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Marker assisted introgression of genes governing resistance to bacterial blight and blast diseases into an elite Basmati rice variety, 'Pusa Basmati 1509'

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Abstract In the present study, we introgressed two genes each governing resistance to major diseases of rice namely, bacterial blight (BB) (*xa13* and *Xa21*) and blast (*Pi2* and *Pi54*) into a popular Basmati cultivar, Pusa Basmati 1509 (PB 1509) through marker assisted backcross breeding (MABB). Through foreground selection, seven plants homozygous for all the four genes were selected from a large population of 1832 BC_2F_2 plants and subjected to background selection coupled with phenotypic selection for agronomic and grain quality traits of the recurrent parent. BC_2F_2 selections were further advanced to BC_2F_4 generation to develop near-isogenic lines (NILs). Six NILs from the BC_2F_4 families with an RPG recovery ranging from 82.5% to 90.5% were evaluated in multi-location trials for agronomic performance, grain quality traits and disease resistance. The level of resistance to BB and blast diseases in all the selected NILs was similar to that of the donor parent. Three BB races, race 2, 4 and 6, respectively produced on average 1.8, 2.3 and 2.5 cm of lesion length in the NILs as against 15.6, 18.1 and 20.8 cm in PB1509. Further, the NILs recorded a disease score of 1.0 and 1.7 for two blast isolates *Mo*-nwi-38 and *Mo*-nwi-kas, respectively, as against the score of 4.0 in PB 1509 for both the isolates. The NILs were similar to PB 1509 for major agronomic and grain quality traits with the advantage of resistance to BB and blast diseases.

Keywords Marker assisted backcross breeding · Bacterial blight · Blast · Resistance · Basmati rice

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Introduction

Basmati is a quality rice from the Indian subcontinent, preferred by consumers worldwide. Among the Basmati growing countries, India is the world leader in production and export of Basmati rice, being cultivated in ~ 2.2 million hectares with an annual production of ~ 8 million tonnes of milled rice and earning of about INR 328,041 million (~ 4608 million USD) from the yearly export (APEDA 2019). The traditional Indian Basmati cultivars are photosensitive with longer duration, prone to lodging, susceptible to diseases and pests and low yielding (Singh et al. 2011, 2018a). Basmati rice genetic improvement is, therefore, fundamentally aimed at improving yield, agronomic traits and disease resistance while maintaining its unique grain and cooking quality. The genetic diversity of Basmati rice is in India is limited, as it is cultivated in a restricted area around north-western Indo-Gangetic plains and adjoining Himalayan valley (Nagaraju et al. 2002). There are no known sources of resistance in Basmati germplasm for diseases such as bacterial blight (BB) and blast. Therefore, the genes for resistance are to be mobilised from the non-Basmati gene pool without compromising its unique grain and cooking quality. Although Basmati varieties with improved agronomic performance have been developed by traditional breeding, their sensitivity to biotic stresses has not improved remarkably (Singh et al. 2011). Moreover, conventional breeding requires a significantly longer time for incorporating multiple resistance genes.

Marker assisted backcross breeding (MABB) is one of the widely used molecular breeding strategies for the incorporation of resistance gene(s) from a suitable donor into a susceptible cultivar (Singh et al. 2019). MABB has been successfully utilized in transferring major genes/ quantitative trait locus (QTL) governing resistance against several diseases such as BB (Abenes et al. 1993; Joseph et al. 2004; Pradhan et al. 2015), blast (Hittalmani et al. 2000; Khanna et al. 2015a, b; Xiao et al. 2017) and sheath blight (Singh et al. 2012). Significant advantages of MABB include precise introgression of target alleles of desired genes with less turnover time, which is about five years for varietal development and release as against over ten years required by traditional breeding (Singh et al. 2019).

BB caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and rice blast caused by *Magnaporthe oryzae* are two major rice diseases that are notoriously known for causing significant yield losses of up to 65% and 50%, respectively, as well as deterioration of grain quality (Mew 1987; Kihoro et al. 2013; Ellur et al. 2016b). Although there are 44 resistance genes reported against Xoo (Kim 2018), the gene combination of *xa13* (on chromosome 8) and *Xa21* (chromosome 11) together have been found to provide a broad-spectrum resistance against the Xoo races prevalent in Basmati growing regions of India (Gopalakrishnan et al. 2008; Ellur et al. 2016b). Likewise, out of more than 100 resistant genes reported for blast (Ellur et al. 2016a), two genes namely, *Pi2* and *Pi54* located on chromosome 6 and 11, respectively, are known to confer broad-spectrum resistance against the *M. oryzae* isolates prevalent in the Basmati growing regions of India. Introgressed together, these genes synergize the spectrum of resistance against the blast pathogens making them ideal for development of blast resistant Basmati cultivars (Singh et al. 2012; Khanna et al. 2015a).

Pusa Basmati 1509 (PB 1509), a recently released Basmati rice variety in India, is a cultivar characterised by its shorter duration with a maturity span of 120 days in addition to its exceptional grain and cooking qualities (Singh et al. 2014a). It is highly popular among farmers because of its shorter growth period that saves a significant quantity of irrigation water by saving at least three to four irrigations. Nevertheless, PB 1509 is highly susceptible to the two major diseases affecting rice namely BB and blast. The disease sensitivity of PB 1509 brings in considerable loss to farmers in the event of a disease outbreak, which occurs sporadically in Basmati growing areas. Chemical control of these diseases results in significant chemical residues in grains (EFSA 2016), causing apprehension in export as well as in domestic markets. Therefore, introgressing broad-spectrum resistant genes against BB and blast in PB 1509 offers an environmentally friendly, sustainable solution to improve yield level under disease incidence, as well as in producing grains relatively safer for human consumption. In this study, we report successful introgression of four resistance genes, two each for BB (*xa13* and *Xa21*) and blast (*Pi2* and *Pi54*) into the background of PB 1509 to develop near isogenic lines (NILs) that has combined resistance for both the diseases together with all agronomic

features of the popular cultivar such as early duration, high yield and Basmati grain quality.

Materials and methods

Plant materials

PB 1509 and Pusa 1790 were used as recurrent and donor parents, respectively (Fig. 1). PB 1509 is a climate-smart, water-saving, fertiliser responsive, short duration, semi-dwarf (95–100 cm), non-lodging, non-shattering, high yielding and premium quality Basmati rice variety developed from the cross, Pusa 1301/PB 1121 and released during 2013 (Singh et al. 2014a). The cooked kernels are uniform, non-sticky with very good kernel length after cooking (19.1 mm), having intermediate amylose content (21.24%) and highly aromatic. The donor parent, Pusa 1790 possesses four resistance genes, *xa13* and *Xa21* against BB, and *Pi2* and *Pi54* against blast. It was developed by crossing two NILs of the aromatic rice restorer line PRR78, namely Pusa 1601 and Pusa 1609. Pusa 1601 possesses BB resistance genes, *xa13* and *Xa21*, while Pusa 1609 harbours blast resistant genes, *Pi2* and *Pi54*. Pusa 1790 is an elite donor with Basmati type grain and cooking quality similar to that of its recurrent parent, PRR 78 (Singh et al. 2014b). The cross, PB 1509*2/Pusa 1790 is designated as Pusa 1847.

Fig. 1 A representative picture of the recurrent parent, Pusa Basmati 1509, a popular Basmati cultivar highly susceptible to BB and blast diseases and the donor, Pusa 1790, a gene pyramided line of PRR 78, carrying four resistant genes, two each governing resistance to BB and blast diseases

In the present study, the development of PB 1509 NILs has been achieved through shuttle breeding approach, wherein two generations are grown annually, one followed by the other at two different favourable locations to achieve rapid breeding turnover per year (Borlaug 2007). The first location is the experimental farm of ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, (28° 35′ N, 77° 12′ E, 228.16 m above mean sea level, MSL) with deep friable, medium textured, sandy loam to loam, non-calcareous soil. The second location, at the experiment station of the ICAR-IARI Rice Breeding and Genetics Research Centre (RBGRC), ICAR-IARI, Aduthurai in Tamil Nadu (11° 00′ N, 79° 28′ E, 20 m above MSL), is with alluvial clay soil and enjoys weather condition suitable for round the year rice cultivation with average annual rainfall of 1150–1250 mm. After the *Kharif* season (June–Nov) harvest at New Delhi, the generation advancement was carried out at Aduthurai during the late *Rabi*/Offseason (Dec–May), before returning the materials to New Delhi for the next *Kharif* season. Selection for Basmati characteristics exclusively conducted at New Delhi, which falls in the traditional Basmati growing region of India.

Breeding strategy

We have adopted a MABB approach combining foreground, background and phenotypic selection (Fig. 2). The recurrent parent, PB 1509 was crossed with the donor, Pusa 1790 during the offseason of 2012–13 at Aduthurai, and the F_1s were grown during the following season (*Kharif* 2013) at New Delhi. True F_1s were identified using the gene linked or gene-based markers, namely AP5930, RM206, xa13prom and pTA248 respectively, for the genes *Pi2, Pi54, xa13* and *Xa21* (Table 1). In the first backcross, the recurrent parent, PB 1509 was used as female to cross with the true F_1 plants heterozygous for all the four genes to produce BC_1F_1 generation. The BC_1F_1 plants were grown at Aduthurai during 2013–14 and the plants heterozygous for all four markers were identified by foreground selection. These plants were further backcrossed to the recurrent parent to generate BC_2F_1 population. The BC_2F_1 were also subjected to

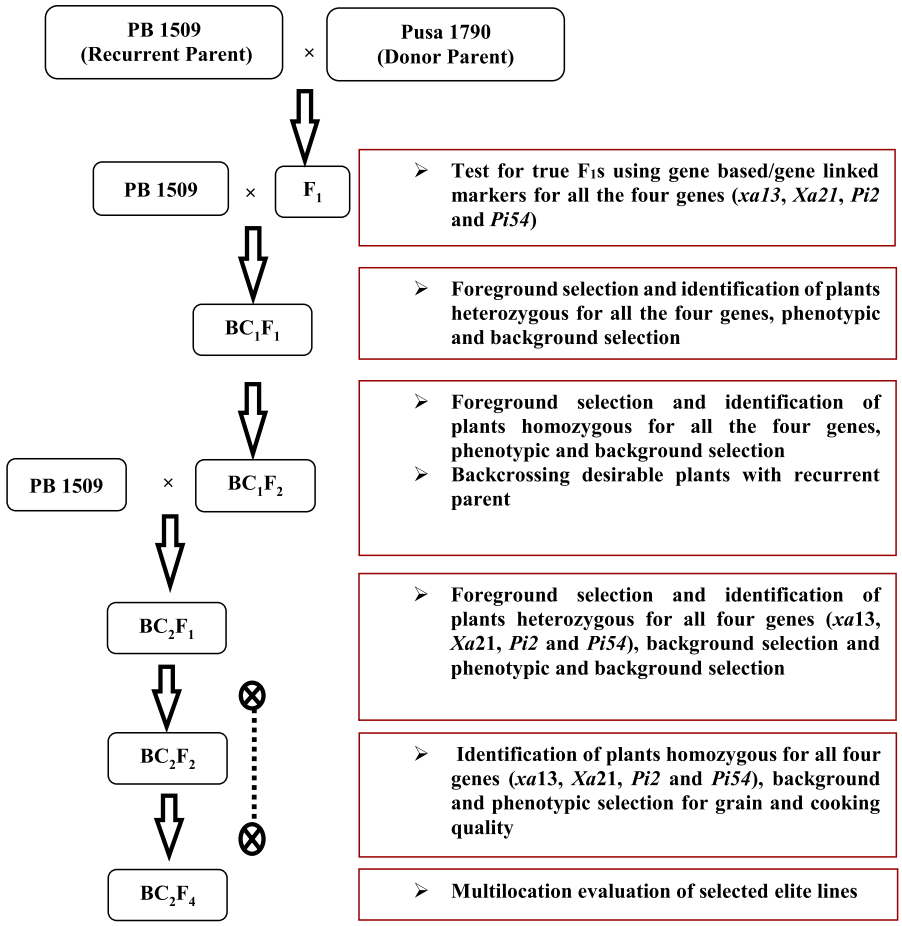

Fig. 2 Scheme of marker assisted backcross breeding (MABB) involving foreground, background and phenotypic selections for development of the BB and blast resistant NILs of PB 1509

foreground selection for all four target genes. A preferential selection for phenotypic similarity to PB 1509 was exercised at this level. The selected BC_2F_1s that were phenotypically similar to PB 1509 were selfed to generate BC_2F_2 population. Subsequently, a large population of BC_2F_2 was grown from which lines homozygous for all the four genes were selected.

A panel of 744 genome-wide sequence tagged microsatellite site (STMS) markers were used for parental polymorphism survey between the recurrent and donor parents. A set of 76 markers identified to be polymorphic. Based on the physical location of these polymorphic markers, a total of 64 evenly spaced STMS markers covering the entire genome were used for background selection among introgressed lines. The background selection followed a reductive strategy to economise the breeding process (Ellur et al. 2016b). Additionally, stringent phenotypic selection for morpho-agronomic traits in tandem with background selection was utilised to maximise the RPG recovery (Gopalakrishnan et al. 2008). The RPG recovery was estimated as per the standard formula (Singh et al. 2018b).

After two backcrosses, pedigree selection was followed from BC_2F_2 onwards to generate PB 1509 NILs. BC_2F_3 families were generated from the selected BC_2F_2 plants homozygous for all gene linked/gene-based markers by selfing. The BC_2F_3 families were then evaluated for agronomic performance along with the parents and one plant per family was selected based on the maximum RPG recovery and phenotypic similarity to the recurrent parent. For recording familywise data, five uniform looking plants were selected and harvested, while one best-looking plant was harvested separately for further advancement. The BC_2F_4 generation was grown from this plant and the bulked seeds were used for multi-location testing at Delhi and Karnal. At the BC_2F_4 generation, improved PB 1509 NILs homozygous for all the four genes namely, *xa13, Xa21, Pi2* and *Pi54* were evaluated for

Table 1 Details of markers used in the study for introgression of resistance genes and recovery of key Basmati characteristics in the NILs

Gene/QTL*	Markers	Trait	Type	Chr	Primer sequence (5′–3′)	References
xa13	xa13-prom	BBR	G	8	F: ggccatggctcagtgtttat	Basavaraj et al. (2010)
					R: gagctccagctctccaaatg	
Xa21	pTA 248	BBR	G	11	F: agacgcggaagggtggttcccgga	Ronald et al. (1992)
					R: agacgcggtaatcgaagatgaaa	
Pi54	RM206	BLR	Q	11	F: cccatgcgtttaactattc	Sharma et al. (2005)
					R: cgttccatcgatccgtatatgg	
Pi2	AP5930	BLR	Q	6	F: atgaaagaaaggagtgcag	Fjellstrom et al. (2004)
					R: acagaattgaccagccaag	
Aro8-1	RM80	ARO	Q	8	F: ttgaaggcgctgaaggag	Amarawathi et al. (2008)
					R: catcaacctcgtcttcaccg	
Badh2	NKSBAD2	ARO	G	8	F: ggttgcatttactgggagttatg	Amarawathi et al. (2008)
					R: caaacaaaggtttaaagacacct	
elr11-1	RM181	KER	Q	11	F: cagctagtgagctcctagtg	Amarawathi et al. (2008)
					R: gctaacccaccaacttattc	
elr11-1	RM209	KER	Q	11	F: atatgagttgctgtcgtgcg	Amarawathi et al. (2008)
					R: caacttgcatcctcccctcc	
asv6-1	RM3	ASV	Q	6	F: acactgtagcggccactg	Amarawathi et al. (2008)
					R: cctccactgctccacatctt	
asv6-1	RM217	ASV	Q	6	F: atcgcagcaatgcctcgt	Amarawathi et al. (2008)
					R: gggtgtgaacaaagacac	

Chr chromosome, *BBR* bacterial blight resistance, *BLR* blast resistance, *ARO* aroma, *KER* kernel elongation ratio, *ASV* alkali spreading value, *G* gene-based marker, *Q* QTL linked marker, *F* forward primer, *R* reverse primer

*First four markers were used for the foreground selection targeting the introgression of resistance genes; the remaining markers were used to confirm the background recovery of key Basmati quality traits

disease resistance, agronomic performance and grain quality parameters.

Screening for disease resistance

The testing for BB resistance under artificial inoculation was conducted at New Delhi, while the blast screening was carried out at two locations, (i) New Delhi under artificial conditions, and (ii) Malan under uniform blast nursery (UBN). For the field level BB resistance screening at New Delhi, the NILs at BC_2F_4 stage were tested along with the parents using virulent isolates of Xoo namely 'race 2', 'race 4' and 'race 6' (Ellur et al. 2016a, b) maintained at the Division of Plant Pathology, ICAR-IARI, New Delhi. Plants at the maximum tillering stage were inoculated with the bacterial culture maintaining a density of 10^9 cells/ml. The maximum tillering stage indicated the end of late vegetative stage in rice phenology, when all of the tillers have emerged, which has occurred at about 60 days after sowing in both the parents used in the present study. The artificial inoculation was carried out as per the procedure of Kauffman et al. (1973). The lesion length was measured 21 days after inoculation adopting the standard protocol (Mew 1987).

For blast resistance evaluation at New Delhi, the NILs and the parents were artificially inoculated using a virulent isolate of *M. oryzae* (*Mo*-nwi-kas) obtained from the Basmati growing region of Kashmir. Plants were inoculated with blast conidia at three-leaf stage (21 days old). Uniformity in seedling growth could be maintained for the identical exposure to the disease inoculum, as both the donor and recipient parents were similar in maturity (120 days' duration) and had similar seedling growth habits. The inoculum comprising $\sim 5 \times 10^4$ conidia per ml was mixed with

0.02% Tween 20 and used for inoculating the test entries. Test entries after inoculation were kept overnight at high humidity and darkness to promote the fungal growth (Singh et al. 2012). At the UBN in Malan (32° 06′ N, 76° 25′ E, 950 m above MSL), the natural *M. oryzae* inoculum load was augmented through inoculation of a virulent isolate (*Mo*-nwi-38) on the NILs and parents grown along with infector rows of susceptible genotype, HR12 at the three-leaf stage. Data for blast score was recorded after 7 days and scored using Bonman's scale (Bonman et al. 1986).

Evaluation for agro-morphological and grain quality traits

A multi-location evaluation for testing the agronomic performance and grain quality parameters was conducted at two locations, New Delhi and Karnal. The experimental site at Karnal was the research farm of ICAR-IARI Regional Station (29°42′ N, 76°49′, 254 m above MSL). The NILs along with the parental checks were evaluated in a randomised complete block design with three replications. Data on agro-morphological traits such as plant height (PH), days to 50% flowering (DFF), panicle length (PL), filled grains per panicle (FGP), spikelet fertility % (SF), thousand grain weight (TGW) and grain yield/plant (GY) were collected from five plants per entry using standard procedures (IRRI 2013). Head rice recovery (HRR) was estimated by dehulling 200 g of raw rice kernels in a Satake® laboratory dehuller THU35B (Japan) and the decorticated grains were further milled using a Satake® Testing Mill TM05C (Japan). The HRR was computed as the ratio of the weight of whole polished grains to the weight of the raw grains and expressed in percentage (Lapis et al. 2019). To determine the grain parameters such as kernel length before cooking (KLBC), kernel width before cooking (KWBC) and length–width ratio (LWB) and cooking quality characteristics, namely, kernel length after cooking (KLAC), kernel width after cooking (KWAC) and elongation ratio (ER), ten grains from each entry was used to record observations as described previously (Khanna et al. 2015a, b) using e-vision, Annadarpan (CDAC, Kolkata). For this, ten whole milled kernels were selected and soaked for 30 min in 10 ml of distilled water taken in test tubes. The tubes were then lowered into a boiling water bath for 8–10 min, completely immersing the lower part of the tube containing rice kernels. The cooked kernels were cooled to room temperature after transferring the contents into a petri plate and data was recorded. The alkali spreading value (ASV) was determined by spreading six milled whole kernels in a petri plate and submerging them in 1.7% KOH for 23 h at 30°c. The ASV per sample was recorded as per the 1–7 score scale (Little et al. 1958).

Molecular validation

All the PB 1509 NILs were validated for the presence of all four resistance genes using their respective target markers with the parents as control. Additionally, molecular validation was done for key Basmati traits such as aroma, elongation ratio and alkali spreading value (ASV). The loci associated with aroma on chromosome 8 was targeted by two markers, RM80 for *aro8-1* QTL and nksbad2 for the *badh2* gene (Amarawathi et al. 2008). The QTL for elongation ratio, *elr11-1* was validated using the flanking markers RM1812 and RM209, while the QTL for ASV, *asv6-1* was tested using the flanking marker pairs, RM3 and RM217. The details of the markers used are given in Table 1.

Statistical analysis

The data were analysed for statistical significance using analysis of variance and mean comparison by the least significant difference test (Fisher 1935). The analysis for blast resistance score was carried out independently at different locations, because of the use of different screening methods and pathogen isolates prevalent in respective locations. All the analyses were done using STAR v. 2.0.1 (IRRI 2014).

Results

Foreground, background and phenotypic selection

The recurrent parent, PB 1509 was crossed with the donor parent, Pusa 1790 to generate 40 F_1 seeds. Out of these, six true F_1s heterozygous for all the gene linked/ gene based markers used for foreground selection were identified and crossed to the recurrent parent to produce 262 BC_1F_1 plants. Foreground

selection using the markers xa13prom, pTA248, AP5930 and RM206 respectively for *xa13, Xa21, Pi2* and *Pi54* genes on the BC_1F_1s resulted in the identification of fifteen plants heterozygous for all the four resistance genes. The selected plants were assessed for morphological similarity with PB 1509 and those phenotypically desirable plants were genotyped with 64 markers polymorphic between the parents to determine the extent of recurrent parent genome (RPG) recovery. The background recovery of the plants ranged from 57% to 71%. One of the BC_1F_1 plants with maximum RPG recovery was backcrossed with PB 1509 to generate 142 BC_2F_1s. A similar selection approach was adopted and nine BC_2F_1 plants heterozygous for all the four genes with more than 82% RPG recovery were identified, which were further selfed to produce 1832 BC_2F_2s (Fig. 3). The foreground selection in BC_2F_2 plants resulted in the identification of seven BC_2F_2 plants homozygous for all four genes.

Seven BC_2F_3 families were grown from these selected plants and one family was rejected based on poor agronomic performance. Of the remaining six BC_2F_3 families, one best plant each per family were used for developing six BC_2F_4 PB 1509 NILs. The foreground markers were further used to confirm the presence of all the four resistance genes (*xa13, Xa21, Pi2, Pi54*) in the PB 1509 NILs (Fig. 4). The RPG recovery in the NILs ranged from 82.54% to 90.48%. The NIL 2 (P1847-12-77-3-10) showed maximum RPG recovery of 90.48%, followed by NIL 5 (P1847-12-1698-25-13) with 88.89%. Graphical genotype generated using STMS marker based genotyping indicated residual heterozygosity present on the chromosomes 1 and 6, while there were smaller donor segments observed in chromosomes 1, 3, 4 and 6 (Fig. 5).

BB and blast disease resistance of the improved lines

Under artificial inoculation with three different Xoo races, race 2, 4 and 6, the recurrent parent, PB 1509 showed high susceptibility to BB with an average lesion length of 18.06 ± 3.23 cm, as against Pusa 1790 that showed highly resistant reaction with the average lesion length of 1.63 ± 0.89 cm (Table 2). The disease reaction against all the Xoo races by PB 1509 NILs and the donor parent was comparatively similar (Fig. 6a). PB 1509 NILs showed an average lesion length of ~ 2.0 cm and thus were grouped as resistant (Mew 1987) in contrast to the high susceptibility observed in the recurrent parent, PB 1509.

For blast disease, the recurrent parent, PB 1509 recorded a highly susceptible reaction with a disease score of '4' on Bonman's scale when screened in the UBN using the virulent isolate *Mo*-nwi-38 as against the highly resistant reaction with a disease score of '0' by the donor parent, Pusa 1790 (Table 2). The improved PB 1509 NILs also showed highly resistant (score 0) to resistant (score 2) reactions for this isolate in the UBN. A very similar disease reaction was observed among the NILs and parental lines under artificial blast inoculation done at New Delhi using *Mo*-nwi-kas isolate (Fig. 6b).

Evaluation of agronomic and grain quality traits among the improved lines

The analysis of variance (ANOVA) indicated significant variation for genotypes, location and genotype x location components, for several agronomic traits. Among the agronomic traits, plant height, days to flowering, filled grains per panicle and plant yield has significant genotype x location interactions (Table 3). For grain quality, the genotypic variation among NILs and the recurrent parent was non-significant for kernel elongation ratio, aroma and alkali spreading value. The further mean separation carried individually for the locations showed that the NILs yielded either on par or better than the recurrent parent, PB 1509 (Table 4). The yield of PB 1509 at New Delhi was 57.88 q/ha, while the improved NILs produced 55.5 q/ha on average, with a range of 53.7 q/ha (NIL 1, P1847-12-51-2-9) to 59.3 q/ha (NIL 5, P1847-12-1698-25-13). Similarly, at Karnal, PB 1509 registered a yield of 56.3 q/ha, while the improved NILs had an average yield of 60.2 q/ha with a range of 54.9 q/ha (NIL 2, P1847-12-77-3-10) to 67.8 q/ha (NIL 1, P1847-12-51-2-9). At Delhi, NIL 6 (P1847-12-1729-27-14) showed significantly higher yield over PB 1509, while it performed at par with PB 1509 at Karnal. While at Karnal, NIL 1 (P1847-12-51-2-9) outperformed the recurrent parent. In general, the yield performance of the recurrent parent was good at New Delhi conditions, while most of the improved NILs performed better at Karnal conditions. Similar pattern was seen for other agronomic traits as well.

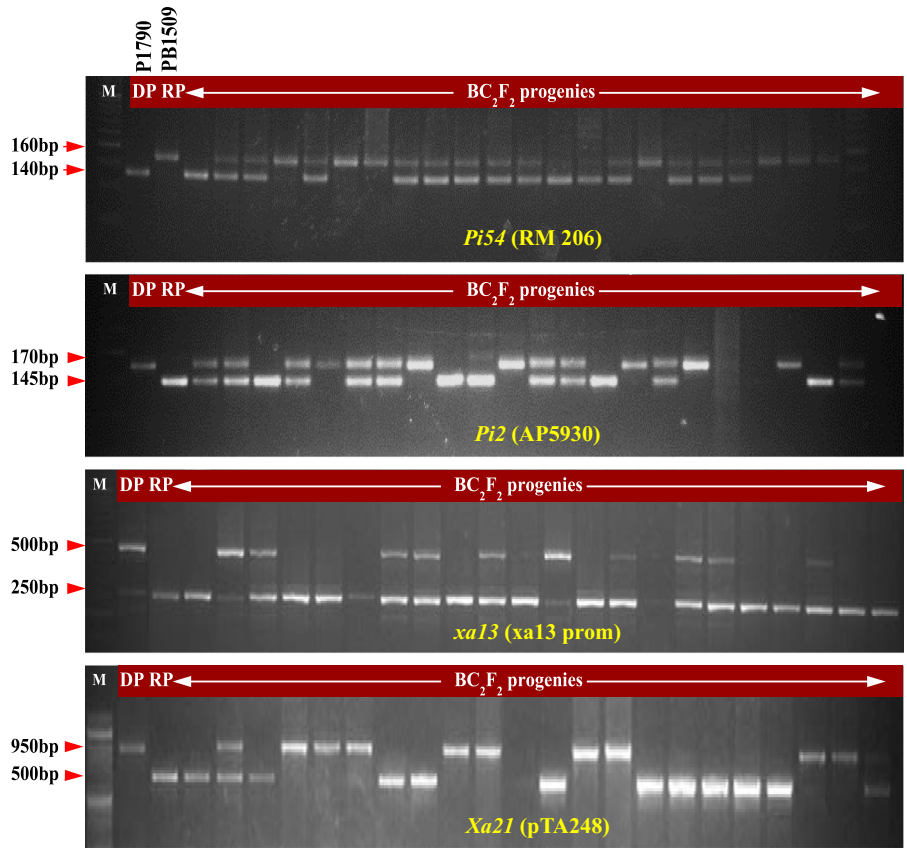

Fig. 3 A representative PCR amplification profile of the markers for the resistance genes governing BB and Blast diseases among the individuals of BC_2F_2 generation along with the parents. DP, donor parent; RP, recurrent parent; M, molecular-weight size marker (DNA ladder)

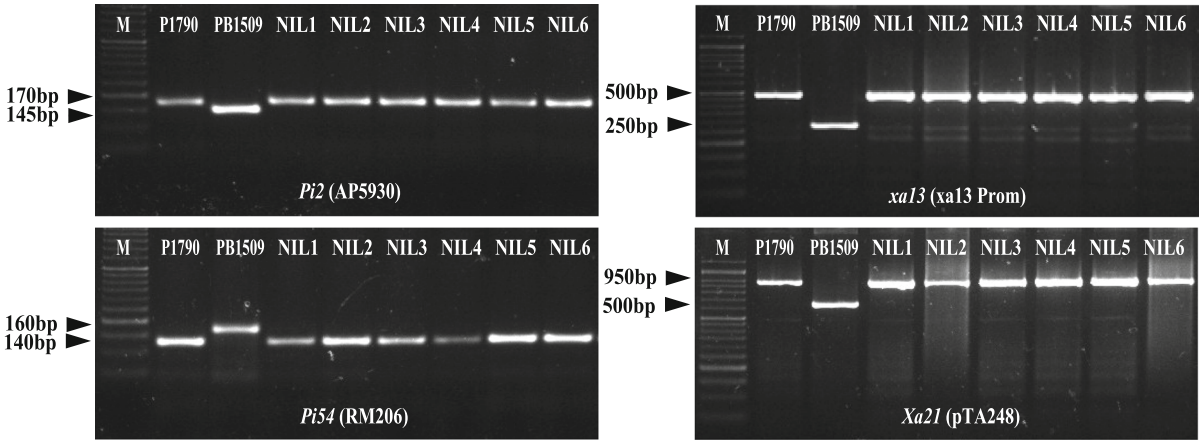

Fig. 4 Confirmative amplification profile of foreground selection markers for two genes each for BB and blast resistance in the six gene-pyramided NILs; M, molecular-weight size marker (DNA ladder)

Grain quality analysis indicated a non-significant difference between the recurrent parent and most of the NILs at both New Delhi and Karnal (Fig. 7). While KER, aroma and ASV registered no difference, other traits such as HRR, KLBC, KWBC and the LWR showed little but significant variation in some of the NILs across both the locations (Table 5). However, there was no statistically significant variation between the NILs and the recurrent parent for KLAC and KWAC at New Delhi. All the improved lines were

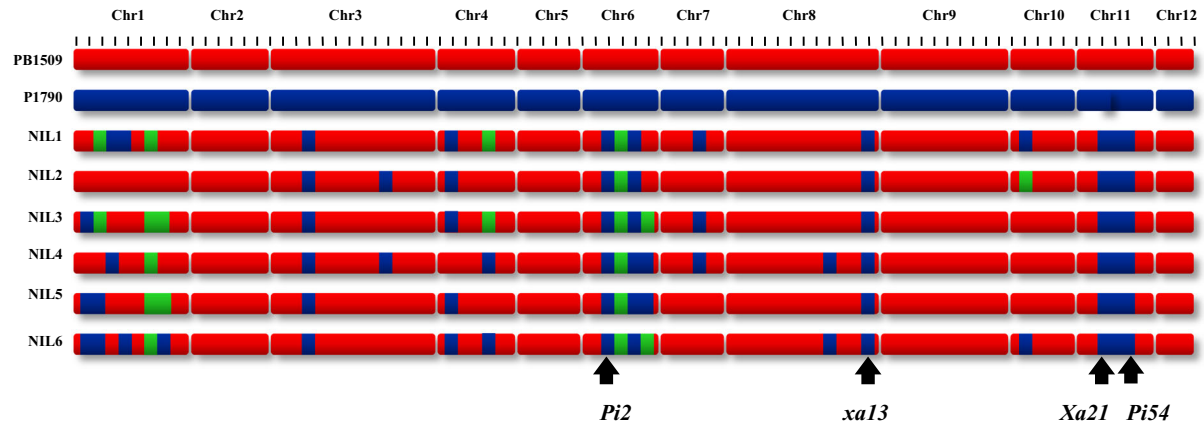

Fig. 5 Chromosomal segments alignment across NILs including donor parent (Pusa 1790) and recurrent parent (PB 1509) showing recurrent parent genome recovery and introgression of resistance genes for blast (*Pi2* and *Pi54*) and bacterial blight (*xa13* and *Xa21*). PB1509, Pusa Basmati 1509; P1790, Pusa 1790; NIL 1, P1847-12-51-2-9; NIL 2, P1847-12-77-3-10; NIL 3, P1847-12-90-10-11; NIL 4, P1847-12-1279-23-12; NIL 5, P1847-12-1698-25-13; NIL 6, P1847-12-1729-27-14; Chr1 to Chr12 are the chromosomes. Red colour indicates recurrent parent chromosome segments, while blue colour denotes the donor segments. Green colour represents heterozygotic loci

Table 2 Disease reaction of the four-gene introgressed NILs in the PB 1509 background against three races of *Xoo* and two isolates of *Magnaporthe oryzae*

Genotypes*	Mean BB lesion length (cm)			Blast score		RPG (%)
	Race 2	Race 4	Race 6	*Mo*-nwi-38	*Mo*-nwi-kas	
NIL 1	2.92 ± 1.18^{a}	2.42 ± 1.13^{a}	2.15 ± 0.55^{a}	2^{a}	0^{a}	82.5
NIL 2	1.59 ± 0.53^{a}	2.42 ± 0.91^{a}	1.72 ± 0.44^{a}	2^{a}	2^{b}	90.5
NIL 3	3.11 ± 0.93^{a}	2.33 ± 0.68^{a}	1.64 ± 0.43^{a}	2^{a}	0^{a}	84.1
NIL 4	2.94 ± 1.13^{a}	2.50 ± 0.61^{a}	1.70 ± 0.50^{a}	2^{a}	2^{b}	84.1
NIL 5	2.00 ± 0.65^{a}	2.14 ± 0.51^{a}	1.88 ± 0.51^{a}	0^{b}	0^{a}	88.9
NIL 6	2.11 ± 0.54^{a}	1.90 ± 0.88^{a}	1.87 ± 0.55^{a}	2^{a}	2^{b}	88.6
PB 1509	20.83 ± 4.18^{b}	18.06 ± 3.12^{b}	15.58 ± 2.01^{b}	4^{c}	4^{c}	100.0
Pusa 1790	1.86 ± 0.48^{a}	2.02 ± 0.54^{a}	2.02 ± 0.61^{a}	0^{b}	0^{a}	0.0

Lesion length ± Standard error, *BB* bacterial blight, *BB* screening was done at New Delhi, Blast screening done at uniform blast nursery (UBN) in Malan used *Mo*-nwi-38 race, while field screening at New Delhi used 'Kashmir' isolate (*Mo*-nwi-kas), *RPG* recurrent parent genome recovery %; The means followed by same letters are statistically not different at $p < 0.05$ by least significant difference test

*NIL 1, P1847-12-51-2-9; NIL 2, P1847-12-77-3-10; NIL 3, P1847-12-90-10-11; NIL 4, P1847-12-1279-23-12; NIL 5, P1847-12-1698-25-13; NIL 6, P1847-12-1729-27-14

highly aromatic with an aroma score of two, while the ASV was six as that of PB 1509. At Delhi, head rice recovery (HRR) of three NILs, NIL 1 (P1847-12-51-2-9) with 53.9%, followed by NIL 5 (P1847-12-1698-25-13) with 52.4% and NIL 4 (P1847-12-1279-23-12) with 51.5% were on par with PB 1509 (50.6%), while the remaining lines showed slightly lesser HRR. These NILs also showed similar patterns of HRR in Karnal as well. Additionally, two more NILs, NIL 3 (P1847-12-90-10-11) and NIL 6 (P1847-12-1729-27-14) also exhibited similar HRR as that of PB 1509 at Karnal location. However, NIL 2 (P1847-12-77-3-10) showed lower HRR at both the locations. For KLBC, five NILs were found to be on par with the recurrent parent at New Delhi as well as at Karnal, except for NIL 1 which showed consistently shorter kernel length at both the environments. Further, a similar trend was observed for KWBC, where in five NILs except for

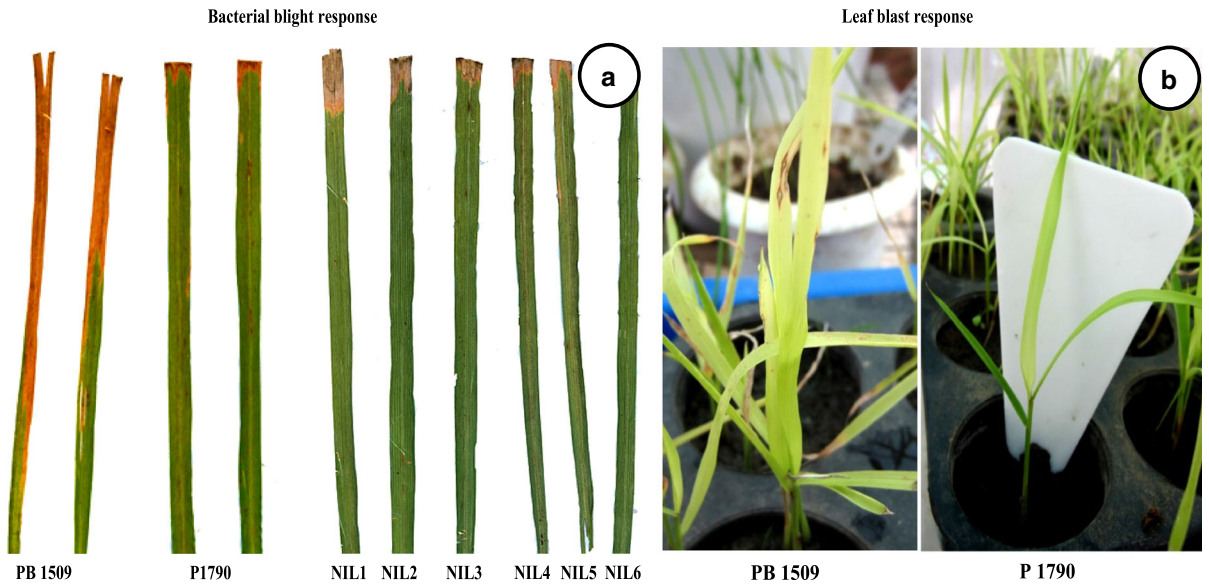

Fig. 6 Disease reactions of **a** BB pathogen, *Xanthomonas oryzae* pv. *oryzae* race 2 and **b** blast pathogen, *Magnaporthe oryzae* isolate *Mo-nwi-kas* on the recurrent (PB 1509) and donor (P1790) parents. The improved NILs also showed significant disease resistance for BB when challenged with race 2 inoculation. Details are provided in Table 2

NIL 2 under New Delhi conditions and four NILs except NIL 1 and NIL 6 were on par with the KWBC of PB 1509, the recurrent parent. Overall, the cooked grain quality of the improved NILs and PB 1509 were comparable at both the locations, except for one NIL (NIL 2) that showed location specific superiority over PB 1509. Five of the improved NILs showed statistically similar cooked kernel length (16.2–17.3 mm) at Karnal with that of PB 1509 (17.1 mm), except for NIL 2 (P1847-12-77-3-10) which showed increased cooked kernel length (19.0 mm) at this location.

The confirmatory evaluation of the improved NILs for target genes and additional genes associated with grain quality parameters such as aroma, elongation ratio and alkali spreading value using specific markers linked the loci governing this trait indicated that all the improved NILs had recovered the alleles of PB 1509 for all these traits (Table 6).

Discussion

Severe susceptibility of PB 1509 to BB and blast diseases is a major concern of Basmati growers in India, although it has gained popularity among the farmers on account of its excellent yield, grain quality, and shorter duration. To address this concern, a combination of four genes, two each for both the diseases were chosen for introgression through MABB. The two genes selected for BB resistance, *xa13* and *Xa21*, have widely been used in marker assisted improvement of popular rice varieties including Basmati rice varieties such as Improved Pusa Basmati 1 (Gopalakrishnan et al. 2008), Pusa Basmati 1718 (Singh et al. 2018c) and Pusa Basmati 1728 (Singh et al. 2017). These improved lines have helped farmers immensely in tackling sporadic outbreak of BB in Basmati growing regions of India. The gene *xa13* is a recessive allele of the gene *Os8N3*, a Nodulin family gene, which is activated by the infection of Xoo possessing a gene *pthXo1* for its virulence. The *xa13* transcript is unresponsive to pthXo1, resulting in resistance (Antony et al. 2010). The second gene, *Xa21* encoding for a receptor like protein kinase (Song et al. 1995; Park and Ronald 2012), is activated by a protein from Xoo known as RaxX, a tyrosine sulphated protein, which in turn triggers defence response in plants (Pruitt et al. 2015). Both the genes are very effective in inciting immune responses in rice against most common races of Xoo. Similarly, the blast resistance genes, *Pi2* and *Pi54,* are known to act synergistically when present together to enhance the

Table 3 Combined analysis of variance (ANOVA) for agronomic and grain quality data derived from the multilocation evaluation of PB1509-NILs

Trait	Genotype (G)	Location (L)	G × L	Error
PH	194.40**	233.00**	26.50**	2.80
TN	3.90**	ns	ns	0.89
PL	5.00**	22.41**	ns	0.47
DFF	49.41**	219.43**	10.32**	0.42
FGP	588.96**	ns	ns	25.93
SF	5.19**	52.57**	ns	2.62
PLY	14.38**	304.56**	7.10*	2.70
YLD	24.94**	151.32**	44.78**	6.59
HRR	107.57**	ns	21.72**	7.66
KLBC	0.73**	ns	0.23**	0.05
KWBC	0.09**	ns	0.06**	0.01
LWR	1.61**	ns	0.42**	0.08
KLAC	2.06**	18.77**	1.08**	0.29
KWAC	0.24**	2.05**	0.13**	0.03
KER	ns	0.19**	ns	0.01
ARO	ns	ns	ns	0.00
ASV	ns	ns	ns	0.00

PH plant height in cm, *TN* number of tillers, *PL* panicle length in cm, *DFF* days to 50% flowering, *FGP* filled grain per panicle, *SF* spikelet fertility in %, *PLY* grain yield per plot in kg, *YLD* projected yield in q/ha, *HRR* head rice recovery in %, *KLBC* kernel length before cooking in mm, *KWBC* kernel width before cooking in mm, *LWR* length by width ratio, *KLAC* kernel length after cooking in mm, *KWAC* kernel width after cooking in mm, *KER* kernel elongation ratio, *ASV* alkali spreading value

*, ** Significant at 5% and 1% level, respectively; *ns* non-significant

spectrum of resistance against a wide range of *M. oryzae* races (Ellur et al. 2016a). *Pi2* and *Pi54* are resistant (R) genes on chromosome 6 and 11 of rice, respectively, and encode for nuclear binding site-leucine rich repeats (NBS-LRR) domains that are known to trigger effector induced immune response on pathogen infection (Marone et al. 2013; Zhang et al. 2018). NILs carrying both *Pi2* and *Pi54* in the background of Pusa Basmati 1121 and Pusa Basmati 6 were found highly resistant with 100% incompatible reaction between the NILs and the blast isolates from across India (Ellur et al. 2016a). Recently, introgression of these blast resistance genes together through MABB resulted in the release of two improved Basmati rice cultivars, Pusa Basmati 1609 and Pusa 6 (Pusa 1612) for effectively combating blast outbreak (Singh et al. 2019).

In the present study, we have developed improved PB 1509 NILs with resistance to both BB and blast, diseases which are prevalent in Basmati growing regions of India. The high level of resistance shown by the improved PB 1509 NILs for both the diseases denoted that the genes deployed for improvement are robust, and are relatively free from background interactions. The screening under artificial inoculation in the field and UBN had shown a significant level of resistance to both the diseases which could help in realising better yields in the event of a disease outbreak. In earlier studies, MABB could be used for successful transfer genes governing either BB or blast resistance singly or in combination, into several rice cultivars in India such as Pusa Basmati 1 (Gopalakrishnan et al. 2008; Khanna et al. 2015a), Pusa Basmati 1121 (Ellur et al. 2016a, b), Pusa Basmati 6 (Ellur et al. 2016a) and BPT 5204 (Krishnan et al. 2019). Many of these efforts culminated in the release of improved varieties such as Improved Pusa Basmati 1, Pusa 1592, Pusa Basmati 1609, Pusa Sugandh 6, Pusa Basmati 1637, Pusa Basmati 1718, Pusa Basmati 1728 and Pusa Samba 1850 (Krishnan et al. 2019; Singh et al. 2019).

Although transferring of dual disease resistance was the prime objective of this study, MABB could also help in the effective development of improved NILs with similar agronomic, grain and cooking qualities identical to that of the recurrent parent, PB 1509. We could achieve the introgression of all four genes from the donor parent through precise foreground selection. When all the four genes are independently assorting, theoretically, at least 16 plants are required to obtain a plant heterozygous for all four genes in a backcross made with a true F_1 and the homozygous recurrent parent. In practical terms, this translates into a large backcross population. We have therefore used a sufficiently large BC_1F_1 and BC_2F_1 population (262 and 142, respectively) to recover plants heterozygous for all four genes. While handling the large backcross population, the reductive screening approach used for foreground selection was found highly useful and efficient. In this method, we have used one marker at a time in a stepwise fashion to reduce the population size to be screened. The plants heterozygous for the marker alleles for the first gene was subjected to screening for the second gene, after

Table 4 Agronomic performance of improved PB 1509 lines at New Delhi and Karnal location during the *Kharif* 2016

Genotypes*	Agro-morphological traits#							
	PH	TN	PL	DFF	FGP	SF	PLY	YLD
A. New Delhi								
NIL 1	111.2^{a}	10.0^{ab}	28.2^{ab}	93.0^{a}	84.6^{b}	85.9^{bc}	2.4^{a}	53.7^{a}
NIL 2	99.6^{b}	12.1^{a}	27.7^{bc}	83.0^{d}	80.8^{b}	81.3^{d}	2.5^{a}	54.6^{a}
NIL 3	110.7^{a}	10.1^{b}	28.2^{b}	93.0^{a}	103.3^{a}	83.4 ^{cd}	2.5^{a}	55.8^{a}
NIL 4	108.7^{a}	10.6^{ab}	26.7^{c}	91.0^{a}	85.1^{b}	87.3^{ab}	2.4^{a}	54.2^{a}
NIL 5	110.7^{a}	10.7^{b}	28.0^{b}	86.0^{c}	106.2^{a}	85.6^{bc}	2.7^{a}	59.3^{a}
NIL 6	111.8^{a}	10.5^{b}	29.8^{a}	89.0^{b}	105.1^{a}	83.2 ^{cd}	2.5^{a}	55.4^{a}
PB 1509	102.0^{b}	11.5^{ab}	27.9^{b}	84.0^{d}	89.9^{b}	90.5^{a}	2.6^{a}	57.9^{a}
Mean (NILs)	108.8	10.7	28.1	89.2	94.2	84.5	2.5	55.5
B. Karnal								
NIL 1	123.7^{a}	12.3^{ab}	30.8^{ab}	96.0^{a}	91.2^{b}	85.9^{bc}	3.4^{a}	67.8^{a}
NIL 2	102.3^{e}	12.4^{a}	29.4^{bc}	89.0^{c}	86.8^{b}	81.3^{d}	2.8^{c}	54.9^{c}
NIL 3	113.6^{c}	10.7^{b}	28.8^{b}	93.0^{b}	104.2^{a}	83.4 ^{cd}	3.1^{bc}	62.4^{ab}
NIL 4	110.4 ^{cd}	10.7^{ab}	28.0^{c}	95.0^{b}	89.2^{b}	87.3^{ab}	2.9^{ab}	58.5^{bc}
NIL 5	118.5^{b}	9.8^{b}	29.5^{b}	95.0^{b}	108.1^{a}	85.6^{bc}	3.0^{abc}	60.2^{bc}
NIL 6	111.9^{c}	9.7^{b}	31.0^{a}	93.0^{b}	106.4^{a}	83.2 ^{cd}	2.9^{abc}	57.2^{bc}
PB 1509	106.3^{de}	11.7^{ab}	29.8^{b}	90.0^{c}	92.5^{b}	90.5^{a}	2.5^{abc}	56.3^{bc}
Mean (NILs)	113.4	10.9	29.6	93.5	97.7	84.5	3.0	60.2
CD (0.05)	4.4	1.7	1.3	1.7	9.4	3.0	4.3	4.3

PH Plant height in cm, *TN* number of tillers, *PL* panicle length in cm, *DFF* days to 50% flowering, *FGP* filled grain per panicle, *SF* spikelet fertility in %, *PLY* grain yield per plot in kg, *YLD* projected yield in q/ha

*NIL 1, P1847-12-51-2-9; NIL 2, P1847-12-77-3-10; NIL 3, P1847-12-90-10-11; NIL 4, P1847-12-1279-23-12; NIL 5, P1847-12-1698-25-13; NIL 6, P1847-12-1729-27-14

#Means followed by same letters are not significantly different at $p < 0.05$ by Tukey's honestly significant test

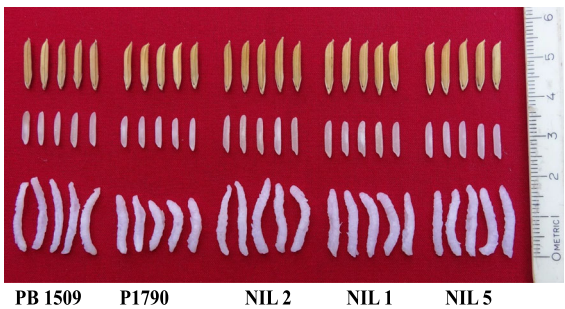

Fig. 7 Grain quality of the parents (Recurrent parent—PB 1509, Donor parent—P1790) and the improved NILs namely, NIL 2, P1847-12-77-3-10; NIL 1, P1847-12-51-2-9; NIL 5, P1847-12-1698-25-13. The NILs show comparable morphology of raw, milled and cooked kernels as that of the recurrent parent, PB 1509. Note that in the donor parent Pusa 1790, cooked kernel elongation is less than that of PB 1509

which the plants heterozygous for both the genes were subjected to foreground selection for the third gene and finally the plants heterozygous for three genes were subjected to the screening for the fourth gene. This has resulted in easy identification of plants heterozygous for all the four genes. The same strategy was used in BC_2 generation as well. Furthermore, for stacking of multiple resistance genes into a single background, gene based/ tightly linked markers were found to be very advantageous in saving time and resources (Khanna et al. 2015a, b; Ellur et al. 2016a, b). In this report also, we could achieve effective foreground selection for all four genes by the use of gene-based markers such as *xa*13prom and pTA148, respectively for *xa*13 and *Xa*21, and gene-linked markers, RM206 and AP5930 for *Pi54* and *Pi*2.

For effecting background selection, although we have used a large number of genome-wide SSR markers, the polymorphism between the parents was about 10%. Compared to earlier MABB programmes from our lab and reported from elsewhere (Sundaram et al. 2008; Gopalakrishnan et al. 2008; Singh et al. 2013; Khanna et al. 2015a; Ellur et al. 2016b), the parental polymorphism obtained in this study was relatively low. The key reason for this was the use of two parents belonging to Basmati lineage, PB 1509 and Pusa 1790. Tracing back into the pedigree, it could be found that the pedigree of both the parents shared common ancestors such as Basmati 370, Sabarmati, Improved Sabarmati and Pusa Basmati 1 at different generation levels (Fig. 8). PB 1509 was developed by crossing Pusa Basmati 1121 with Pusa 1301, both of

Table 5 Grain and cooking quality traits of the NILs and recurrent parent, PB 1509 produced at Delhi and Karnal during *Kharif* 2016

Genotypes*	Grain quality traits (raw and cooked)#								
	HRR	KLBC	KWBC	LWR	KLAC	KWAC	KER	ARO	ASV
A. New Delhi									
NIL 1	53.9^{a}	8.1^{c}	1.7^{ab}	4.9^{b}	15.5^{a}	2.0^{a}	1.9^{a}	2.0^{a}	6.0^{a}
NIL 2	41.6^{d}	9.0^{a}	1.4^{b}	6.2^{a}	16.0^{a}	2.0^{a}	1.8^{a}	2.0^{a}	6.0^{a}
NIL 3	46.6$^{b–d}$	8.9^{ab}	1.7^{ab}	5.3^{b}	16.1^{a}	2.1^{a}	1.8^{a}	2.0^{a}	6.0^{a}
NIL 4	51.5$^{a–c}$	8.6$^{a–c}$	1.9^{a}	4.5^{b}	15.9^{a}	2.1^{a}	1.9^{a}	2.0^{a}	6.0^{a}
NIL 5	52.4^{ab}	8.6$^{a–c}$	1.7^{ab}	5.1^{b}	16.1^{a}	2.1^{a}	1.9^{a}	2.0^{a}	6.0^{a}
NIL 6	44.9 ^{cd}	8.9^{ab}	1.9^{a}	4.7^{b}	15.7^{a}	2.3^{a}	1.8^{a}	2.0^{a}	6.0^{a}
PB 1509	50.6$^{a–c}$	8.3^{bc}	1.8^{a}	4.7^{b}	15.5^{a}	2.0^{a}	1.9^{a}	2.0^{a}	6.0^{a}
Mean (NILs)	48.5	8.7	1.7	5.1	15.9	2.1	1.9	2.0	6.0
B. Karnal									
NIL 1	48.3^{a}	8.3^{c}	2.0^{a}	4.2^{c}	16.3^{b}	2.9^{a}	2.0^{a}	2.0^{a}	6.0^{a}
NIL 2	38.9^{b}	9.7^{a}	1.7^{b}	5.3^{a}	19.0^{a}	2.1^{b}	2.2^{a}	2.0^{a}	6.0^{a}
NIL 3	50.9^{a}	8.7^{bc}	1.7^{b}	5.8^{ab}	17.3^{b}	2.3^{b}	2.0^{a}	2.0^{a}	6.0^{a}
NIL 4	54.5^{a}	8.4^{bc}	1.7^{b}	5.1^{b}	16.2^{b}	2.9^{a}	1.9^{a}	2.0^{a}	6.0^{a}
NIL 5	50.7^{a}	9.0^{b}	1.7^{bc}	5.4^{ab}	17.3^{b}	2.3^{b}	1.9^{a}	3.0^{b}	6.0^{a}
NIL 6	48.0^{a}	8.4^{bc}	1.9^{a}	4.5^{c}	16.4^{b}	2.9^{a}	2.0^{a}	2.0^{a}	6.0^{a}
PB 1509	47.3^{a}	8.4^{bc}	1.6^{b}	5.4^{ab}	17.1^{b}	2.3^{b}	2.0^{a}	2.0^{a}	6.0^{a}
Mean (NILs)	48.6	8.8	1.8	5.1	17.1	2.6	2.0	2.2	6.0
CD (0.05)	7.25	0.4	0.2	0.7	1.4	0.5	0.1	0.4	0.0

HRR head rice recovery in %, *KLBC* kernel length before cooking in mm, *KWBC* kernel width before cooking in mm, *LWR* length by width ratio, *KLAC* kernel length after cooking in mm, *KWAC* kernel width after cooking in mm, *KER* kernel elongation ratio, *ARO* aroma, *ASV* alkali spreading value

*NIL 1, P1847-12-51-2-9; NIL 2, P1847-12-77-3-10; NIL 3, P1847-12-90-10-11; NIL 4, P1847-12-1279-23-12; NIL 5, P1847-12-1698-25-13; NIL 6, P1847-12-1729-27-14

#Means followed by the same letters are not significantly different at $p < 0.05$ by Tukey's honestly significant test

which shared a common ancestry with Improved Sabarmati. While Pusa Basmati 1121 inherited the lineage through its grandparent, Pusa 145; Pusa 1301 was developed from the cross between Improved Sabarmati and Khalsa 7, a landrace. Pusa 145, the grandparent of Pusa Basmati 1121 had Improved Sabarmati both in its maternal and paternal lineage.

Besides, Improved Sabarmati itself is a derivative of Basmati 370. Sabarmati was originally developed through backcross breeding from the cross, T(N)1/ Basmati 370//5*Basmati 370 (Singh 2000). And one more backcrossing of Sabarmati with Basmati 370 resulted in Improved Sabarmati. Similarly, Pusa 1790 was derived by crossing two NILs of PRR78, namely, Pusa 1601 and Pusa 1609. PRR 78 was developed by crossing Pusa 3A with Haryana Basmati, of which the former is a backcross derived line of Pusa Basmati 1, a popular semi-dwarf Basmati rice cultivar. Pusa Basmati 1 also share the ancestry with Improved Sabarmati through its parent Pusa 150. Maximum RPG recovery of 90.48% could be achieved in the BC_2F_4 generation in the present study, which could amount to a recurrent genome similarity of 99% with PB 1509, if all the monomorphic and recovered polymorphic markers are considered. In earlier reports, Gopalakrishnan et al. (2008) and Arunakumari et al. (2016) reported background recovery of 86.9% and 92% estimated based on 69 and 109 polymorphic STMS markers, respectively while improving two popular rice cultivars, Pusa Basmati 1 and MTU1010 in that order. Sundaram et al. (2008) suggested that approximately 50 polymorphic STMS markers would be

Table 6 Marker profile of the improved PB 1509 lines and the parents for genes/QTLs governing disease resistance (BB and blast) and grain quality (aroma, elongation ratio and ASV). '+' indicates the homozygosity for desirable alleles of the gene(s)/ QTLs and '−' indicates the homozygosity for undesirable alleles

Genotypes*	Genes					QTLs		
	Pi2[§]	*Pi54*[§]	*xa13*[§]	*Xa21*[§]	*badh2*[a]	*aro8-1*[a]	*elr11-1*[a]	*asv6-1*[a]
NIL 1	+	+	+	+	+	+	+	+
NIL 2	+	+	+	+	+	+	+	+
NIL 3	+	+	+	+	+	+	+	+
NIL 4	+	+	+	+	+	+	+	+
NIL 5	+	+	+	+	+	+	+	+
NIL 6	+	+	+	+	+	+	+	+
PB 1509	−	−	−	−	+	+	+	+
Pusa 1790	+	+	+	+	+	+	+	+

Pi2 and *Pi54*, blast resistance genes; *xa13* and *Xa21*, BB resistance genes; *badh2*, aroma gene; *aro8-1*, QTL for aroma; *elr11-1*, QTL for elongation ratio; *asv6-1*, QTL for ASV

[§] Indicates the genes that are introgressed in the study

[a]Indicates genes/ QTLs tested for confirming the presence of key Basmati traits among the NILs

*NIL 1, P1847-12-51-2-9; NIL 2, P1847-12-77-3-10; NIL 3, P1847-12-90-10-11; NIL 4, P1847-12-1279-23-12; NIL 5, P1847-12-1698-25-13; NIL 6, P1847-12-1729-27-14

sufficient to recover the yield and quality characteristics of the recurrent parent.

MABB augmented with rigorous phenotypic selection especially for grain quality parameters has been successfully demonstrated in Basmati rice improvement for disease resistance, such as BB (Joseph et al. 2004; Gopalakrishnan et al. 2008; Basavaraj et al. 2010; Ellur et al. 2016b), blast (Singh et al. 2012, 2013; Khanna et al. 2015a, b; Ellur et al. 2016a) and both the diseases combined (Singh et al. 2014b; Sagar et al. 2018). Phenotypic selection is a critical step in MABB to accelerate recovery of the recurrent parent phenome effectively (Singh et al. 2011, 2019), particularly when a non-Basmati donor is used for the Basmati varietal improvement. Often use of non-Basamti parental lines leads to potential impairment of Basmati grain and cooking quality. However, in this study, the donor Pusa 1790 was an elite Basmati quality rice restorer, which however had a significant difference in cooked kernel length from that of PB 1509. Although Pusa 1790 is a long-slender grain genotype, it exhibits relatively less cooked kernel elongation and marginal swelling as compared to that of PB 1509. PB 1509 grains, on the other hand, are extra-long slender with exceptional cooked kernel elongation, which is typical of premium Basmati rice (Singh et al. 2014b). Nevertheless, both the parents possessed grain aroma, and therefore no segregation for aroma was observed in the progenies. Molecular analysis for the *badh2* locus showed that both the parents carried the same aroma allele having an 8 bp deletion, which drives the production of 2-Acetyl-1-Pyrroline (2-AP). Another trait used for the visual selection was the leaf trait. The recurrent parent had a narrow erect leaf with dark green canopy, while the donor parent had a broad semi-erect leaf with a light green canopy. The rigor in phenotypic selection could eliminate undesirable plant types such as plants with tall and with broader leaves at early stages of breeding, while the selection for grain and cooking parameters could help in accruing the recurrent parent phenome quickly (Fig. 6). Further, validation of PB 1509 alleles for QTLs such as *aro8-1* associated with aroma, *elr11-1* for elongation ratio and *asv6-1* for alkali spreading value could ensure the retention premium Basmati traits in the improved NILs. These genes/ QTLs governing the Basmati rice characteristics were mapped earlier from a mapping population developed using Pusa Basmati 1121 as one of the parents (Amarawathi et al. 2008). Pusa Basmati 1121 is one of the most popular Basmati cultivars of India (Singh et al. 2018a, b, c). The presence of these alleles was

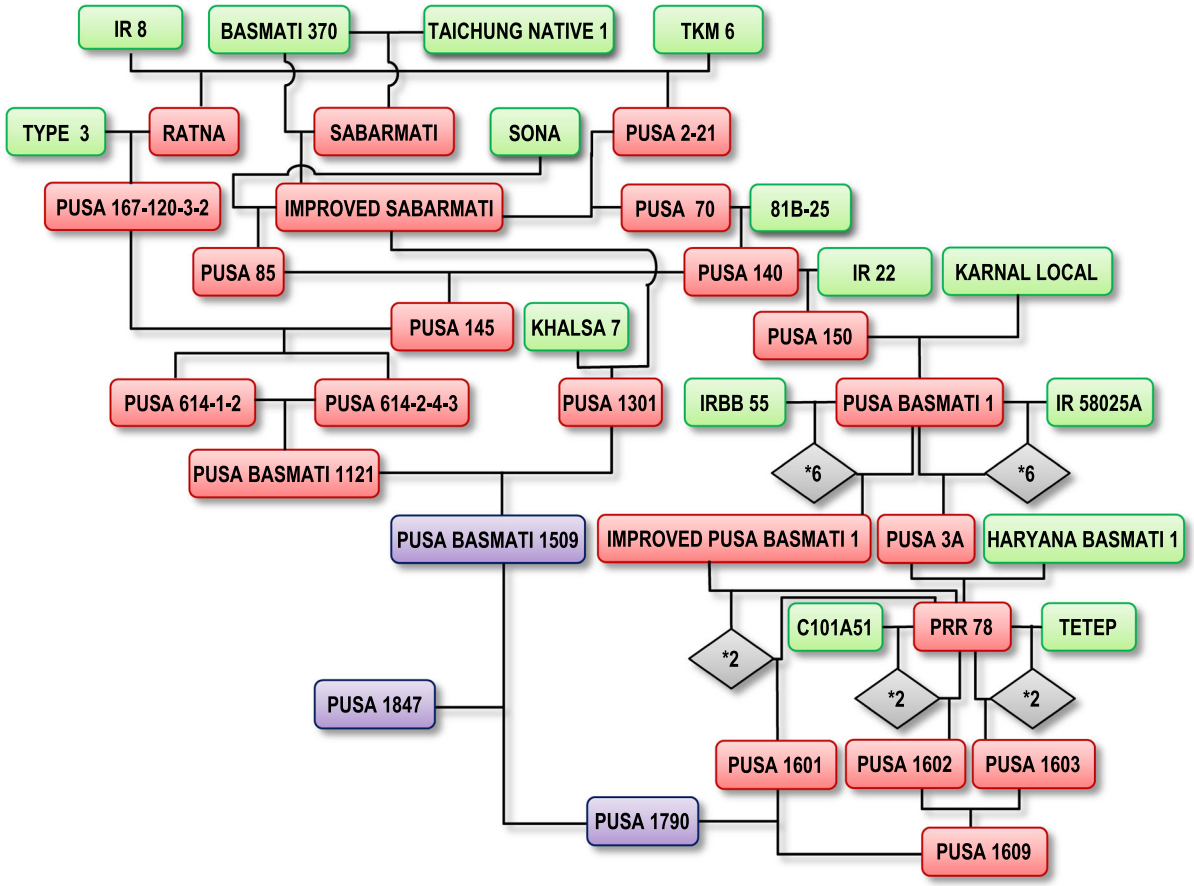

Fig. 8 Pedigree of the improved NILs (Pusa 1847) of Pusa Basmati 1509, indicating immediate and common ancestors of the parental lines, PB 1509 and Pusa 1790. Green boxes indicate founder parents and red boxes indicate derived lines. The NILs and their parents are coloured purple

also confirmed in PB 1509, hence recovery of these loci could assure Basmati grain and cooking quality in the NILs.

Conclusion

The present study reports successful introgression of four resistant genes into a popular short duration Basmati rice cultivar, PB 1509 through MABB targetted against the two most important diseases of rice, namely BB and blast. The newly developed NILs exhibited broad spectrum resistance against common races of both the pathogens (Xoo and *M. oryzae*) under artificial and field screening. They have also showed remarkable phenotypic similarity as that of the recurrent parent combining superior yield and Basmati quality. The improved PB 1509 NILs are further tested in national Basmati trials, which would enable their release for commercial cultivation in India. These BB and blast resistant improved PB 1509 NILs would provide an economical and sustainable assurance for the farmers against losses due to BB and blast diseases in the event of any untoward outbreak of these diseases in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Authors' contribution AKS and GKS conceptualised the project; AKS and GKS led the experiment and did evaluation and mid-course corrections; VS, GD, RKE, MN and GKS generated the plant materials and conducted the field experiments; VS, KKM, GP and RR performed the disease screening experiments; VS, GKS, PKB, HB and KKV did the data curation and analyses; VS, GKS, KKV and AKS wrote the paper. All the authors have read and approved the final manuscript.

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