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RESEARCH ARTICLE

MARKER-ASSISTED INTROGRESSION OF GENES CONFERRING RESISTANCE AGAINST BACTERIAL BLIGHT & BLAST INTO RPHR-1005, THE ELITE RESTORER LINE OF THE POPULAR RICE HYBRID, DRRH-3

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ABSTRACT

RPHR-1005 is a stable, elite restorer line of rice possessing highly desirable medium-slender grain type and is the male parent of the popular public bred hybrid DRRH-3. However, both RPHR-1005 and DRRH-3 are highly susceptible to major diseases like bacterial blight (BB) and blast. As genes conferring effective resistance are available against both BB and blast along with availability of gene-specific markers, the present study was carried out to introgress a major BB resistance gene, *Xa21* and a major blast resistance gene, *Pi2* into the genetic background of RPHR-1005 through marker-assisted backcross breeding. RPBio Patho-1, a breeding line in the genetic background of the popular variety, Samba Mahsuri and possessing *Xa21* and *Pi2* served as the donor. Marker-assisted backcross breeding strategy was deployed for targeted introgression of the two resistance genes into RPHR-1005. This involved two rounds of backcrossing and at each backcross generation, foreground selection was carried out using PCR based molecular markers specific for *Xa21* (i.e. pTA248) and *Pi2* (i.e. AP5659-5) along with the markers specific for the major fertility restorer genes, *Rf3* (i.e. DRRM-RF3-10) and *Rf4* (i.e. DRCG-RF4-14) and background selection was done using a set of 61 parental polymorphic SSR markers spread across the rice genome. At BC_2F_2, a single plant possessing all the targeted genes along with maximum recurrent parent genome recovery (~ 93.4%; plant # RP-9-27-79-179) was selected and advanced through selfing and pedigree-based selection for morphological traits. AT BC_2F_4, three lines, viz., RP-9-27-79-179-74-9, RP-9-27-79-179-74-79 and RP-9-27-79-179-74-105, possessing high level of resistance against BB and blast along with complete fertility restoration and all the elite features of RPHR-1005 were identified.

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INTRODUCTION

Plant material: A recently developed near isogenic line (NIL) RPBio Patho-1 (Prasad *et al*., 2011) with medium slender grain type, *Indica* rice line possessing good grain yiel and the BB and blast resistance genes, *Xa21* and *Pi2,* respectively was used as the donor. RPHR-1005, an elite restorer line, derived from the cross Samba Mahsuri/SC3126-3-2-4 (Ramesha *et al*., 2010), possessing medium-slender grain type with stable fertility restoration and the male parent of the popular rice hybrid, DRRH-3. In addition, Taichung Native 1(TN1), HR12 were used as susceptible checks for phenotypic screening for BB and blast resistance, respectively.

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Marker-assisted introgression of *Xa21* and *Pi2* into RPHR-1005: RPHR-1005 was crossed with RPBio Patho-1 and the F_1s obtained were analyzed using *Xa21* specific marker co-dominant, pTA248 (Ronald *et al*., 1992) for identification of 'true' F_1s, which were then backcrossed with RPHR-1005 to generate BC_1F_1s. They were then subjected for foreground selection using the markers, pTA248 (for *Xa21*) and AP5659-5 (for *Pi2*; Fjellstorm *et al*., 2006) and those plants which were positive for both the genes were analyzed using the molecular markers specific for the major fertility restorer genes, *Rf3* and *Rf4*, viz., DRRM-RF3-10 and DRCG-RF4-14, respectively (Balaji *et al*., 2012). This helped us to identify plants positive for *Xa21* and *Pi2* and also possessing restorer alleles at both *Rf3* and *Rf4* in homozygous condition. The PCR protocol as recommended in Ronald *et al*. (1992), Fjellstorm *et al*. (2006) and Balaji *et al*. (2012) was adopted for marker analysis with

respect to *Xa21, Pi2, Rf3* and *Rf4*. BC_1F_1 plants, which were positive for all the four target genes, were then subjected for background analysis using SSR markers, which were polymorphic among the donor and recurrent parents using the procedure prescribed in Sundaram *et al*. (2008). A single 'positive' (i.e. possessing *Xa21, Pi2, Rf3* and *Rf4*) BC_1F_1 plant possessing maximum recovery of RPHR-1005 genome was identified and backcrossed with RPHR-1005 to develop the BC_2F_1s. They were then subjected for foreground selection for *Xa21* and *Pi2* and background selection using the remaining polymorphic SSR markers as described earlier and a single 'positive' BC_2F_1 plant possessing maximum recovery of recurrent parent genome was identified and selfed to generate BC_2F_2s, They were then analyzed with *Xa21* and *Pi2* specific markers to identify homozygous plants and using the remaining parental polymorphic SSR markers a single BC_2F_2 plant possessing *Xa21* and *Pi2* in homozygous condition and maximum recovery of RPHR-1005 genome was then advanced further by selfing through pedigree-based morphological selection for key traits like plant height, grain type, panicle exsertion etc. till BC_2F_4 wherein the lines were subjected for phenotypic screening against BB and blast diseases and also for key agro-morphological traits.

Screening for blast resistance: The local pathogen, *Magnaporthae oryzae,* a virulent fungal isolate (SPI-28) from Institute of Rice Research (IIRR), Hyderabad, India (Madhan Mohan *et al*. 2011), was used to screen the donor and recurrent parent along with backcross derived lines of RPHR-1005 for blast resistance under *in vivo* conditions following uniform blast nursery (UBN) method at Indian Institute of Rice Research, Hyderabad, India. In inoculation test, this isolate was found to be highly virulent on rice differential HR12 and other blast differentials. The pathogen strain was cultured and stored (Prasad *et al*., 2011). The young seedlings at four-leaf stage were inoculated with the fungal conidial suspension at a concentration of 1 x 10^5 conidia/ml with the help of hemocytometer. The parents along with the improved lines of BC_2F_4 population were evaluated for their reaction to blast disease. The plants were sown in rows and were surrounded with the densely sown spreader rows of susceptible cultivar HR12.The seedlings at four-leaf stage were sprayed with spore suspension of a highly virulent isolate of *M. grisea* (SPI-28). High relative humidity was maintained for disease development. Data entry scores were recorded three times using a scale of 0-9 (IRRI, 1996) at 10 days intervals starting from 30 days after sowing. The lines with scores of 0-3 were considered resistant, 4-5 as moderately resistant, 6 as moderately susceptible and 7-9 as susceptible.

Screening for BB resistance: The parents and improved lines of BC_2F_4 population was screened for BB resistance through artificial inoculation during kharif (wet season) under field condition, IIRR, Hyderabad. Plants were inoculated with the bacterial suspension at a density of 10^9 cell/ml at maximum tillering stage using the most virulent DRR isolate DX-020. 4-5 young leaves in each plant were inoculated through clip inoculation method (Kauffman *et al*., 1973) and the disease reaction was scored 21 days after inoculation (DAI). Disease response was observed by measuring the mean lesion length of two inoculated leaves at 14 day interval after inoculation and scored as follows. Plants with an average lesion length of up to 6cm were considered resistant and those with lesion lengths above 6 cm were scored as susceptible.

Evaluation of agro-morphological characters: Thirty-day-old seedlings of the selected backcross-derived lines at BC_2F_4 were transplanted in the field along with the donor and recurrent parents. Standard agronomic practices were followed to develop promising lines, which were evaluated during the wet season (Kharif) in 2014. Data was recorded for the agronomic traits, viz. days to 50% flowering (DFF), mean plant height (cm), number of productive panicles per plant, panicle weight (g), No of grains per panicle, panicle length (cm), grain yield per plant (g), 1000-seed weight (g) and panicle exertion.

RESULTS

Targeted introgression of *Xa21* and *Pi2* into RPHR-1005 through marker-assisted backcross breeding (MABB)

MABB strategy was deployed to incorporate the resistance genes, *viz. Xa21* for BB and *Pi2* for blast into RPHR-1005 by crossing the donor parent (RPBio Patho-1) and the recurrent parent RPHR-1005. A total of 42 'true' F_1s (i.e. heterozygotes) were identified and backcrossed with RPHR-1005 to generate 360 BC_1F_1 seeds. Foreground analysis of the BC_1F_1 plants using the gene-specific markers, pTA248 (for *Xa21*) and AP5659-5 (for *Pi2*) revealed that 22 plants to be heterozygous for both the genes (Figure 1A and B; Table 1). They were then for the presence of restorer alleles with respect to *Rf3* and *Rf4* in homozygous condition using the gene-specific markers (DRRM-RF3-10 and DRCG-RF4-14) and a total of five 'positive' BC_1F_1 plants were thus identified (Figure 1C and 1D). Among these, a single plant (# RP-9-27) was selected for further backcrossing as it was observed to possess a maximum recovery of recurrent parent genome (~ 73.7%; as inferred through background selection using 61 parental polymorphic SSR markers) and a total of 134 BC_2F_1 plants were thus produced. Foreground selection among the BC_2F_1 plants revealed a total of eight plants possessing *Xa21* and *Pi2* in heterozygous condition, which were then subjected to background genome recovery analysis. A single BC_2F_1 plant with maximum recurrent parent genome recovery (~ 86.8%) was identified and selfed to develop a total of 560 BC_2F_2s. Marker-assisted screening of these plants identified 35 double positive plants (*Xa21+ Pi2*) and among these, a single plant possessing maximum recurrent parent genome recovery (~93.4%) was identified and forwarded by selfing through pedigree method involving morphological trait-based selection till BC_2F_4, wherein three promising advanced backcross derived lines (ABLs) were identified (viz., RP-9-27-79-179-74-9, RP-9-27-79-179-74-79 and RP-9-27-79-179-74-105). They were then subjected for phenotypic evaluation for disease resistance, yield, fertility restoration, heterosis and other agro-morphological parameters as given below.

Phenotypic screening for blast and BB resistance

The three ABLs possessing *Xa21 + Pi2* were evaluated for their resistance to blast in the Uniform Blast Nursery (UBN) beds (Figure 2A, Table 2).

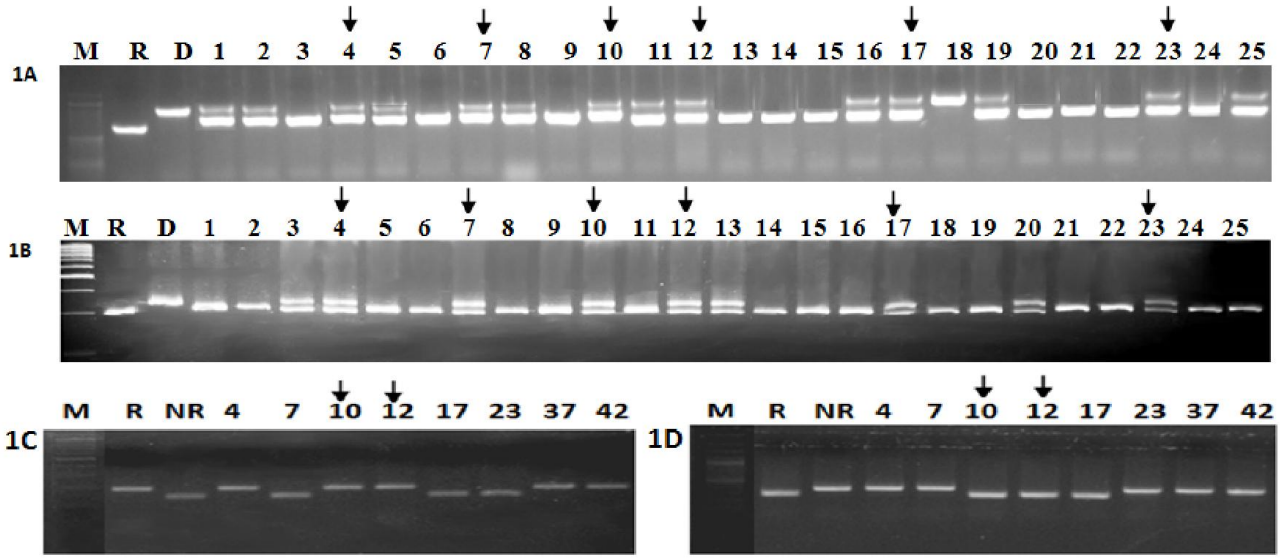

Figure 1. Marker-assisted foreground selection at BC_1F_1 generation for *Xa21* (1A), *Pi2* (1B), *Rf3* (1C) and *Rf4* (1D). While heterozygous plants were selected for *Xa21* and *Pi2* based on gene-specific markers, plants homozygous for the restorer allele (determined based on marker analysis) were selected with respect to *Rf3* and *Rf4*. as Lanes M: 100bp molecular weight ladder ; D-Donor parent (RPBio Patho-1), R-Recurrent/Restorer parent (RPHR1005); NR-Non Restorer (DRR17B) 1-25 – BC_1F_1 plants; Arrows indicate 'positive plants'. Plant # 10 and 12 were positive for all the four genes

Table 1. Details of foreground and background selection among the backcross plants derived from the cross RPHR-1005/RPBio Patho-1

S. No.	Generation	No. of plants screened	Foreground Selection		Background selection			Best plant selected based on background selection
			No. of plants positive for *Xa21, Pi2*	No. of plants positive for *Xa21, Pi2* along with *Rf3* and *Rf4*	No. of SSR makers screened among the foreground selection positive plants	No. of parental polymorphic SSR markers which are homozygous for RPHR-1005 allele in the best plant	(%) recovery of Recurrent parent genome	
1	F_1	48	42	-	-	-	-	-
2	BC_1F_1	360	22	5	61	45	73.7%	RP-9-27
3	BC_2F_1	134	8	8	16	8	86.8%	RP-9-27-79
4	BC_2F_2	560	35	35	8	4	93.4%	RP-9-27-79-179

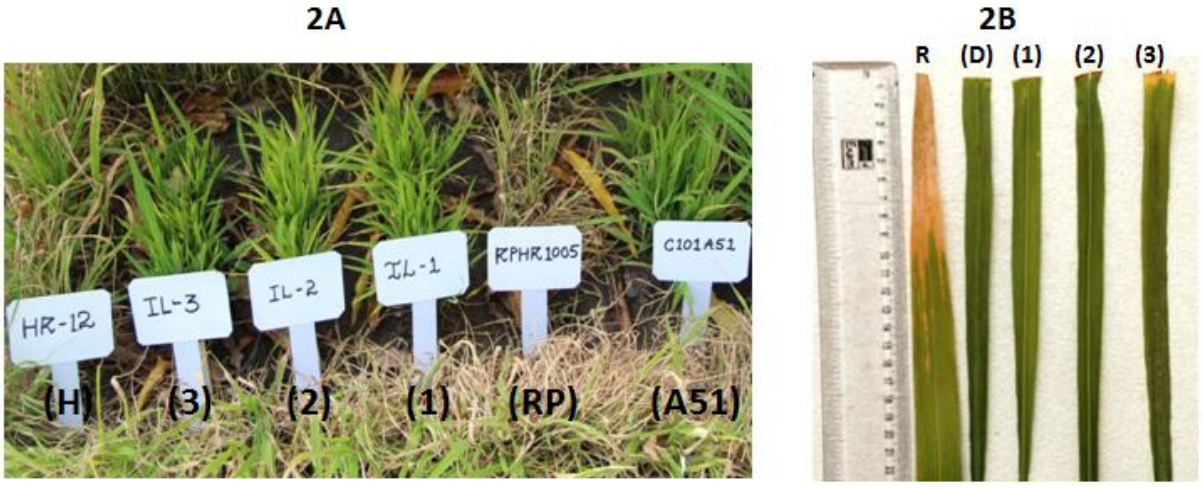

Figure 2. Phenotypic screening of BC_2F_4 lines against rice blast and bacterial blight diseases

2A: Screening of selected BC_2F_4 lines against blast disease following UBN Method. (RP) RPHR-1005 – recurrent parent (susceptible), (A51) C101A51- resistant check; (H) HR-12 –Susceptible check and all the back cross derived lines IL-1 to IL-3 (i.e., RP-9-27-79-179-74-9, RP-9-27-79-179-74-79 and RP-9-27-79-179-74-105) were highly resistant. 2B: Screening of the parents, (R) RPHR-1005 and (D) RPBio Patho-1 and selected BC_2F_4 lines, viz., (1) RP-9-27-79-179-74-9 , (2) RP-9-27-79-179-74-79 and (3) RP-9-27-79-179-74-105 for BB resistance. All the advanced backcross derived lines were highly resistant to the disease

Table 2. Reaction of the three selected pyramided BC_2F_4 lines after inoculation with bacterial blight and blast pathogen

S.No	Rice line	Resistance genes genotyped by flanking markers*				Reaction against bacterial blight#		Reaction against blast#	
		Pi2	*Xa21*	*Rf3*	*Rf4*	DX-020		SPI-28	
		AP5659-5	pTA248	Rf3-10	Rf4-14	Score	R/S	Score	R/MR/S
1	RPHR1005	--	--	++	++	9.0 ± 0.0	S	9	S
2	RPBio Patho-1	++	++	--	--	1.7 ± 0.3	R	0	R
4	RP-9-27-79-179-74-9	++	++	++	++	2.7 ± 0.7	R	1	R
5	RP-9-27-79-179-74-79	++	++	++	++	2.3 ± 0.3	R	1	R
6	RP-9-27-79-179-74-105	++	++	++	++	1.7 ± 0.3	R	1	R

* ++ Possessing homozygous resistant allele at the particular gene locus, based on screening with gene-specific marker,
-- Possessing homozygous susceptible allele at the particular gene locus, based on screening with gene-specific marker
R, Resistant; S, Susceptible

Table 3. Details of agronomic performance of the parents and improved lines of RPHR-1005 at BC_2F_4 under field conditions

S.No	Designation	Days to 50% flowering (DFF)	Mean plant height (cm)	No. of productive panicles/plant	Panicle weight (gm)	No. of grains/ panicle	Panicle length (cm)	1000 Grain weight (gm)	Grain Yield per plant (gm)	Panicle exertion	RPG (%)
1	RPHR-1005	105.3 ± 0.3	91.0 ± 0.3	17.3 ± 0.3	2.48 ± 0.03	191.3 ± 1.45	15.7 ± 0.4	16.7 ± 0.1	26.9 ± 0.1	PE	-
2	RPBio Patho-1	96.0 ± 1.2	68.8 ± 0.2	15.7 ± 0.3	1.88 ± 0.03	182.3 ± 1.45	19.2 ± 0.4	17.1 ± 0.1	25.3 ± 0.4	PE	-
3	RP-9-27-79-179-74-9	100.3 ± 0.3#	89.9 ± 0.1	15.7 ± 0.3	2.24 ± 0.07	183.0 ± 1.15	16.8 ± 0.6#	16.8 ± 0.2#	26.0 ± 0.1	FE	92.5
4	RP-9-27-79-179-74-79	102.7 ± 0.7#	88.8 ± 0.5	17.7 ± 0.9	2.45 ± 0.03	190.3 ± 1.45	19.2 ± 0.4#	16.9 ± 0.4#	26.0 ± 0.1	FE	91.2
5	RP-9-27-79-179-74-105	101.0 ± 0.6#	91.5 ± 0.6	21.0 ± 0.6#	2.88 ± 0.02#	205.7 ±1.76#	20.3 ± 0.3#	17.7 ± 0.2#	27.3 ± 0.3#	FE	93.4

DFF: Days to 50% flowering, PH: Mean plant height (cm), NT: Number of tillers/plant, PL: Panicle length (cm), 1000GW (gm): 1000 grain weight (gm), Y/P: Yield per plant (gm), Panicle exertion: FE- Full exertion, E-Exertion, PE-Partial exertion, RPG: Recurrent parent genome recovery (%).

The susceptible check, HR12 and the recurrent parent RPHR-1005 (Score-9) were highly susceptible to blast, while the resistant check, RPBio Patho-1/C101A51 (Score-0) and all the ABLs were found to be highly resistant to the disease with a score of 1. The ABLs were then evaluated for BB resistance (Figure-2B, Table-2). The recurrent parent, RPHR-1005 showed high susceptibility to the disease with a lesion length of > 15 cm, while the donor, RPBio patho-1 was observed to be highly resistant with a lesion length ranging from 1-3 cm. As expected, all the ABLs showed a high level of resistance to BB with a lesion length of < 3 cm indicating that successful introgression of *Xa21* in these lines.

Agronomic characterization of the improved lines

The selected three ABLs (RP-9-27-79-179-74-9, RP-9-27-79-179-74-79 and RP-9-27-79-179-74-105) which exhibited high level of resistance to BB and blast were evaluated for key agro-morphological traits *viz.* plant type, grain quality, panicle number, grain yield, 1000 grain weight, number of tillers, panicle exertion and panicle type (Table 3). The ABL (RP-9-27-79-179-74-105 with an RPG 93.7%) displayed grain yield slightly higher than (27.3 ± 0.3 gm) that of the recurrent parent (i.e. RPHR; 26.9 ± 0.1 gm), while other ABLs (RP-9-27-79-179-74-9 and RP-9-27-79-179-74-79 with an RPG 92.5%, 91.2% respectively) displayed grain yield per plant equivalent to that of the recurrent parent. One of the three ABLs, i.e., RP-9-27-79-179-74-105 (91.5 ± 0.6 cm) was observed to be significantly taller than RPHR-1005 (91.0 ± 0.3 cm). No significant variation was observed with respect to the number of productive tillers/plant, no. of panicles and panicle length among the three ABLs as compared to RPHR-1005. One ABL, i.e. RP-9-27-79-179-74-105 found to be better than that of the RPHR-1005 as it had better panicle exertion (Table 3).

DISCUSSION

Despite the fact that hybrid rice technology was introduce more than one and half decades in India, the spread of rice hybrids in the country is limited to about 2.5 Mha out of 44.5 Mha area under rice (Mishra *et al*., 2003). This is due to several reasons like low level of heterosis, poor grain quality and high level of susceptibility of the hybrids to several pests and diseases. The problem of grain quality was perceived to be severe as most of the first generation hybrids in India were based on IR58025A, which has significantly lower amylase content (hence sticky) and also possesses aroma, which is not desired in many parts of India. Second generation hybrids like DRRH-2, DRRH-3, KRH4 do not have many of these negative quality traits and particularly

DRRH-3 possesses highly desirable medium-slender grain type along with high level of heterosis. Despite its superior grain and yield qualities, DRRH-3 and its female parent, APMS6A and male parent RPHR-1005 are highly susceptible to two major rice diseases, i.e. BB and blast, which significantly reduce the yield of rice varieties and hybrids (Ram Singh *et al.* 2013, Guvvala *et al*., 2013). Hence the present study was carried out with an objective to improve the RPHR-1005 and its derived hybrid (i.e. DRRH-3) for resistance against BB and blast, while retaining the premium grain quality and other good features of RPHR-1005 through MABB coupled with stringent phenotypic selection. A major, dominant gene each conferring broad spectrum of resistance against BB (*Xa21*) and blast (*Pi2*) were introgressed into the restorer parent as the hybrid derived from such improved restorers also are expected to possess resistance against the two deadly diseases of rice, as the genes selected in the present study are dominant in nature.

Earlier, we developed BB resistant versions of the varieties, Samba Mahsuri (Sundaram *et al*., 2008), Triguna (Sundaram *et al*., 2009), the restorer line KMR-3R (Hari *et al*. 2011), the maintainer lines, IR58025B (Hari *et al*., 2013), DRR17B (Balachiranjeevi *et al*., 2015) through MABB. PRR78, a popular elite basmati restorer line was improved for resistance against BB and blast by Basavaraj *et al*. (2010) and Singh *et al*. (2012), respectively by implementing an approach similar to that used in the present study. In addition to use of markers for selecting *Xa21* and *Pi2,* we also adopted a positive selection strategy involving MAS for fertility restoration trait and for quick recovery of the genome of RPHR-1005, thus limiting the total number of backcrosses to just two. Thus through this study, three improved breeding lines RPHR-1005 possessing good plant type, excellent resistance against BB and blast along with medium-slender grain type, suitable for use as a restorer parents in three-line breeding system have been developed. Test crossing of these improved lines of RPHR-1005 with, APMS6A, the female parent of DRRH-3 revealed that the newly developed hybrids were completely fertile and possessed heterosis levels identical or better than DRRH-3 (data not shown).

MABB has been observed to be an efficient technique for precise transfer of one or few target genes in the genetic background of an elite variety or parental line. The task is even more challenging, when target genes have to be introgressed into parental lines as such an effort requires that the genetic background of the parental line does not undergo gross changes during such introgression, which could make them unfit for use as restorers or maintainers. Further, it is also necessary to select dominant genes for such introgression as selection of recessive resistance genes entails introgression into both the parents (i.e. maintainer and CMS line and their restorer lines), as introgression into CMS line is a two step process involving introgression first into the maintainer line and then later conversion of maintainer to CMS line each step needing at least 3-5 seasons for completion. Thus for the present study, we prudently selected the restorer line of DRRH-3 (i.e. RPHR-1005) for improvement of its biotic stress resistance through introgression of two major dominant genes (i.e. *Xa21* and *Pi2*). There are a few reports wherein breeders have improved hybrid rice parental lines for either resistance against bacterial blight (Khush *et al*., 1989, Chen *et al*., 2001; Shanti *et al*., 2010; Hari *et al*., 2011) or blast (Singh *et al*., 2012) or both (Narayanan *et al*., 2004; Singh *et al*., 2011; Zhan *et al*., 2012; Hari *et al*., 2013; Balachiranjeevi *et al*., 2015). In the present study, we adopted an approach similar to that of Hari *et al*. (2013), wherein markers were used not only for introgression of target resistance genes, but also for fertility restoration (i.e. for both *Rf4* and *Rf3*). Through stringent MAS, we were able to recover the good traits of RPHR-1005 along with identification of a breeding line (RP-9-27-79-179-74-105) possessing highly desirable taller plant type and higher yield as compared to RPHR-1005. This was possible by coupling stringent MAS in the initial stages of backcrossing and morphology and phenotype based selection in the later stages, which resulted in development of backcross derived disease resistant lines which are equivalent to or superior than RPHR1005.

Similar to several earlier studies, in the present study, the PCR based markers specific for *Xa21* (i.e. pTA248) and *Pi2* (i.e. AP5659-5) were deployed for foreground selection. Both the markers were observed to be highly efficient in identification of BB and blast resistant lines, respectively and no-false positives were observed (Table 1). In addition to these two markers, we also used molecular markers specific for the major fertility restorer genes, *Rf3* and *Rf4* for foreground selection in the first generation of backcrossing. This is because the donor parent (RPBio Patho-1) was earlier observed to be devoid of restoring allele at the both the loci (data not shown) and it was essential to ensure that the backcross derived lines possess both the restorer genes. Further, we also deployed a modest number of (i.e. 61) of parental polymorphic SSR markers for background selection and background genome analysis. At BC_2F_2 generation, the background genome recovery varied from 91.2% to 93.7% among the three selected plants and all of them were identical or slightly better than RPHR-1005 in most of the agro-morphological features and grain type. The analysis further indicated that donor genome segment is limited to < 2Mb on either side of the target resistance genes. This strategy of deployment of markers for background selection coupled with phenotype based selection ensured that the advanced backcross derived plants did not have any adverse linkage drag from the donor parent.

For the present study, we selected a single dominant gene each conferring resistance against BB (i.e. *Xa21*) and blast (i.e. *Pi2*). Even though there are few previous reports about breakdown of resistance conferred by a single BB resistance gene (Mew *et al*. 1992, Khush *et al*., 1989) in rice, till date there is no report about large-scale breakdown of resistance conferred by either *Xa21* or *Pi2* from India or abroad. Further, as per a recent report (DRR annual report, 2008-14), NILs of Samba Mahsuri and Swarna possessing only *Pi2* displayed resistance across multiple locations in India. This was evident, when the improved lines of RPHR-1005 were screened under UBN, wherein all of them were highly resistant against blast disease (Table-2). The same was true with BB resistance also wherein a single gene, i.e. *Xa21* was able to confer good level of resistance in the advanced backcross derived lines of RPHR-1005. Even though, we do not expect that resistance conferred by either *Xa21* or *Pi2* will breakdown in the immediate future, we have still, crossed backcross derived lines of RPHR-1005

possessing *Xa21* + *Pi2* with those possessing *Xa33* + *Pi54* (developed through another study at our Institute) so that pyramided lines of RPHR-1005 possessing *Xa21* + *Xa33* + *Pi2* + *Pi54* can be developed. Presently these lines are at intercross F_3 generation and they will be screened for BB and blast resistance shortly.

Among the improved lines of RPHR-1005 (Table-3), no apparent yield penalty associated with the presence of BB (*Xa21*) & blast (*Pi2*) resistance genes was noticed. This indicates that cultivation of the BB and blast resistant, improved lines would be of great advantage in BB and blast endemic areas. Among the improved lines of RPHR-1005, RP-9-27-79-179-74-105 with 93.4% RPG recovery (Table-3), was identified as best restorer line and is being used a potential parent for developing superior hybrids by crossing it with multiple WA-CMS lines. The improved lines of RPHR-1005 (possessing BB and blast resistance along with improved panicle exertion) and their derived hybrids are being further evaluated for their agronomic performance through station trials at IIRR, Hyderabad, India. The best hybrids will be identified and nominated for multi-location trails under All India Coordinated Rice Improvement Project (AICRIP) for their evaluation and possible release for the benefit of rice farmers.

In conclusion, through the present study, we have developed improved versions of the elite restorer line, RPHR-1005 possessing resistance against BB, blast along with complete fertility restoration and MS grain type.

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