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RESEARCH ARTICLE

Marker-assisted characterization and evaluation of improved Basmati rice (*Oryza sativa* L.) parental lines for multiple disease resistance

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Abstract

The development of improved parental lines with inbuilt resistance to major biotic stresses is a crucial step in developing promising rice hybrids since the yield advantage from the hybrids can be affected by a disease outbreak. The present study was carried out to characterize a set of improved Basmati parental lines (BPL) consisting of 59 putative maintainers and 107 restorers which were derived from multi-parent crosses involving their diverse maintainers and restorer founder parents for the resistance to bacterial blight (BB), blast and sheath blight diseases. Among the BPLs, 71 entries displayed resistance to BB, including 32 restorers and three maintainers carrying both *Xa21* and *xa13* resistant alleles. Moreover, 85 genotypes exhibited resistance to blast disease, with 35 of them possessing the resistance allele of both *Pi2* and *Pi54* genes. Overall, 108 BPLs harbored resistant alleles for BB and blast, while 60 genotypes were found to possess the resistant allele(s) of one or more genes governing resistance to both blast and BB diseases. As many as 20 restorers possessed resistance alleles of all four genes (*xa13, Xa21, Pi2*, and *Pi54*). As many as 22 genotypes including nine restorers and 13 maintainers were identified as resistant to sheath blight disease. However, none of them possessed the resistant allele of the QTL, *qSBR11-1*. Analysis of allelic frequencies revealed enrichment of favorable resistant alleles in this improved Basmati rice genotype panel compared to founder parents. The amalgamation of multiple genes conferring disease resistance against diverse diseases in these parental lines can bolster hybrid breeding by developing superior hybrids with inherent resistance to these major diseases.

Keywords: Bacterial blight, blast, sheath blight, restorers, maintainers, hybrid breeding

Introduction

Basmati rice is endowed with a distinct combination of favorable aroma, texture, high-volume expansion and palatability of cooked rice, which fetches a premium price for the produce in domestic as well as overseas markets (Gopalakrishnan et al. 2008). In 2023-24, India earned rupees 48,389 crores through the export of Basmati rice (https://agriexchange.apeda.gov.in/indexp/Product_description_32head.aspx?gcode=0601). Owing to the geographic specificity of cultivation that is attached to its trade quality, the Government of India conferred a Geographical Indication tag to Basmati rice in 2016. Among

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the different options for improving crop productivity, hybrid breeding offers immense scope for increasing Basmati rice production through the exploitation of heterosis. Parallel to the popularisation of rice hybrids in India, several challenges have emerged in the adoption of this technology, and one such challenge is the susceptibility of hybrids to various biotic stresses.

The introgression of gene(s) governing resistance to major diseases in the parental lines of hybrids is important for the realization of the yield advantage of the hybrids by avoiding yield losses due to these diseases (Virmani and Kumar 2004). Major diseases such as bacterial blight (BB) and blast outbreaks can result in yield loss of up to 70% (Mew and Vera Cruz 2001), and 50% (Scardaci et al. 1997), respectively. Whereas, sheath blight disease can cause yield loss ranging from 20 to 50% in rice (Groth and Bond 2007). Therefore, enriching the Basmati rice parental lines with alleles of genes governing resistance to multiple diseases can save the hybrids from epidemics (Singh et al. 2012). Although a large number of genes governing resistance to BB and blast diseases have been identified, the gene combinations of *xa13* and *Xa21* for BB, and *Pi2* and *Pi54* for blast have been very effective in managing *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae* races are prevalent in the Basmati growing regions of India (Gopalakrishnan et al. 2008; Elluret al. 2016; Sagar et al. 2020).

So far 48R genes have been identified in rice that confer resistance against bacterial blight (Wang et al. 2020), several of which have been utilized for the genetic improvement of hybrid rice across the world. Zhai et al. (2002) transferred *Xa21* into a widely used restorer line of *indica* hybrid rice (Minghui 63) in China. Basavaraj et al. (2010) improved the parental lines of popular superfine aromatic hybrid PRH10, namely, Pusa 6B and PRR78, by incorporating two genes for BB resistance namely, *xa13* and *Xa21* through marker-assisted backcross breeding. Singh et al. (2012a) incorporated *Pi2* and *Pi54* from two different donor lines into a restorer parent, PRR78 through MABB. Ramalingam et al. (2020) incorporated *Pi54* gene into the genetic background of two popular restorer lines, which were widely used in the rice hybrid breeding programs in South India.

Besides BB and blast diseases, sheath blight is one of the most important fungal diseases, causing significant yield loss in rice (Pan et al. 1999). Several QTLs controlling resistance to sheath blight disease have been mapped in rice (Senapati et al. 2022). Channamallikarjuna et al. (2010) identified a major QTL, *qSBR11-1* which governs sheath blight resistance. *qSBR11-1* has been introgressed into 'Improved Pusa Basmati1' and CO51 to develop several near-isogenic lines resistant to the disease (Singh et al. 2012b; Senthilvel et al. 2021). However, as far as our knowledge there are no reports of improvement for sheath blight disease resistance in hybrid rice breeding.

All the earlier reports are on marker-assisted backcross breeding for the introgression of these resistance genes into the parental lines of popular hybrids. There has not been a systematic breeding program to develop parental lines enriched with resistance alleles of the genes governing resistance to BB and blast-in-the-line breeding programs of hybrids. Threrefore the present study was carried out to address this gap wherein, a set of 166 improved Basmati parental lines including 59 maintainers and 107 restorers which were developed from a set of maintainer and restorer founder parents were genotyped with gene/QTL-based or linked markers as well as phenotyped for their resistance to BB, blast and sheath blight to identify improved multiple disease stress resistant Basmati rice parental lines.

Materials and methods

Plant material

Genes governing resistance to BB, blast, and sheath blight were genotyped in 13 restorer founders and six maintainer founders, which formed the source of resistance in the improved Basmati parental lines (Table 1). Genotyping was also carried out in the 166 improved Basmati parental lines including 59 maintainers and 107 restorers, which were derived from the aforementioned founder parents through a systematic selective intermating within maintainer founders and restorer founders. The detailed strategy for the development of these improved basmati rice parental lines is described in Abhijith (2023). Based on standard methodologies, the resistance to BB, blast, and sheath blight was phenotyped in 166 Basmati parental lines.

Table 1. List of the aromatic rice varieties/breeding lines used as founder parents for the development of Basmati restorer population (BRP) and Basmati maintainer population (BMP)

Restorer founder parents (RFP)	Maintainer founder parents (MFP)
Pusa 1790	Pusa 1401
PRR50019	Pusa 1608
PB 1121	Pusa 21B
Pusa 1826	ANP416
Punjab Basmati 3	IET12014
PRR50012	Pusa 25B
JGL11609	
PKV Makarkand	
PB 1509	
-	
Pusa 1568	
PB 1609	
Pusa 1601	

Genotyping for biotic stress resistance genes

Founder parents, as well as improved Bbasmati parental lines (BPLs), were genotyped for genes/QTL governing resistance to three major diseases of rice namely, blast (*Pi2* and *Pi54*), and bacterial blight (*xa13* and *Xa21*) and sheath blight (*qSBR11-1*) using gene-based/ gene linked markers for their allelic status (Table 2). Isolation of genomic DNA was carried out from the leaves of young seedlings of each plant using the standard CTAB procedure (Murray and Thompson 1980). The DNA was quantified using 0.8% agarose gel in 1X TAE buffer with diluted uncut genomic DNA as standard. The polymerase chain reaction for all the markers was carried out with steps of initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation (at 94°C for 30 seconds), annealing (at 55°C for 30 seconds), and 72°C for 1-minute of extension. The final extension was carried out at 72°C for 5 minutes. The PCR products were resolved on 3.5% metaphor® agarose gel prepared by dissolving 17.5 g of fine agarose powder in 500 mL of 1X TAE buffer. The PCR products were run until the bands were separated clearly. The banding pattern was then visualized using a gel documentation system (Bio-Rad Laboratories Inc., USA). Finally, the amplified products were scored based on agarose gel electrophoresis.

Phentoypic screening for resistance to bacterial blight disease

In order to evaluate BPLs for disease resistance, phenotypic screening for response to bacterial blight disease was carried out at ICAR-IARI, New Delhi during *Kharif* 2019. The BPLs and checks were inoculated with the bacterial suspension at a density of 10^9 cells/ mL using specific isolate 'IARI-Kaul' of *Xanthomonas oryzae* pv. *oryzae* (Xoo) maintained at the Division of Plant Pathology, ICAR-IARI (ICAR-Indian Agricultural Research Institute), New Delhi. Inoculation was done at the maximum tillering stage and carried out through the leaf clipping method (Kauffman et al. 1973). The extent of infection was estimated by damage caused by the pathogen through lesion length measured at 21 days after inoculation. Entries with an average lesion length of less than 6cm were considered resistant and those of more than 6cm were considered susceptible.

Phenotypic screening for resistance to blast disease

The BPLs were raised in Uniform Blast Nursery (UBN) at CSKHPKV, Palampur during *Kharif* 2019 under natural blast epiphytotic conditions. Rows of raised beds measuring 50 cm in length and 10 cm in width were prepared and each BPL was planted in individual rows. To aid the uniform spread of disease, a spreader row of the susceptible check was planted along border rows as well as after every five rows. Scoring was carried out based on the IRRI standard evaluation system. Entries with scores of 0 to 3 were considered resistant; scores of 4 to 6 were considered moderately resistant; those with scores of 6 to 7 were regarded as moderately susceptible while the lines with scores of 7 to 9 were considered susceptible (IRRI 1996).

Phenotypic screening for resistance to sheath blight disease

Phenotypic screening for sheath blight was carried out at ICAR-IARI, New Delhi during *Kharif* 2020 following the typha-bit protocol of Bhaktavatsalam et al. (1978). A virulent strain of *Rhizoctonia solani*, R-359 (ON383512) was multiplied on the shoots of *Typha angustata*. The inoculation was carried out by placing typha bits between tillers above the water line in the field. The observation was recorded 21 days after inoculation by measuring the length of the lesion. The relative lesion height (RLH) was calculated by dividing lesion height by total plant height and multiplying with 100. Genotypes were classified based on the RLH value. Genotypes with RLH less than 20% were classified as resistant, 20-30% were moderately resistant, 31 to 45%

Table 2. Details of the markers used for screening the presence of biotic stress resistance genes

Trait	Gene	Marker name	Primer sequence (5' – 3')	Marker distance	References
Bacterial blight	*Xa21*	pTA248	F-AGACGCGGAAGGGTGGTTCCCGGA	0.1cM	Ronald et al. (1992)
			R-AGACGCGGTAATCGAAGATGAAA		
	xa13	xa13 prom	F-GGCCATGGCTCAGTGTTTAT	Gene-based	Sundaram et al. (2011)
			R-GAGCTCCAGCTCTCCAAATG		
Blast	*Pi2*	AP5930	F-CATGAAAGAAAGGAGTGCAG	0.1cM	Fjellstrom et al. (2006)
			R-ACAGAATTGACCAGCCAAG		
	Pi54	RM206	F- CAATCTCCAAAGTTTTCAGG	0.6 cM	Sharma et al. (2005)
			R-GCTTCAATCACTGCTAGACC		
Sheath blight	*qSBR11-1*	RM224	F-ATCGATCGATCTTCACGAGG	-	Channamallikarjuna et al. (2010)
			R-TGCTATAAAAGGCATTCGGG		

Table 3. Allelic status of target genes for resistance to Bacterial Blight blast and sheath blight diseases in the founder parents

Founder parents	*Pi2*	*Pi54*	*xa13*	*Xa21*	*qSBR11-1*
PB 1509	B	B	B	B	B
Pusa 1568	B	B	B	B	B
PB 1609	A	A	B	B	B
Pusa 1601	B	B	A	A	B
P1790	A	A	A	A	B
PKVM	B	B	B	B	B
JGL11609	B	B	B	B	B
PRR50012	B	B	B	B	B
PRR50019	B	B	B	B	B
Punjab Basmati 3	B	B	A	A	B
SGW223	B	B	B	B	B
PB 1121	B	B	B	B	B
Pusa 1826	B	B	B	B	B
Pusa 21B	B	B	A	A	B
Pusa 25B	B	B	A	A	B
ANP416	B	B	B	B	B
IET12014	B	B	B	B	B
Pusa 1608	B	A	B	A	B
Pusa 1401	B	B	B	B	B

A =Resistance allele and B =Alleles for susceptibility

were moderately susceptible, 46 to 65% were susceptible, and greater than 65% were classified as highly susceptible (IRRI 1996).

Statistical analysis

Assessment of the shift in allelic frequencies of favourable alleles in the improved BPLs was done applying statistical procedures.The allelic frequency of each of the genes, *xa13, Xa21, Pi2,* and *Pi54* was estimated by using the following formula for founder parents and BPL populations, separately:

$$\text{Allele frequency of A1 allele} = (\text{Number of A1 Homozygotes} \times 2 + \text{Number of Heterozygotes}) \div (2 \times \text{Total Number of Individuals})\text{Allele frequency of A1 allele}$$

Where A1 = favorable allele governing resistance to either BB or blast diseases. The shift is then assessed by calculating the difference in the allelic frequencies of BPL with founder parents for each gene.

The Upset plot, a tool for visualizing intersecting sets was used to identify sets of genotypes with common and unique combinations of biotic stress-resistant genes using an R package, UpsetR (Conway et al. 2017).

Results

Assessing the allelic status of biotic stress resistance genes in the founder parents

The genotyping of the founder parents for genes governing bacterial blight resistance (*xa13* and *Xa21*) and blast resistance (*Pi2* and *Pi54)* genes using gene-based or gene-linked markers (Fig. 1), showed that seven out of 19 founders parents possessed one or more alleles governing resistance to bacterial blight resistance (*xa13* and *Xa21*) and blast resistance (*Pi2* and *Pi54*). The presence of the resistance allele of *Pi2* was observed in two restorer founder parents (RFPs) and the *Pi54* resistance allele was amplified in three founder parents including two RFPs and one maintainer founder parent (MFP). In the case of BB, the resistance allele of *xa13* was observed in five founder parents consisting of three RFPs and two MFPs, while for *Xa21* resistance allele was amplified in three RFPs and three MFPs. The founder parents were also screened for a major QTL controlling sheath blight resistance, *qSBR11-1* using the QTL-linked marker, RM224. However, none of the founder parents was found to possess the resistant allele for this QTL (Table 3).

Phenotypic response of BPLs for bacterial blight (BB), blast and sheath blight diseases

The response of the BPLs to bacterial blight disease at ICAR-IARI, New Delhi was recorded during *Kharif* 2019. Leaf damage caused by the *Xanthomonas oryzae* pv. *oryzae* in the inoculated leaves were measured through the length of the lesion. The screening revealed that 71 improved maintainer genotypes showed resistant reaction (lesion length less than 6 cm), out of which 27 genotypes were found to possess the resistance alleles of both the *xa13* and *Xa21* genes. Eighteen genotypes possessed the resistance allele of *xa13* alone, and 17 genotypes possessed only the resistance allele of *Xa21*. About nine genotypes did not possess the resistance allele of either of these genes but exhibited resistant reactions. In 95 improved restorer genotypes, the lesion length exceeded more than 6cm and was classified as susceptible to bacterial blight. Out of 166 BPLs subjected to screening for BB disease, 32 restorers and 3 maintainers were found to possess the

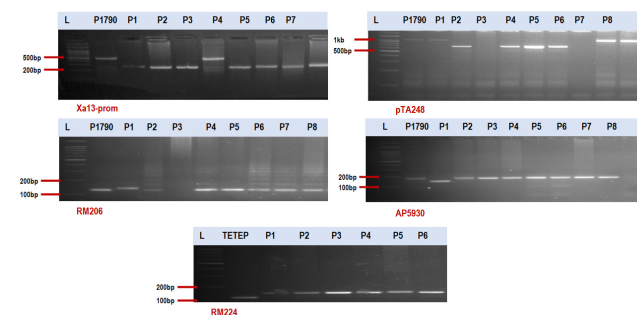

Fig. 1. Representative gel pictographs of various markers used for genotyping for biotic stress resistance genes in the improved population along with the resistant check. L: 100Kb Ladder

resistance alleles of both *Xa21* and *xa13* genes. The presence of the resistance allele of *Xa21* alone was observed in 10 restorers and 16 maintainers, and the presence of the resistance allele of *xa13* alone was detected in 21 restorers and six maintainers (Table 4).

The BPLs were evaluated for blast resistance in Uniform Blast Nursery (UBN) at Palampur, Himachal Pradesh, and scoring was done at the seedling stage (Fig. 2). A total of 85 genotypes showed resistant reactions with a score of 0 to 3, while 34 of them exhibited moderate resistance to blast disease. Moderately susceptible reactions were shown by 24 genotypes, while 23 genotypes were susceptible. Genotyping of the BPLs based on the analysis with gene-based markers for *Pi2* and the gene-linked marker for *Pi54* genes showed that 49 genotypes (all are restorers) possessed the resistance allele of *Pi2*, while 66 genotypes including 60 restorers and six maintainers were found to possess the resistance allele of *Pi54*. Since both these genes are dominant, their presence in the restorer parent alone will be sufficient to ensure blast resistance in the rice hybrids. A total of 35 genotypes were found to carry the resistance allele of both the resistance genes (all are restorers) and all of these genotypes exhibited resistance in the phenotypic screening conducted in the UBN, with the exception of one genotype, which demonstrated moderate resistance. The lines that carry exclusively the resistance allele of *Pi2* and *Pi54* were compared for their phenotypic response to blast. Fourteen restorer genotypes were found to possess the resistant allele of *Pi2* alone, out of which, 13 genotypes were scored as resistant and one was found to be moderately resistant. However, genotypes possessing solely the resistance allele of the *Pi54* gene for blast resistance displayed diverse reactions to blast disease, ranging from a resistant to a susceptible response (Table 4).

Out of 166 BPLs, 108 genotypes were observed to possess the resistance allele for one or more genes governing blight and/or blast resistance (Fig. 3). A total of 60 genotypes were identified to carry the resistance allele of one or more genes for both blast and blight resistance. Notably, a subset of 20 entries was pinpointed to possess all four relevant genes, with the entirety of these lines being identified within the restorer set.

The BPLs were also evaluated for response to sheath blight disease during *Kharif* 2020 at ICAR-IARI, New Delhi. The relative lesson height (%) was recorded after 21 days of inoculation at the maximum tillering stage. The genotypes varied for their disease reactions ranging from resistant to susceptible (Fig. 4). As many as 22 genotypes (nine restorers and 13 maintainers) were identified as resistant to sheath blight (Table 4). Moderate resistance was recorded in 82 genotypes including 43 restorers and 39 maintainers. A total of 53 BPLs registered moderate susceptibility, whereas nine genotypes fell within the susceptible classification.

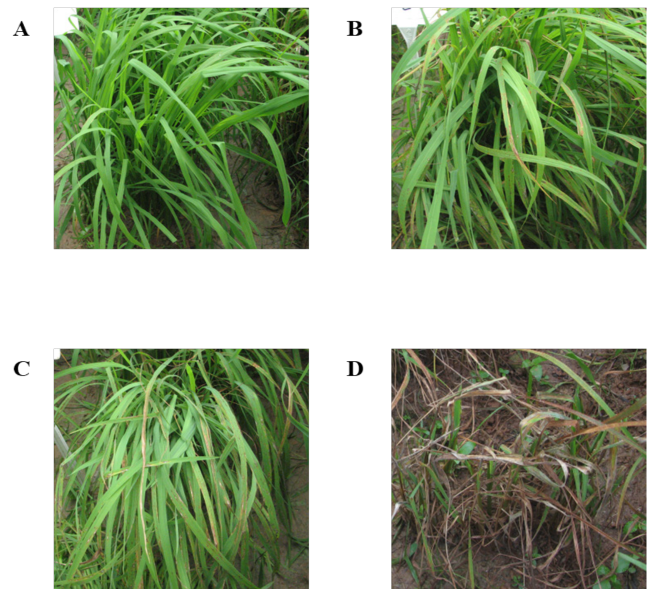

Fig. 2. Representative photographs of genotypes showing different disease reaction for blast under Uniform blast nursery. A = Resistant; B = Moderately resistant; C= Moderately susceptible and D= Highly susceptible

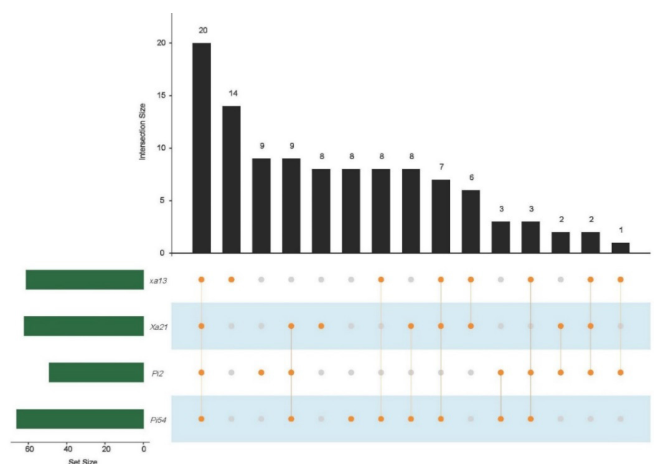

Fig. 3. Upset plot indicating the frequencies of the BPLs possessing one or more genes for resistance to biotic stresses such as BB, blast and sheath blight diseases. Black bars represent the number of entries possessing specific gene combinations. Orange dots connected by lines indicate gene combinations and green set bars indicate the number of genotypes with a specific gene

The BPLs were screened using an SSR marker linked to the major QTL for sheath blight, i.e., *qSBR11-1*. However, none of the BPLswere found to carry the resistance allele of the *qSBR11-1* (Table 3).

Enrichment of favorable resistant alleles of genes governing BB and blast resistance in the BPLs

The allelic frequency of the resistance allele of *xa13* showed an increase of 0.10 (Fig. 5), while that of the allelic frequency of the resistance allele of *Xa21* showed an improvement

from 0.32 in the founder population to 0.37 in the improved parental line population, which marks a shift of 0.05. A significant change in the allelic frequency was observed in the case of *Pi54*, which was observed in the 40% of improved genotypes in contrast, only 16% of the *Pi54* allele was reported in the founders, showing a significant improvement of 0.24 in its allele frequency over founder parents. A similar trend was also observed for *Pi2,* where the allelic frequency of the resistance allele improved by 0.19 (Figure 5) and marked a shift from 0.11 in founder parents to 0.30 in BPLs.

Discussion

Pyramiding multiple genes governing resistance to different diseases can strengthen hybrid breeding by aiding the development of parental lines with inbuilt resistance to major diseases. Several reports suggest the increased incidence of diseases in the hybrids as compared to their corresponding parental lines (Virmani 1994; Guzman and Oard 2019). Among the genes governing resistance to bacterial blight and blast diseases, *xa13* and *Xa21* against Xoo races, and *Pi2* and *Pi54* against *M. oryza* have been found to confer broad-spectrum resistance to these diseases in the Basmati growing regions of India (Gopalakrishnan et al. 2008; Ellur et al. 2016; Sagar et al. 2020).

The improved Basamati parental lines were derived from a systematic intermating followed by pedigree selection between a set of restorer founders which led to the development of 107 improved Basmati restorers and a similar process utilizing a set of maintainer founders resulted in a set of 59 improved Basmati maintainer lines. Therefore, the allelic status of the founder parents for biotic stress resistance genes is crucial in assessing the allelic status of biotic stress resistance genes of the improved population. The representation of *xa13, Xa21,* and *Pi54* is observed in both the maintainer and restorer founders, however, *Pi2* was found in none of the maintainer founders. A major QTL for sheath blight resistance, *qSBR11-1* was also checked for its presence in the founder parents, since, *qSBR11-1* is located in the vicinity of *Pi54* on chromosome 11 (Singh et al. 2012). However, none of the founder's parents possessed the QTL.

Fig. 4. Representative photographs showing the differences in disease reaction for sheath blight disease of rice. A= Resistant; B= Moderately resistant and C= Moderately susceptible

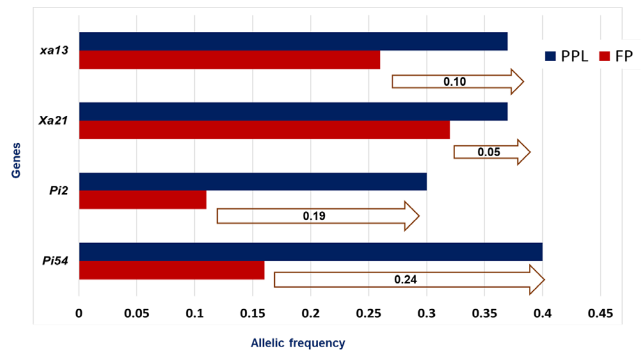

Fig. 5. The shift in the allelic frequency of resistance alleles in the improved Basmati parental lines (BPL) over that of founder parents (FP) for two genes governing resistance to BB (*xa13, Xa21*) and blast diseases (*Pi2, Pi54*), respectively

A total of 108 of these basmati parental lines were found to possess one or more gene(s) for resistance to either of these diseases and 60 BPLs were carrying one or more gene(s) for both bacterial blight and blast diseases. These improved parental lines with resistance genes to both BB and blast can be a valuable resource in developing multiple disease-resistant hybrids. Resistance genes with the dominant mode of action are preferred over recessive genes in hybrid breeding since their presence in either of the parents could provide resistance in the resulting F_1 hybrid, whereas, in the case of recessive genes, they have to be incorporated into both the parents, which is resource consuming (Zhang et al. 2006; Basavaraj et al. 2010). Out of four genes that have been considered for utilization in the improvement of the Basmati rice parental lines, the genes, *Xa21, Pi2,* and *Pi54* show the dominant mode of action, and there is no necessity to incorporate the resistance alleles in both parents. On the other hand, the gene *xa13* is recessive and its presence is observed in 42 improved genotypes in the Basmati restorer set and 19 improved genotypes in the Basmati maintainer set. Therefore, to utilize the gene effectively we need to choose appropriate restorers and maintainers that carry this gene. To further enrich the allelic frequency of *xa13* in each pool, a crossing program within restorer and maintainers can be designed and then selected for the progenies that carry the *xa13* gene.

The screening for sheath blight resistance revealed a spectrum of disease reactions ranging from resistant to susceptible in the putative parental lines even though the QTL, *qSBR11-1* was absent which warrants the need to identify the genomic region governing the resistance reaction for sheath blight in the panel. Since the panel consists of diverse Basmati breeding lines, a genome-wide association study can be conducted to identify the novel genomic variants governing sheath blight in this population. Members of this population have already been employed to map novel genomic regions governing agronomic and grain quality traits for Basmati rice improvement in a

Table 4. Allelic status of the genes governing resistance to biotic stresses (BB, blast and sheath blight) in the improved Basmati rice parental lines and their disease reactions

S. No.	Genotypes	*xa13*	*Xa21*	*Pi2*	*Pi54*	*qSBR11-1*	BB Lesion length (cm)	Blast Score	Sheath blight (RLH %)
C1	Pusa 1790 (Resistant check)	A	A	A	A	B	0.63	0-1	22.76
C2	Pusa 6B (Susceptible check)	B	B	B	B	B	8.13	9	42.48
1	GPR3	B	A	A	A	B	2.07	0-1	29.60
2	GPR4	A	B	A	A	B	1.40	0-1	25.18
3	GPR19	A	B	B	B	B	1.65	5	29.15
4	GPR20	B	B	A	A	B	7.43	0-1	23.02
5	GPR21	B	B	A	A	B	9.50	0-1	18.30
6	GPR23	B	B	B	B	B	8.17	4	14.27
7	GPR32	A	B	B	B	B	3.63	5	22.02
8	GPR35	A	B	A	B	B	7.25	0-1	12.67
9	GPR38	B	B	B	B	B	6.35	4	28.50
10	GPR39	B	B	A	B	B	16.00	1	21.85
11	GPR45	B	B	B	B	B	7.80	4-5	20.02
12	GPR47	B	B	A	B	B	5.50	0-1	18.98
13	GPR52	B	B	A	B	B	6.33	0-1	24.67
14	GPR60	B	A	A	B	B	1.10	1	21.33
15	GPR62	B	A	A	B	B	4.50	0-1	19.07
16	GPR67	A	A	A	B	B	6.05	0-1	23.01
17	GPR70	A	A	A	A	B	7.10	0-1	20.96
18	GPR74	A	A	B	B	B	8.53	0-1	28.79
19	GPR77	A	A	A	A	B	7.63	0-1	21.74
20	GPR78	A	A	A	A	B	4.67	0-1	24.97
21	GPR80	A	A	A	A	B	6.50	0-1	24.37
22	GPR82	A	A	A	A	B	6.00	0-1	20.06
23	GPR86	A	A	A	A	B	4.50	0-1	21.51
24	GPR87	A	A	A	A	B	4.00	1	22.44
25	GPR92	A	A	A	A	B	4.83	0-1	23.83
26	GPR96	A	A	A	A	B	7.00	0-1	31.94
27	GPR100	A	A	A	A	B	5.50	0-1	26.89
28	GPR102	A	A	A	A	B	7.20	0-1	26.30
29	GPR104	A	A	B	A	B	5.50	0-1	23.19
30	GPR106	A	A	B	A	B	6.00	0-1	21.41
31	GPR111	B	A	A	A	B	6.10	0-1	23.56
32	GPR112	B	B	A	A	B	9.50	0-1	32.94
33	GPR113	B	B	A	B	B	6.10	0-1	31.09
34	GPR114	B	B	A	B	B	5.30	0-1	45.90
35	GPR115	B	A	A	A	B	5.80	0-1	24.37
36	GPR117	B	B	A	B	B	8.70	0-1	20.70

37	GPR118	B	A	A	A	B	5.47	0-1	25.48
38	GPR119	B	A	A	A	B	7.23	0-1	21.33
39	GPR120	A	A	B	A	B	6.90	1	47.79
40	GPR131	A	B	A	A	B	8.43	1	16.38
41	GPR136	A	B	A	A	B	5.03	1	21.90
42	GPR142	A	A	A	A	B	3.20	1	25.35
43	GPR144	A	A	A	A	B	2.73	1	30.14
44	GPR146	A	A	A	A	B	5.07	1	30.64
45	GPR147	A	A	A	A	B	5.00	1	26.00
46	GPR148	A	A	A	A	B	5.73	1	26.84
47	GPR149	A	A	B	A	B	4.60	1	31.48
48	GPR150	A	A	A	A	B	5.90	1	20.59
49	GPR151	A	A	A	A	B	4.33	1	26.18
50	GPR155	B	A	A	A	B	10.20	1	42.12
51	GPR157	B	A	A	A	B	9.60	1	30.26
52	GPR160	A	A	A	A	B	3.80	1	23.57
53	GPR164	B	A	B	A	B	8.03	5	31.11
54	GPR166	B	B	B	B	B	7.90	6	29.00
55	GPR173	B	A	B	A	B	3.25	5-6	20.44
56	GPR175	B	B	B	B	B	8.10	6	17.83
57	GPR176	B	B	B	B	B	5.20	5-6	19.47
58	GPR194	B	B	B	B	B	5.77	6-7	34.61
59	GPR195	B	B	A	B	B	14.50	0-1	16.02
60	GPR196	B	B	B	B	B	8.07	6	24.37
61	GPR202	B	B	B	B	B	4.27	7	35.66
62	GPR206	B	B	B	B	B	6.30	7	30.61
63	GPR216	B	A	A	A	B	1.90	2	31.16
64	GPR222	B	B	A	B	B	3.40	2-3	29.49
65	GPR231	B	B	A	B	B	4.13	2-3	33.17
66	GPR234	B	A	B	A	B	4.60	1	44.00
67	GPR237	B	B	B	A	B	6.00	5-6	31.40
68	GPR238	B	A	B	B	B	12.00	4-5	41.27
69	GPR239	A	B	B	A	B	4.00	5-6	34.32
70	GPR240	A	B	B	A	B	9.88	6	39.86
71	GPR241	B	B	B	B	B	10.30	6	49.04
72	GPR247	B	B	B	B	B	8.97	0-1	34.48
73	GPR248	B	B	B	B	B	6.90	1	30.72
74	GPR251	B	B	B	B	B	6.95	7	35.72
75	GPR255	B	B	B	B	B	7.80	6-7	40.14
76	GPR258	B	B	B	B	B	7.87	6-7	48.84
77	GPR259	B	B	B	B	B	7.40	6-7	37.71

78	GPR260	B	B	B	A	B	6.20	6-7	33.20
79	GPR262	B	B	B	B	B	16.00	1	31.51
80	GPR263	B	B	B	B	B	7.23	1	45.12
81	GPR267	B	B	B	B	B	7.20	2	51.62
82	GPR283	B	B	B	B	B	8.35	5-6	49.07
83	GPR290	A	A	B	B	B	0.37	6	28.34
84	GPR292	A	B	B	B	B	6.37	6	30.20
85	GPR293	A	A	B	B	B	0.70	5-6	28.34
86	GPR296	B	A	A	A	B	1.35	5-6	34.98
87	GPR298	B	A	B	A	B	3.35	5	41.59
88	GPR301	B	A	B	B	B	4.70	5	43.53
89	GPR303	B	A	B	A	B	0.30	4	35.30
90	GPR305	B	A	B	A	B	4.23	2-3	44.10
91	GPR308	B	A	B	A	B	6.33	2-3	49.82
92	GPR310	B	A	B	A	B	4.17	4	39.15
93	GPR312	A	B	B	A	B	1.15	5-6	40.81
94	GPR313	A	A	A	A	B	0.53	0-1	41.85
95	GPR314	A	A	B	A	B	1.15	5	44.72
96	GPR315	A	A	B	A	B	1.27	5-6	51.70
97	GPR318	A	A	A	B	B	2.50	5	30.90
98	GPR319	A	A	B	A	B	1.27	5	38.20
99	GPR321	B	B	B	A	B	6.13	6-7	39.13
100	GPR324	B	B	B	A	B	6.32	6-7	30.95
101	GPR329	B	B	B	B	B	7.22	6-7	22.81
102	GPR331	B	B	B	A	B	7.90	6-7	36.41
103	GPR334	B	B	B	A	B	10.17	6-7	31.16
104	GPR335	B	B	B	B	B	7.20	6	30.76
105	GPR338	B	B	B	B	B	6.13	7	38.40
106	GPR339	B	B	B	B	B	8.57	8	34.36
107	GPR341	B	B	B	A	B	9.50	8	38.39
108	GPM16	A	B	B	A	B	4.27	0-1	44.91
109	GPM20	A	B	B	A	B	6.78	0-1	37.96
110	GPM21	A	B	B	A	B	4.77	0-1	36.01
111	GPM22	A	B	B	A	B	0.23	0-1	23.06
112	GPM23	A	B	B	A	B	2.93	1	26.46
113	GPM25	B	B	B	B	B	6.20	6	17.18
114	GPM26	B	B	B	B	B	9.90	6	20.71
115	GPM27	A	B	B	B	B	5.40	3	23.69
116	GPM28	A	B	B	B	B	0.13	2	27.31
117	GPM29	A	B	B	B	B	3.85	5	16.63
118	GPM30	A	B	B	B	B	6.53	5	22.98

119	GPM31	B	B	B	B	B	8.12	5-6	33.83
120	GPM33	A	B	B	B	B	6.67	6	30.32
121	GPM35	B	B	B	B	B	13.00	6	21.31
122	GPM36	B	B	B	B	B	4.00	5-6	23.09
123	GPM37	B	B	B	B	B	12.70	5	29.52
124	GPM40	B	B	B	B	B	7.50	4-5	19.28
125	GPM45	B	B	B	A	B	6.75	5-6	21.37
126	GPM48	B	B	B	B	B	7.42	0-1	19.65
127	GPM49	B	B	B	B	B	15.53	0-1	26.40
128	GPM53	B	B	B	B	B	6.77	0-1	22.12
129	GPM55	B	B	B	B	B	8.55	0-1	30.42
130	GPM60	B	B	B	B	B	18.17	0-1	28.01
131	GPM61	B	B	B	B	B	7.33	0-1	29.08
132	GPM67	A	B	B	B	B	3.11	0-1	28.15
133	GPM68	B	B	B	B	B	13.00	0-1	27.54
134	GPM69	A	B	B	B	B	15.75	0-1	25.06
135	GPM71	B	B	B	B	B	6.50	0-1	27.67
136	GPM72	B	B	B	B	B	9.25	0-1	29.33
137	GPM73	B	B	B	B	B	9.50	0-1	23.65
138	GPM75	B	B	B	B	B	9.87	0-1	26.31
139	GPM77	B	B	B	B	B	7.07	0-1	26.06
140	GPM78	B	B	B	B	B	7.53	0-1	17.37
141	GPM80	A	B	B	B	B	4.67	0-1	26.96
142	GPM81	A	B	B	B	B	3.67	0-1	27.89
143	GPM82	A	B	B	B	B	3.27	0-1	24.64
144	GPM83	B	B	B	B	B	7.13	9	24.97
145	GPM85	B	B	B	B	B	7.93	9	23.04
146	GPM86	B	B	B	B	B	8.93	9	24.17
147	GPM87	B	B	B	B	B	7.27	9	16.00
148	GPM88	B	A	B	B	B	6.20	8	25.03
149	GPM91	B	A	B	B	B	2.54	7-8	23.42
150	GPM93	B	B	B	B	B	8.17	7-8	23.34
151	GPM94	B	B	B	B	B	6.83	6-7	26.72
152	GPM97	B	A	B	B	B	6.80	8	28.68
153	GPM98	B	B	B	B	B	6.23	8	17.19
154	GPM100	A	B	B	B	B	5.78	9	25.01
155	GPM101	A	A	B	B	B	0.57	9	21.67
156	GPM105	A	A	B	B	B	0.93	8	18.83
157	GPM106	A	A	B	B	B	2.93	7	17.57
158	GPM109	B	B	B	B	B	7.70	7	26.08
159	GPM113	B	B	B	B	B	9.83	7	24.82

160	GPM114	B	B	B	B	B	7.30	7	22.02
161	GPM115	B	B	B	B	B	7.95	7	30.19
162	GPM117	B	B	B	B	B	7.83	6	19.25
163	GPM118	B	B	B	B	B	6.33	3-4	25.01
164	GPM119	B	A	B	B	B	0.63	3-4	15.10
165	GPM124	B	A	B	B	B	6.63	4	18.10
166	GPM127	B	A	B	B	B	4.20	4-5	12.09

A = Resistance allele and B= Allele for susceptibility; GPR =; GM =

previous study (Abhijith et al. 2022). The comparison of allelic frequencies of these key disease-resistance genes between BPLs and their founder parental sets revealed that the allelic frequencies of favorable resistance alleles of these four disease-resistant genes have been improved significantly in the improved Basmati rice genotype, as compared to their founder parental set.

Authors' contribution

Conceptualization of research (SGK); Designing of the experiments (SGK, AKP); Contribution of experimental materials (SGK, AKP); Execution of field/lab experiments and data collection (AKP, SGK, PKB, KKM, RR, GP, BMB, MN); Analysis of data and interpretation (AKP, KKV, SGK, RKE, HB, AKS); Preparation of the manuscript (AKP, SGK, KKV).

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