf and possess the characteristics of IRRI's new plant type breeding line *i.e.* fewer tillers and larger panicles than the current modern high yielding varieties (Bharaj *et al*., 1994). Embryo-sac fertility showed more than 93% in the $S5^n$ gene–harboring hybrids, whereas embryo-sac fertility showed relatively low in control hybrids between typical *indica* and *japonica* cultivars without the $S5^n$ gene, suggesting that $S5^n$ can overcome the sterility between *indica–japonica* hybrids (Yang *et al*., 2012). F_1 hybrids, *indica* × *japonica* (*I* × *J*) showed hybrid sterility and spikelet fertility percentage ranged from 4 to 97% (Revathi P *et al*., 2015).

CONCLUSION

The present study demonstrates differential expression of hybrid fertility between improved maintainer line with *WC* gene and original parent without *WC* gene in terms of both pollen and spikelet fertility. The new materials developed showed overcome the hybrid sterility in the inter-subspecific crosses and would be prove to useful for *indica-japonica* hybrid technology to increase in yield potential.

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