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Crossability among backcross progenies harbouring different resistant genes for rice blast and bacterial blight diseases and elite genotype HPR 2143 of rice

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Abstract

Rice blast and bacterial leaf blight are the two major diseases affecting rice productivity in north-western Himalayan region of India. Exploitation and utilization of major resistance (R) genes is an effective way to control these diseases. The selected elite BC_2F_2 progenies of four crosses, HPR2143/DHMAS164, HPR2143/PB1, HPR2143/IRBB54 and HPR2143/PR114 were used as a source for blast resistance genes *Pita* and *Pi9*, and bacterial leaf blight resistance genes *Xa21* and *Xa38* respectively. In order to pyramid 2 genes for each disease, hybridization among these backcross derivatives containing single genes was attempted. Overall 346 spikelets were emasculated and pollinated to combine genes, *Pita* and *Pi9*, out of which 136 seeds were set with a mean crossability of 39.31 per cent. Similarly to combine genes, *Xa21* and *Xa38*, 335 spikelets were emasculated and pollinated in which 127 seeds were set with an overall crossability of 37.91 per cent. Overall crossability over both sets of crosses (*Pita* × *Pi9*) and (*Xa21* × *Xa38*) was recorded to be 38.61 per cent that was comparatively low as compared to earlier documented reports.

Key words: Rice, Hybridization, *Pita*, *Pi9*, *Xa21*, *Xa38*.

Rice (*Oryza sativa* L.) is one of the oldest domesticated crops, which provides food for more than half of the world's population and constitutes a major source of carbohydrate for urban as well as rural population (Khush 2005). It is cultivated on all the continents except Antarctica, over an area of more than 150 million ha, but most rice production takes place in Asia (Akhtar *et al.* 2010). Rice plays an important role in feeding the world's population, as it is the most important global staple food occupying maximum area under cultivation and contributes nearly 44 per cent of the total food grain (http://www.worldriceproduction.com, 2014-2015) and 43 per cent calorie requirement for more than 70 per cent Indians. The production of rice must be doubled to meet the requirement of the increasing population. This can be done only by enhancing the productivity and preventing losses caused by insect-pest and diseases of rice (Hossain 1996; Mishra *et al.* 2003).

In India, rice is a staple food for more than 70 per cent of population, where around 4,000 varieties and hybrids are grown to fulfill varied consumer preferences (Singh *et al.* 2012). India, a country encompassing centre of origin of rice, is enriched with massive genetic diversity and is home to at least 50,000 landraces of rice (Shikari *et al.* 2014). However, two major diseases of rice namely, rice blast (RB) caused by the heterothallic ascomycete *Magnaporthe oryzae* (Hebert) Barr. and bacterial leaf blight (BLB) disease caused by the gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), are the most serious diseases of rice causing severe yield losses throughout the world (Ou 1985). These two diseases occur in more than 80 rice growing countries which results in yield losses estimated to be more than 50 per cent (Ou 1985; Mew 1989). These 2 diseases also affect rice productivity in north-western Himalayan region. Under such a scenario, disease resistant varieties offer a low cost, environmentally safe and sustainable means of controlling these diseases. Various combinations of Pi9 and its allelic resistance genes with Pish, Pik^h, Pita, $Pita^2$ have been identified as potential sources of blast resistance for deployment in north-western Himalayan states including Himachal Pradesh (Rathour *et al*. 2011). Screening of rice isogenic lines carrying different bacterial leaf blight (BB) resistance genes against Xoo isolates collected from various rice growing regions of

Himachal Pradesh has indicated that genes xa5, xa13 and Xa21 are effective in this region. A new BLB resistance gene Xa38, identified from O. nivara has also been found to be effective against all the prevalent Xoo pathotypes in the neighboring state of Punjab (Cheema et al. 2008) and is likely to provide good level of resistance against Xoo virulences in Himachal Pradesh as well. Among the BLB resistance genes identified so far, the dominant gene, Xa21, originally discovered from an accession of the wild rice, Oryza longistaminata, confers broad spectrum resistance to many Xoo isolates in India and elsewhere (Ronald *et al.* 1992). Deployment of host plant resistance is considered to be the best strategy for disease management. Breeding rice varieties with multiple disease resistance genes will broaden the resistance spectrum and therefore increase the resistance durability for the varieties (Ji Zhi-juan *et al.* 2016).

In view of the above, there is an urgent need to broaden the rice gene pool by introgressing new genes from diverse sources in order to meet various challenges which affects rice production (Brar and Khush 1997). The genus *Oryza* includes two cultivated (2 *n* = 24, AA) and 22 wild species (2 *n* = 24, 48) representing the AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, and HHJJ genome types. These wild species are reservoirs of many useful genes, particularly for resistance to major biotic and abiotic stresses (Sanchez *et al.* 2014). To achieve precise transfer of genes from wild species, strategies involving a combination of conventional plant breeding methods with tissue culture and molecular approaches have become important (Brar and Khush 2002; Brar and Khush 2006). The transfer of genes from wild to cultivated rice can be achieved through hybridization, embryo rescue, backcrossing, and selection processes (Jena and Khush 1986; Khush 1977).

Breeding rice with the quality of broad-spectrum and durable disease resistance is the principal goal of rice improvement (Kou and Wang 2010). Exploitation and utilization of major resistance (R) genes is an effective way to control various diseases such as bacterial blight and rice blast (Li *et al.* 2012). The present study was aimed at estimation of the success rate of hybridization while combining blast resistance genes (Pi9 and Pita) and bacterial blight resistance genes (Xa21 and Xa38) in the backcross progenies of rice having different single resistance genes for rice blast and bacterial leaf blight in the background of elite parent, HPR 2143.

Materials and Methods

Plant material

The rice genotypes DHMAS164 (*Pita*), PB1 (*Pi9*), IRBB54 (*Xa21*) and PR114 (*Xa38*) were used as donors of resistance genes against rice blast and bacterial leaf blight diseases, whereas rice cv. HPR2143 was used as recipient parent. Already developed crosses between recurrent parent HPR2143 and resistance donors and their further backcrosses (BC_2F_2) developed through an ongoing programme in the Department of Agricultural Biotechnology, CSKHPKV Palampur at that time were used as starting material for the present study. The seeds of selected elite BC_2F_2 progenies of four crosses, HPR2143/DHMAS164 (*Pita*), HPR2143/PB1 (*Pi9*), HPR2143/IRBB54 (*Xa21*) and HPR2143/PR114 (*Xa38*) were sown in small separate plots (in nursery beds) in the cage house of the Department of Agricultural Biotechnology, CSK HPKV Palampur. Standard agronomic practices were followed to raise the good healthy crop.

Research methodology

Combining 2 genes each for resistance against rice blast and bacterial leaf blight through crossing of lines carrying single genes

To combine two blast resistance genes (*Pita*+*Pi9*), already selected gene-positive homozygous plants (selected by foreground selection) for genes *Pita* and *Pi9* were crossed by using emasculation and pollination procedures described below. Similarly to combine two bacterial leaf blight resistance genes (*Xa21*+*Xa38*), selected plants positive and homozygous for single genes i.e. *Xa21* and *Xa38* were crossed.

Emasculation

Healthy female plants were selected and tagged in the crossing block for making crosses. In the evening hours (3.00 PM to 5.00 PM), emasculation was carried out. Productive tillers with healthy panicles were selected and leaf sheaths were removed carefully. Further, top florets that had completed anthesis and the young florets at the bottom were gently removed from panicles. Only the middle florets that will flower on the next day were used for crossing. The top 1/3rd portion of florets was clipped-off in a slanting position with sharp scissors and the six anthers present in each spikelet were removed with the help of fine tip forceps carefully, without damaging the stigma. Much care was taken to remove the anthers without leaving any remnants anther parts. To prevent contamination from the undesired foreign pollen, the emasculated spikelets were covered with a butter paper bag and labeled properly.

Pollination

Pollination was done on subsequent morning between 9.00 to 11.00 AM when the stigma was receptive. Matured anthers were collected from protected male parent in petri dish and dusted on the stigma of emasculated female flower very gently with the help of brush and forceps after temporarily removing butter paper bag from them. The pollinated spikelets of panicles were again covered with fresh butter paper bags to protect from cross pollination. Coloured thread was tied at the base of the panicle to identify the crossed ones. The crossed seeds were collected 30 days after pollination from the plants and sun dried, counted and placed in small envelops.

In both the crosses, selected gene-positive homozygous plants were randomly used as male and female parents achieving different combinations of hybrid progenies. For cross 1 (*Pita*+*Pi9*), a total of 346 spikelets were pollinated in 10 combinations using both *Pi9* and *Pita* gene harbouring progenies as female parent. Similarly for cross 2 (*Xa21*+*Xa38*), a total of 335 spikelets were artificially pollinated in 12 combinations using both *Xa21* and *Xa38* harbouring backcross progenies as female parent. Data were collected from different cross combinations on number of spikelets pollinated and total number of seed set. The number of crossed seeds collected for cross 1 (*Pita*+*Pi9*) and cross 2 (*Xa21*+*Xa38*) were recorded in reciprocal combinations using each gene harbouring progenies as female parent. The data on reciprocal combinations were pooled to calculate overall crossability among backcross progenies disease-wise.

Crossability was expressed as the percentage of seed set in hand pollinated spikelets and was calculated as follows:

$$\text{Crossability} = \frac{\text{Total number of seed set}}{\text{Total number of spikelets pollinated}} \times 100$$

Results and Discussion

To recombine and pyramid 2 genes conferring resistance to rice blast, progenies positive for gene *Pita* were crossed with selected homozygous positives progenies for the gene, *Pi9* in reciprocal manner. Likewise, plants which were homozygous positives for genes *Xa21* and *Xa38* were crossed with each other to produce F_1. In both the crosses, the selected plants were randomly used as male and female parents.

For cross 1 (*Pita* × *Pi9*), a total of 346 spikelets were artificially pollinated in 10 different combinations (Table 1 & Table 2). In first five combinations, where *Pita* gene containing progenies were used as female parent, the crossability ranged from 35.29 to 46.43 per cent with a mean crossability of 40.31 per cent. Overall 177 spikelets were emasculated and pollinated in which 71 putative hybrid grains developed and matured. On an average 35.4 spikelets/panicle were pollinated in which 14.2 seeds/panicle were set (Table 1).

Similarly in reciprocal 5 combinations (using progenies containing gene *Pi9* as the female parent), a total of 169 spikelets were artificially emasculated and pollinated in 5 combinations in which 65 putative hybrid seeds developed (Table 2). In these combinations the crossability ranged from 32.14 to 45.95 per cent. On an average 33.8 spikelets/panicle were pollinated in which 13 seeds/panicle were set.

For cross 2 (*Xa21* × *Xa38*), a total of 335 spikelets were artificially pollinated in 12 different combinations. In first six combinations, where progenies having gene, *Xa21* were used as a female parent, the crossability ranged from 26.32 to 51.72 per cent with a mean crossability of 38.13 per cent (Table 3). On an average 26 spikelets/panicle were pollinated in which 10.17 seeds/panicle were set. Overall 156 spikelets were emasculated and pollinated in which 61 putative hybrid grains developed and matured.

In reciprocal 6 combinations (using progenies containing gene *Xa38* as the female parent), a total of 179 spikelets were artificially emasculated and pollinated in 6 different combinations in which 66 putative hybrid seeds developed (Table 4). In these combinations the crossability ranged from 23.80 to 43.90 per cent. On an average 29.83 spikelets/panicle were pollinated in which 11 seeds/panicle were set.

Hybridization is one of the most commonly used breeding methods for improvement, mainly of open pollinated and often cross pollinated crops. The genetic improvement through hybridization and selection has been the main objective in the breeding programmes in rice. Although frequent intervarietal hybridizations are usually attempted under rice breeding programmes throughout the world, the documented literature on its success rate and crossability are scanty. Debbarma and Khanna (2018) in their studies on intervarietal hybridization and genetic diversity of rice by molecular markers reported crossability of various accessions of *O. sativa L*. with the perspective of pollen development. They have reported seed set up to 68.96 per cent in a cross of CAU R-1 with Narendra-359. In present study, the level of overall success rate of hybridization among backcross progenies harbouring genes for resistance to rice blast and bacterial leaf blight ranged from 37.91 to 39.31 per cent respectively with a mean crossability of 38.61 per cent. Overall 346 spikelets were

Table 1. Number of spikelets pollinated, seeds set and crossability (%) among selected BC_2F_2 progenies containing resistance genes *Pita* and *Pi9* against rice blast disease using progenies containing gene *Pita* as female parent

Progeny No. having gene *Pita*	Progeny No. having gene *Pi9*	Number of spikelets pollinated/panicle	No. of seeds set	Crossability (%)
6	52	40	17	42.50
30	61	38	15	39.47
36	63	34	12	35.29
37	64	37	14	37.84
39	66	28	13	46.43
Total		177	71	
Mean		35.4	14.2	40.31
SD		4.67	1.92	4.31
SE (m)±		2.09	0.86	1.93

Table 2. Number of spikelets pollinated, seeds set and crossability (%) among selected BC_2F_2 progenies containing resistance genes *Pi9* and *Pita* against rice blast disease using progenies having gene *Pi9* as female parent

Progeny No. having gene *Pi9*	Progeny No. having gene *Pita*	Number of spikelets pollinated/panicle	No. of seeds set	Crossability (%)
42	6	34	12	35.29
43	30	37	17	45.95
44	36	31	13	41.94
45	37	28	9	32.14
46	39	39	14	35.90
Total		169	65	
Mean		33.8	13	38.24
SD		4.44	2.92	5.58
SE (m)±		1.98	1.30	2.50

Table 3. Number of spikelets pollinated, seeds set and crossability (%) among selected BC_2F_2 progenies containing resistance genes *Xa21* and *Xa38* against bacterial blight disease using progenies having gene, *Xa21* as female parent

Progeny No. having gene *Xa21*	Progeny No. having gene *Xa38*	Number of spikelets pollinated/panicle	No. of seeds set	Crossability (%)
86	129	31	13	41.94
91	139	33	12	36.36
96	146	29	15	51.72
97	149	23	9	39.13
98	150	21	7	33.33
106	153	19	5	26.32
Total		156	61	
Mean		26	10.17	38.13
SD		5.76	3.82	8.56
SE (m)±		2.35	1.56	3.49

Table 4. Number of spikelets pollinated, seeds set and crossability (%) among progenies containing resistant genes *Xa38* and *Xa21* for bacterial blight disease using progeny having gene *Xa38* as female parent

Progeny No. having gene	Progeny No. having gene	Number of spikelets emasculated and pollinated/panicle	No. of seeds set	Crossability (%)
Xa38	***Xa21***			
129	110	10	4	40.00
139	111	41	18	43.90
146	112	21	5	23.80
149	115	49	16	32.65
150	123	31	12	38.71
153	125	27	11	40.74
Total		179	66	
Mean		29.83	11	36.63
SD		13.95	5.65	7.29
SE (m)		5.69	2.30	2.98

Table 5. Number of spikelets pollinated, seeds set and overall crossability (%) among selected backcross progenies carrying different gene combinations (*Pita* × *Pi9*) and (*Xa21* × *Xa38*)

Cross	Total number of spikelets pollinated	Total number of seeds set	Crossability (%)
BC_2F_2 (*Pi9*) × BC_2F_2 (*Pita*) & reciprocals	346	136	39.31
BC_2F_2 (*Xa21*) × BC_2F_2 (*Xa38*) & reciprocals	335	127	37.91
Total	681	263	
Mean	340.5	131.5	38.61
SD	7.78	6.36	0.99
SE (m)±	5.50	4.50	0.72

In interspecific and other wide hybridizations in rice, a comparatively much low and variable rate of success has been documented in the literature. Niruntrayakul *et al.* (2009) found that all cultivated rice and common wild rice (*O. rufipogon)* genotypes were interfertile and produced normal F_1 seeds but with different rates of seed set. Kanya *et al.* (2012) have reported crossing success rate of 6 per cent and 0 per cent in crosses between the two species i.e. *O. longistaminata* and *O. sativa* using *O. sativa* and *O. longistaminata* as female parents, respectively. Similarly, in another findings on wild *Oryza* species by Sitch *et al.* (1989), the results showed that when IR64 was crossed with five *O. nivara* accessions, it showed limited variation in seed set (13.9-28.9 per cent); while crosses with IR36 gave wide variation (9.1- 62.2 per cent). On the other hand crosses of IR54 with *O. rufipogon* IRGC Acc. 100907 gave lower seed sets (32.4 per cent) than that with Acc. 103817 (73.0 per cent). In crosses with IR64, Acc. 100907 gave the highest seed set (53.4 per cent) indicating clearly the genotypic effects on cross compatibility. Many factors including low pollen fertility (Causse and Ghesquiere 1991), deterioration of hybrid embryos about three days after fertilization (Sano 1989), emasculation errors and pollination processes are important and determine the success rate of hybridization in rice (Kanya *et al*. 2012).

Conclusion

Based on the observations and results of the study, it can be concluded that crossability between backcross progenies carrying *Pi9* and *Pita,* and *Xa21* and *Xa38* genes was 39.31 per cent and 37.91 per cent,

respectively. Despite a low crossing success rate for both the crosses, enough F_1s were produced to combine 2 resistance genes against each disease. The differences in crossability rate can be ascribed to the techniques used for manual hybridization as well as may be due the effect of different genes harboured by the resistant backcross progenies used for hybridization to achieve pyramiding of the 2 resistant genes each.

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