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Introgression of Broad Spectrum Blast Resistance Gene Pi2 Into Mega Variety Swarna Through MABB

Agricultural Science

KEYWORDS : Rice, Swarna, C101A51, Magnaporthe oryzae, Blast resistance gene (Pi2) and Marker assisted back-cross breeding (MABB)

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ABSTRACT

Swarna is one of the most popular high yielding rice varieties in India. It had good grain quality and requires 25% less nitrogen as widely claimed by the farmers released in Andhrapradesh in 1982. It spread across the subcontinent and into Bangladesh, where it was never officially released. Despite, its superior grain and yield qualities swarna is highly susceptible to blast disease. Therefore, in the present study a blast resistant gene Pi2 from donor C101A51 were introgressed into Swarna through marker assisted backcross breeding (MABB) strategy. Twenty five pants were selected possessing Pi-2 gene in homozygous condition through foreground selection by gene specific marker AP5659-5 coupled with stringent phenotypic selection with virulent M.oryzae isolate (SPI-28). Four plants were selected through background selection analysis using polymorphic SSR markers with recurrent parent genome recovery (95%). The indentified plants were morphological similar to recurrent parent in relation to plant height and grain quality. However this introgressed lines yielded more than the recurrent parent.

Introduction

The rice blast disease is caused by an ascomycete fungus *Magnaporthe oryzae*, is a devastating disease, which caused significant yield loss up to 70–80 % during an epidemic (Khush and Jena 2009). Fungicides such as Carbendazim 50 WP, Chlorothalonil 75% WP, Ediphenphos 50% EC, Eprobenfos 48% EC, etc. worth Rs. ~222 crores were used during 2010-11 on rice crop to combat blast disease (Kumar et al. 2013). However, use of chemical pesticides have caused concerns regarding rejection of consignments by the importing nations due to presence of pesticide residues in the produce. Also, use of chemical pesticides is not considered as an eco-friendly approach and therefore, developing genetic resistance is most feasible. More than 100 blast resistance (R) genes and 350 QTLs governing blast resistance have been identified. Among them, 26 blast resistance genes *viz., Pib*, *Pita*, *Pi54*, *Pid2*, *Pi9*, *Pi2*, *Pizt*, *Pi36*, *Pi37*, *Pikm*, *Pi5*, *Pit*, *Pid3*, *pi21*, *Pish*, *Pb1*, *Pik*, *Pikp*, *Pia*, *Pi25*, *Pid3A4*, *Pi35*, *NLS1*, *Pikh*, *Pi54 of* and *Pi54rh* have been cloned and functionally validated (Sharma et al. 2012)

The *Pi2* was orignially identified from a resistant *indica* rice genotype, 5173 and introgressed into the blast susceptible cultivar CO39 and a near-isogenic line (NIL), named C101A51 was developed (Mackill and Bonman 1992). This cultivar was also widely used in breeding programs at the International Rice Research Institute (IRRI) for developing high yielding blast resistant cultivars due to their broad spectrum of disease resistance (Ou, 1985 and IRRI, 1996). Mapping of blast resistance gene was started with the use of limited RFLP probes developed by Kinoshita et al. (1990) and availability of genetic map was studied by Causse et al. 1994. Among the blast resistance genes widely deployed in breeding, *Pi2* has been identified to be one of the most effective, broad-spectrum resistance genes. *Pi2* gene encodes a NBS-LRR (a nucleotide-binding site and leucine-rich repeat) protein (Zhou et al. 2006) and a gene-specific molecular marker, named AP5659-5 has been developed for MAS of the gene (Fjellstrom et al. 2006). Considering these characteristics the *Pi2* was selected as the target blast resistance gene for introgression into Swarna in the present study.

Marker-assisted backcrossing has enormous potential to introduce the blast resistance genes into diverse rice cultivars (Collard and Mackill, 2008; Collard et al., 2008). Introgression of blast resistant genes into advanced improved rice lines is a cost-effective and environmentally friendly approach to combat yield losses (Wen and Gao, 2012). The main advantage of marker- assisted selection is the accuracy of selection of the true plant within the short breeding cycle to produce blast resistant rice varieties. Currently, the blast resistant breeding program has achieved greater success with the advent of marker-assisted selection (Ragimekula et al., 2013). Recently, blast resistance genes *Piz5* and *Pi54* have been introgressed into the genetic background of the PRR78 rice variety from donor parent C101A51 and Tetep, and blast resistant lines were developed with the application of MABC breeding (Singh et al., 2012). The selection was based on foreground markers RM287and RM206. The *Pi1* leaf blast resistance gene has been introgressed into the D521 line derived from the donor line BL122. These examples provide a great opportunity to develop blast resistant rice varieties through MABB breeding. In the present study, the MABB technique was applied to introgress blast resistant gene from the highly resistant rice variety

C101A51 to blast susceptible rice cultivar Swarna.

Materials and Methods

Plant Materials and Breeding Strategy

Swarna variety with high yielding was used as recurrent parent, while C101A51 carrying (*Pi2*) resistance gene was used as donor parent. F_1 seeds were developed from the normal hybridization between Swarna and C101A51.Selected F_1 plant was then backcrossed with Swarna to produce BC_1F_1 seeds. Selected plant carrying resistance gene with highest background parent genome recovery and maximum phenotypic similarity to the recurrent parent were backcrossed with Swarna to generate BC_2F_1 seeds. Foreground and background selection were carried out to select the elite plant from each backcross generation. The BC_2F_1 plants were also subjected to foreground selection followed by phenotypic selection to identify plants homozygous for *Pi2* gene with maximum recovery for RPG. These plants were then selfed to generate BC_2F_2 populations. In the BC_2F_2 generation, plants homozygous for *Pi2* gene were identified and then advanced to the BC_2F_4 generation through the pedigree method of selection.

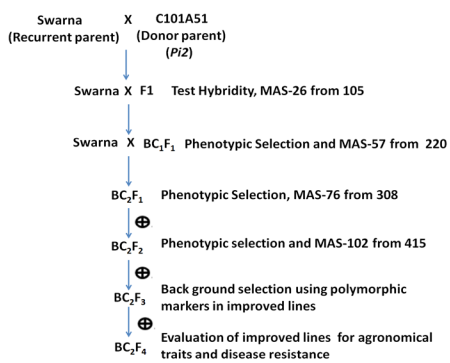

Figure: 1 Flow diagram showing Steps involved in marker assisted back cross breeding program

DNA extraction and PCR Amplification

Total DNA was extracted according to the procedure of Sundaram et al. (2008). Adoption of MAS was facilitated by using ALP marker, AP5659-5 for *Pi2* (Fjellstorm et al. 2006) Table 1). PCR reactions were performed on thermal cycler (AB Bio-systems). Each 10 µl PCR reaction mixture contained 50 ng genomic DNA, 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl2, 2.5 mM dNTPs, 10 µM each of the primer pair and 3 unit Taq DNA polymerase. Template DNA was initially denatured at 94°C for 5 min prior to 35 cycles of denaturation at 94°C (30s), annealing at 55°C (30s), and extension at 72°C (1 m). At the final step, the reaction mixture was incubated at 72°C for 10 min before the completion. The amplified products were then electrophoretically resolved on a 3% agarose gel in 1 × TAE buffer.

Table 1: Marker Details used for introgression

Trait	Gene	Marker	LG	MD (Cm)	Forward sequence	Reverse sequence	Reference
Blast	Pi2	AP5659-5	6	~1.0	CTC-CTTCAGCT-GCTCCTC	TGAT-GACTTC-CAAACG-GTAG	Fjell-storm et al.(2006).

LG: linkage group, MD: map distance

Phenotypic screening of blast disease

The introgressed lines, C101A51 (*Pi2* gene donor), Swarna (recurrent parent) and HR12 (susceptible check) were grown in UBN (Uniform blast nursery) at ICAR-Indian Institute of Rice Research (IIRR), Rajendranagar, India. The most virulent local isolate SPI-28 of *Magnaporthe oryzae* was used for blast screening (Madhan Mohan et al. 2011). The method of inoculum preparation and inoculation was the same as described by (Prasad et al. 2011). The young seedlings at four leaf stage were inoculated with the fungal conidial suspension at concentration of 1 X 10-5 conidia /ml. The inoculated plants were observed after 9 days of inoculation for blast disease lesions. The plants and blast lesion degrees (BLDs) were evaluated on the basis of 0-9 of the IRRISES scale (IRRI, 1996. Roumen et al. 1997).

Agro-morphological traits evaluation

The advanced lines along with parents were planted at a spacing of 15 × 20 cm in a randomized complete block design with three replications, and were evaluated for agronomic traits during Khairf 2014 at the rice fields of Indian institute of Rice Research, Rajendranagar, Hyderabad. Data were recorded for various agronomic traits: days to 50 % flowering (DFF), Days to maturity (DM), plant height (PH), number of tillers (NT), panicle length (PL), Grain per panicle (GP), 1000-grain weight (TW) and yield per plant (Y/P). These traits were recorded from all of the best selected lines of BC2F4 along with the recurrent parent. The procedures for measurement of these traits have been followed by (Abhilash et al. 2015).

Results and Discussion

Marker-assisted foreground selection

Crosses were made between the Swarna and C101A51, and F_1 seeds were collected. The best F_1 plants were screened with foreground markers to identify the true F1 plants carrying the gene of interest in a heterozygous form. The single F_1 gene positive plant was backcrossed with the recurrent parent Swarna and obtained 220 BC_1F_1 seeds. The BC_1F_1 plants were screened for the selection of a heterozygous allele at the *Pi2* locus with AP5659-5 marker along with phenotypic selection. The allele size in base pairs (bp) of both the parents Swarna and C101A51 amplified by marker has been given in Table 2. The best plants of BC_1F1 having an appearance similar to Swarna and carrying the target gene were again crossed with the recurrent parent and 308 plants of the BC_2F_1 generation were selected. These 308 BC_2F_1 plants were screened to identify the plants in heterozygous form with maximum RPG recovery. Selfing was performed in the BC_2F_1 plants, and got BC2F2 seeds were grown. The plants with similarity to Swarna along with homozygous resistant were selected (Figure 2a and 2b). Out of these 76 positive plants with (*Pi2*) were indentified and among these, a single plant possessing maximum recurrent parent genome recovery (~91.5%) was identified and forwarded by selfing till to BC_2F_4, From the four promising advanced backcross lines were identified viz., SA-15-101-5-81-32-5, SA-15-101-5-81-32-29, SA-15-101-5-81-32-51 and SA-15-101-5-81-32-95. They were again then subjected for phenotypic evaluation for disease resistance and also agro morphological parameters.

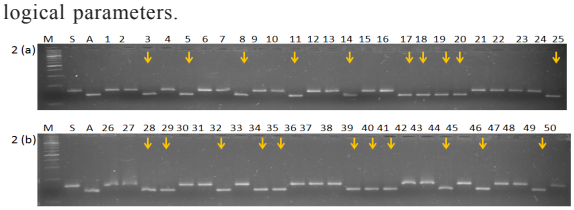

Figure 2. Marker assisted foreground selection at BC_2F_2 generation for Pi2 gene, while arrow Marks showing selection of homozygous plants for Pi2 gene using gene specific marker AP5659-5. as Lanes M: 100bp molecular weight ladder ;S Recurrent parent (Swarna); A, Donor parent (C101A51) and 1-25, 26-50 – BC_2F_2 plants; Arrows indicate 'homozygous positive plants'. Plants # 3,5, 8, 11, 14, 17, 18, 19, 25, 28,29,32, 34, 35, 39, 40, 41, 45, 46 and were positive for Pi2

Table 2. Details of foreground and background selection among the backcross plants derived from the cross Swarna/C101A51

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S. No	Generation	No. of plants screened	Foreground Selection	Background selection			Best plant selected based on background selection
			+ve for *Pi2*	SSRs used analyzed	polymorphic SSRs, homozygous for R' allele	(%) recovery of Recurrent parent genome	
1	F_1	105	91	-	-	-	-
2	BC_1F_1	220	57	59	42	71.1%	SA-15-101
3	BC_2F_1	308	76	6	11	81.3%	SA-15-101-5
4	BC_2F_2	415	102	6	5	91.5%	SA-15-101-5-81

Disease Reaction for Blast

The introgressed lines were screened for resistance to blast disease along with Swarna (Recurrent parent), C101A51 (Donor parent) and HR12 (Susceptible parent) as checks using standard procedures (Figure- 3). The donor parent C101A51 containing (*Pi2*) gene was found to be resistant to the most virulent SPI-28 isolate IIRR and the recurrent parent Swarna (score-9) was susceptible to rice blast. All of four selected improved back crossed lines SA-15-101-5-81-32-5, SA-15-101-5-81-32-29, SA-15-101-5-81-32-51 and SA-15-101-5-81-32-95 observed to be highly resistant to the disease with a score of 1 (Table-3).

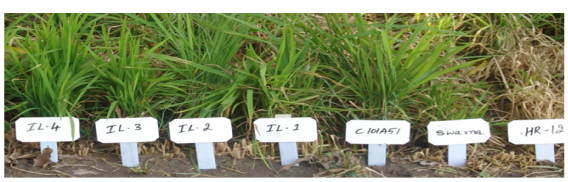

Figure-3: Phenotypic screening of BC_2F_4 introgressed lines of Swarna against blast disease following by UBN Method.HR12 (Susceptible parent), Swarna (Recurrent parent), C101A51 (Donor parent) and BC_2F_4 introgressed lines (IL-1, SA-SA-15-101-5-81-32-5, IL-2, SA-15-101-5-81-32-29, IL-3, SA-15-101- 5-81-32-51 and IL-4, SA-15-101-5-81-32-95

Table 3: Reaction of selected introgressed lines of Swarna to blast

S.No	Rice line	Resistance genes genotyped by linked marker	Reaction against to blast	
		***Pi2* (AP5659-5)**	SPI-28 Score	R/S
1	Swarna	--	9	S
2	C101LA51	++	0	R
3	HR12	--	9	S
4	SA-15-101-5-81-32-5	++	1	R
5	SA-15-101-5-81-32-29	++	0	R
6	SA-15-101-5-81-32-51	++	1	R
7	SA-15-101-5-81-32-95	++	1	R

Agronomical traits of improved lines

The agronomic performance of advanced breeding lines was evaluated during Kharif 2014 (Table 3). The days to heading and days to 50% flowering of introgressed lines were earlier than recurrent parent by 20 days earlier respectively. The advanced line SA-15-101-5-81-32-95 with RPG (95%) displayed, high panicle number, panicle length, grains per panicle, thousand grain weight and yield per panicle are more than recurrent parent Swarna and found to be the best plant in terms of yield advantage. Agro-morphological traits were no significant difference to recurrent parent swarna

Table 3: Agronomical characters of selected four Swarna improved lines

S.No	DFF (Days)	DM (Days)	PH (Cm)	PN	PL (cm)	GP	TGW (gms)	Y/P	% Recovery
Swarna	127.7 ± 1.45	156.3 ± 1.52	79.2 ± 0.23	9.3 ± 0.33	25.0 ± 0.23	159.0 ± 2.08	16.9 ± 0.25	21.2 ± 0.21	
C101A51	88.3 ± 1.67	117.6 ± 1.45	80.3 ± 0.33	8.00 ± 1.00	24.5 ± 0.15	156.7 ± 1.67	16.2 ± 0.17	20.1 ± 0.33	
SA-15-101-5-81-32-5	107.7 ± 0.33	134.6 ± 0.33	79.0 ± 0.49	9.6 ± 0.33	24.8 ± 0.47	162.0 ± 1.37	16.8 ± 0.15	21.3 ± 0.43	93.2
SA-15-101-5-81-32-29	108.3 ± 1.67	136.0 ± 1.00	78.7 ± 0.29	9.0 ± 0.0	25.2 ± 0.32	160.3 ± 1.5	16.9 ± 0.31	21.0 ± 0.07	94.9
SA-15-101-5-81-32-51	107.3 ± 1.45	135.6 ± 0.88	79.6 ± 0.32	9.3 ± 0.17	25.2 ± 0.10	159.6 ± 0.33	16.8 ± 0.17	21.1 ± 0.17	91.5
SA-15-101-5-81-32-95	109.3 ± 0.67	135.67 ± 0.98	79.2 ± 0.12	9.8 ± 0.54	25.9 ± 0.27	163.7 ± 1.86	17.4 ± 0.26	21.6 ± 0.23	96.6

DFF: Days to 50% flowering, DM: Days to maturity, PH: Mean plant height (cm), PN: No.of panicle per plant, PL: Panicle length (cm), TGW (gm): 1000 grain weight (gm), Y/P: Yield per plant (gm) and RPG and Recurrent parent genome recovery (%).

Discussion

Rice production is always constrained by several biotic stresses, among which blast disease impose both several yield and quality losses. These serious and most challenging disease could be overcome by utilizing resistance genes (Tabien et al., 2002). Swarna was released by Andhrapradesh in 1982 developed by Maruteru (ANGRAU) research station and many other states of India (West Bengal, Kerala, Karnataka and Tamilnadu) cultivating in large portion. Swarna Parentage was (Vasista X Mahsuri), matures in 155 days with short plant height, profuse tillering, with short bold grain, requires 25% less nitrogen. High level of susceptibility of Swarna to Blast Saha et al. (2008), caused by the fungus *Magnoporthe oryzae*, is a serious constraint to rice production, which results in major yield loss. Hence, in the present study, an attempt was made to develop high yielding resistant Swarna through MABB approach. Hence a selected dominant resistance gene *Pi2* was selected for introgression into Swarna in the present study.

Jiefeng Jiang et al. (2015) introgressed blast resistance gene *Pi2* into thermo-sensitive genic male sterile (TGMS) lines through MABC breeding approach similar to our study. Introgressed blast resistant gene *Pi-9* into elite Restorer line Luhui17 by marker assisted selection (Wen et al. 2011). (Madhavi and Prasad 2012) introgressed the blast resistance gene *Pi-kh* into Improved Samba Mahsuri from donor parent Tetep. Enhancement of panicle blast resistant in Korean rice cultivar saeilmi by marker assisted back cross breeding. The broad spectrum blast resistant gene *Pi-1* introgressed into an elite hybrid maintainer line, Zhenshan 97 by molecular marker assisted selection (MAS) procedure (Liu et al, 2003). This is the first report on introgression of blast resistance gene *Pi2* into swarna through MABB breeding coupled with phenotypic selection.

The blast resistance gene *Pi2* was identified and located at chromosome 6 and shows a wide-spectrum of leaf blast resistance. Even though there are few previous reports about breakdown of resistance conferred by a single blast resistance gene (Khush *et al*., 1989) in rice, till date there is no report about large-scale breakdown of resistance conferred by *Pi2* from India or abroad. Further, as per a recent re-

ports (DRR annual report, 2008-14), rice line C10A51 possessing *Pi2* displayed resistance across multiple locations in India. In the present study, the selected plants carrying *Pi2* gene phenotyped with blast isolate SPI-28. All introgressed lines SA-15-101-5-81-32-5, SA-15-101-5-81-32-29, SA-15-101-5-81-32-51 and SA-15-101-5-81-32-95 observed to be highly resistant to blast with a score of 1.

Evaluation of the introgressed lines along with the recurrent parent for agronomic performance showed the similarity of these lines to Swarna for most of the traits (Tables 3). The mean values of introgressed lines with Pi2 gene for the traits like panicle number (PN), panicle length (PL) plant height (PH) and grain per panicle were identical or slightly better than Swarna in most of the agro-morphological features and also in grain type. Among the improved lines of Swarna (Table-3), no apparent yield penalty associated with the presence of blast (*Pi2*) resistance gene was noticed. The yield of the introgressed lines ranged from 21.1 to 21.6 gms/plant during Kharif 2014, whereas the recurrent parent Swarna had the values of 21.2 gms/plant. There was a variation of 20 days earliness for 50% flowering and days to maturity in all selected introgressed lines. The earliness in the introgressed lines is understood by the earliness of donor parent C101A51 (95 days and 120 days) respectively.

In the present study, the PCR based marker specific for *Pi2* (AP5659-5) were used for foreground selection. The marker was observed to be highly efficient in identification of blast resistant lines, and no-false positives were observed (Table 1). In addition to this marker, deployed 59 parental polymorphic SSR markers for background selection. At BC_2F_4 generation, the background parent genome recovery varied from 93.3% to 96.6%. That cultivation of blast resistant, introgressed lines would be of great advantage in blast endemic areas. Among the improved lines of Swarna, SA-15-101-5-81-32-95 with 96.6% RPG recovery (Table-3), was identified as best line and is a potential parent for future breeding programmes.

Conclusion

In this study, improved lines of Swarna with resistance to blast disease were developed through marker-assisted backcross breeding selection combined with phenotypic selection. The lines that carried *Pi2* gene in the background of Swarna were either equal to or better than the recurrent parent Swarna in terms of agronomic traits and had the additional feature of being resistant to blast.

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