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Development of Basmati rice genotypes with resistance to both bacterial blight and blast diseases using marker assisted restricted backcross breeding

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Abstract

Marker assisted backcross breeding (MABB) is aimed at introgression of trait(s) into a popular variety to augment specific trait(s) in an otherwise popular variety. While MABB can improve a variety with respect to introgressed trait(s), it offers very little scope for improvement of other traits. Marker assisted restricted backcross breeding (MARBB) is an alternative which can help in identifying transgressive segregants especially, when the donor parent is an elite genotype with several desirable traits. In the present study, restricted backcrossing followed by pedigree selection was used for the development of improved genotypes of Basmati rice with BB and blast diseases using an early maturing Basmati rice variety, Pusa Basmati 1509 as recurrent parent and an elite restorer line, Pusa 1790 as donor. Foreground selection for *xa13*, *Xa21*, *Pi2* and *Pi54* in the backcross progenies was combined with phenotypic selection for agronomic and grain quality traits to ensure premium Basmati grain quality in the progenies. Multi-location yield trial was conducted to evaluate the performance of the improved Basmati rice genotypes with both BB and blast resistance. Pusa 1847-12-62-115-20-6 and Pusa 1847-12-62-190-39-7 recorded significantly higher yields of 68.88 and 62.44 q/ha, respectively, compared to PB 1509 (57.88 q/ha). The improved progenies exhibited resistance to BB with an average lesion length of 2 cm, and blast with scores between 0-2, while PB 1509 was highly susceptible. Another genotype, Pusa 1847-12-62-37-8-3 exhibited head rice recovery (HRR) of 63.99 %, which was significantly higher than in PB 1509 (56.40 %). Marker assisted selection was also effected for fertility restoration genes and improved grain quality traits based on which two improved Basmati rice genotypes pyramided with BB and blast resistance namely, Pusa 1847-12-62-115-20-6 and Pusa 1847-12-62-190-39-7 were found promising, along with improved grain and cooking quality as well as restoration potential, which could be used in breeding better quality hybrids.

Key words: Basmati rice, BB, blast, marker assisted restricted backcrossing breeding, grain and cooking quality

Introduction

Basmati rice grown in Himalayan foothills of Indian sub-continent is a valuable export commodity, which earned foreign exchange worth Rs. 22718/- crores during 2015-16. It is renowned worldwide for its exquisite quality traits featuring a harmonious blend extra-long, superfine grains, length-wise kernel elongation with minimum swelling on cooking, fluffy cooked rice with pleasant aroma, appealing taste and pleasing appearance (Singh and Singh 2009). The heritage of Basmati rice in the northwestern zones of Indian subcontinent has been maintained through its protection under Geographical Indication. Pusa Basmati 1509 (PB 1509) is an elite Basmati rice variety, which is early maturing (120 days), with exceptional grain and cooking quality (Singh et al. 2014). It is very popular among the farmers and is grown in around 1.29 lakh hectares during *kharif* 2017. However, it is highly susceptible to two major diseases of rice namely, blast and bacterial blight (BB), which can cause yield losses upto 65% and 50%, respectively.

For transfer of major genes governing biotic/ abiotic stresses into a popular variety, backcross breeding is most effective. Over the last decade, marker assisted backcross breeding (MABB) has created a paradigm shift in trait introgression in crop

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improvement, especially in Basmati rice improvement (Singh et al. 2011) wherein the number of backcrosses in MABB has been reduced to two to three as compared to at least six in conventional backcross breeding. MABB has been successfully utilized in rice for development of improved rice varieties such as Pusa Basmati 1718 (Singh et al. 2018), Pusa Basmati 1637 (Singh et al. 2017a), Pusa Basmati 1728 (Singh et al. 2017b), Pusa Basmati 1609, Pusa 1592 and Pusa 6 (Pusa 1612) and Improved Samba Mahsuri (Sundaram et al. 2008). While MABB can help in sustaining the production and productivity by recreating the phenotype with trait introgression, its utility for simultaneous improvement of multiple traits remains to be explored. Marker assisted restricted backcross breeding (MARBB) is an effective alternative for simultaneous improvement of multiple traits especially when the donor parent is an elite genotype with additional desirable traits. However, so far, this approach has been used in a limited way for varietal development (Joseph et al. 2004 and Gopalakrishnan et al. 2008). The use of MARBB is limited by choice of the donors used in the breeding programs. When the target gene is in the background of unadpated genotypes such as wild relatives/ prebreeding lines, the number of backcrosses needed is invariably higher. The donors used for MABB in Basmati breeding earlier were all of non-Basmati background making the process of MABB comparatively tedious (Singh et al. 2011; Singh et al. 2012; Ellur et al. 2016a; Ellur et al. 2016b; Khanna et al. 2015; Singh et al. 2017a). However, with the availability of improved donors for various genes governing BB resistance (*xa13, Xa21, Xa38*) (Ellur et al. 2016a, 2016b) and blast resistance (*Pi1, Pi2, Pi5, Pi9, Pi54, Pita,* and *Pib*) in the elite Basmati rice background (Khanna et al. 2015a), the scope for improving multiple traits without impairment of Basmati quality traits has now become possible, wherein the possibility of obtaining superior recombinants along with recovery of Basmati grain and cooking quality characteristics is much higher.

Bacterial blight (BB), caused by *Xanthomonas oryzae pv. oryzae (*Xoo*)*, is one of the most devastating diseases, which can cause severe yield losses (74 to 81%) in rice (Srinivasan and Gnanamanickam 2005). Till date, 42 genes governing resistance to BB have been identified and many of them have been mapped with molecular markers. Marker assisted breeding (MAB) has been adopted extensively in rice to introgress genes for resistance to BB both in India as well as abroad (Abenes et al. 1993; Yoshimura et al. 1995; Zhang et al. 1996; Sanchez et al. 2000; Singh et al. 2001; Gopalakrishnan et al. 2008; Basavaraj et al. 2010; Singh et al. 2012; Singh et al. 2013; Pradhan et al. 2015). *Xa21* and *xa13* on chromosome number 11 and 8, respectively confers broad spectrum resistance to the BB due to their synergistic effect in combating BB pathogen (Lee et al. 2009, Antony et al. 2010; Pradhan et al. 2016), and therefore, is one of the most widely utilized gene combination for marker assisted introgression of BB resistance in rice.

Rice blast disease, caused by *Magnaporthe oryzae*, can cause yield losses of upto 80% (Khush and Jena 2009). Although, it can be managed by chemical control, the detection of fungicide residue above the minimum residue limit (MRL) becomes a potential threat to Basmati rice export. Every year several hundred crores rupees worth of fungicides is used in prophylactic sprays for managing rice blast disease (Khanna et al. 2015a). Moreover, it is not sustainable, economically viable and bio-safe option. Deployment of resistance genes is one of the best means for managing the disease. A large number genes and QTLs have been reported to confer resistance to blast disease, of these, cloning and characterization of 22 genes have been undertaken (Sharma et al. 2012; Lv et al. 2013; Fukuoka et al. 2014; Ma et al. 2015). Among the major blast resistance genes, *Pi54* and *Pi2* have been found to confer resistance to the most of the blast isolates available in the Basmati growing region of India, either singly and/ together (Ellur et al. 2016a). *Pi2* (formerly known as *Piz5*) in chromosome 6 (Zhou 2006), confers broad spectrum resistance against various *M. oryzae* isolates from the hot-spots for blast disease in India (Singh et al. 2012; Singh et al. 2013; Khanna et al. 2015a; Ellur et al. 2016a). *Pi54* (earlier known as *Pi-k^h*) in chromosome11 (Sharma et al. 2005; Sharma et al. 2010) is another broad spectrum blast resistance gene, which is effective against predominant races of the pathogen in India (Sharma et al. 2002). There are several successful report of using marker assisted breeding for transfer of blast resistance genes into different rice cultivars (Hittalmani et al. 2000; Chen et al. 2008; Wen et al. 2011; Singh et al. 2012; Khanna et al. 2015a, b).

Incorporation of major BB and blast resistance genes is an effective strategy to widen the resistance spectrum of genotypes as well as to attain durable resistance. In view of the above, the current research study was carried out with the aim of developing short

duration premium Basmati rice genotypes with resistance for two major diseases namely BB and blast, which has been evaluated and found to be potential for direct release as varieties as well as parental lines in hybrid breeding.

Materials and methods

ICAR- Indian Agricultural Research Institute (ICAR-IARI) is a pioneer in Basmati rice breeding, wherein continuous efforts are being made for the development of short duration Basmati rice variety. The present research was carried out at the experimental farm of ICAR-IARI, New Delhi, located at 28°35'N latitude, 77°12'E longitude and at an altitude of 228.16 m above mean sea level.

Climate and soil

New Delhi is a subtropical region of India with dry hot summer, moderate rain and mild winters. Long summer (early April-August) precedes monsoon season (July-September) with a mean annual rainfall of 710 mm, more than 75 % concentrated during the southwest monsoon period. The crop season of rice is from June to October in the rainy season (provincially called *kharif* season). After harvesting the *kharif* season, the subsequent crop was grown at Rice Breeding and Genetics Research Centre (RBGRC) of ICAR-Indian Agricultural Research Institute (ICAR-IARI) Aduthurai, Tamil Nadu. RBGRC enjoys tropical climate of India, located between 11°00' N and 79° 28'E which receives rain during northeast monsoon, with weather conditions that favor rice cultivation throughout the year. The off season crop of rice is from December to middle of April in the winter season. Experimental farm of ICAR-IARI has Indo-Gangetic alluvium with loam to sandy loam texture, slight alkalinity (pH 7.4), organic C 3.9 g kg^{-1}, total N 0.031 percent, available P (10.2 kg ha^{-1}) and K (279.9 kg ha^{-1}) whereas the soil at RBGRC, Aduthurai, Tamil Nadu is deltaic alluvial clay with pH of 7.5.

Plant materials

PB 1509, an early maturing Basmati rice variety, and Pusa 1790 (P 1790) were used as parents in the present study. PB 1509 was developed from the cross Pusa 1301/Pusa 1121 and released during 2013. It is a short duration (120 days), semi-dwarf (95 to 100 cm), non-lodging, non-shattering, fertilizer responsive and high yielding, Basmati rice variety (Singh et al. 2014). It possesses aromatic extra-long slender grains (8.41mm) with occasional grain chalkiness, very good kernel length after cooking (19.1 mm), desirable ASV (7.0) and intermediate amylose content (21.24%). P 1790 is a promising genotype developed in the genetic background of Pusa Rice Restorer 78 (PRR78). P1790 is highly aromatic, has long slender grain and possess bacterial blight resistance genes *(xa13 and Xa21)* and blast resistance genes (*Pi2* and *Pi54)*. It is semi-dwarf (103-108 cm), produces on an average about 20 tillers/plant, matures in 130 days with an average yield of 62 q/ha. In terms of grain and cooking quality traits, it has long slender grains (7.66 mm) with very occasional grain chalkiness, good kernel length after cooking (14.5 mm), desirable ASV (7.0), intermediate amylose content (23.4%) and high head rice recovery (HRR) (60%). The desirable grain and cooking quality traits along with bacterial blight and blast resistance make P 1790 an ideal donor for MARBB (Singh et al. 2014).

Breeding strategy

Crosses were made between the parents, PB 1509 and P 1790 to produce F_1s. True hybrids were identified using gene linked/ gene-based markers namely, AP5930, RM206, xa13prom and pTA248 for the resistance genes *Pi2, Pi54, xa13* and *Xa21*, respectively (Table 1). The four gene positive plants were backcrossed to PB 1509 as recurrent parent to produce BC_1F_1 progenies. Foreground selection was done to identify heterozygous plants, which were selfed to produce BC_1F_2. It was further advanced to BC_1F_6 through phenotypic selection for agro-morphological, grain and cooking quality, through shuttle breeding strategy at ICAR -IARI, New Delhi and Rice Breeding and Genetics Research Centre (RBGRC), Aduthurai.

Table 1. Details of markers used in foreground selection

Gene	Markers	Trait	Distance	Chr	References
Pi54	RM206	blast	0.8cM	11	Sharma et al. 2005
Pi2	AP5930	blast	0.05cM	6	Fjellstorm et al. 2004
*xa*13	xa13-prom	BB	Gene based	8	Basavaraj et al. 2010
Xa21	pTA 248	BB	Gene based	11	Ronald et al. 1992

Multi-location evaluation of selected lines

The four gene pyramided lines in BC_1F_6 generation along with the parental lines PB 1509 and P 1790 were

evaluated in RCBD at two locations namely at ICAR-IARI, New Delhi and its Regional Station, Karnal, Haryana.

Screening for disease resistance

Screening for blast resistance

The pyramided lines were screened against the virulent isolate, 'Monwi38' collected from the Basmati growing region. Three replicates of lines were screened following Bonman et al. (1986) protocol. Two parents and pyramided lines were inoculated at three leaf stage. The inoculum comprising $\leq 5 \times 10^4$ conidia per ml and 0.02% Tween 20 was used for artificial inoculation. Test entries were kept overnight at high humidity and darkness to promote the fungal growth. Data for blast score was recorded after seven days (Ellur et al. 2016a).

Screening for resistance to bacterial blight disease

The pyramided lines and parents were tested for its resistance to bacterial blight disease with the virulent isolates of Xoo. Plants were inoculated with Xoo at a density of 10^9 cells/mL at maximum tillering stage. Three races namely, race 2, race 4 and race 6 of Xoo provided by Division of Plant Pathology, ICAR - IARI, New Delhi were used and the lesion length was recorded 21 days after inoculation adopting the SES scale (SES-IRRI, 2002).

Evaluation for agronomic performance, grain and cooking quality characteristics

The pyramided lines along with the parents were evaluated in augmented design and the data for agro-morphological traits as described in Khanna et al. (2015a). Data on days to 50 % flowering (DFF), plant height (PH), panicle length (PL), panicle number (PN), filled grains per panicle (FGP), spikelet fertility (%) (SF), thousand grain weight (TGW) and grain yield/ plant (GY) were collected on five random plants per entry. The grain quality traits namely, kernel length before cooking (KLBC), kernel breadth before cooking (KBBC) and length/breadth ratio (L/B) and cooking quality characteristics, namely, kernel length after cooking (KLAC), kernel breadth after cooking (KBAC) and elongation ratio (ER), were recorded on ten grains from each entry were measured using e-vision Annadarpan (CDAC, Kolkata). Alkali spreading value, aroma and amylose content were also estimated using standard procedures (Khanna et al. 2015a).

Molecular characterization of the pyramided lines

Leaf samples from the test genotypes were collected from 10 random plants for each genotype and DNA was isolated using CTAB protocol. Screening for the presence of *Pi2, Pi54, xa 13* and *Xa21* genes were performed in F_1, BC_1F_1 and BC_1F_2 generation and also at BC_1F_6 generation to confirm the presence of all the four genes using foreground markers, AP5930, RM206, xa13prom and pTA248. A total of 744 STMS primer pairs were used for parental polymorphism survey between the parents, PB 1509 and P 1790, out which 76 were found to be polymorphic. However, based on their physical location a total of 48 evenly spaced markers were selected for genome contribution analysis of recurrent parent among the pyramided lines. Selected improved lines were also screened for *Rf3* and *Rf4* genes for fertility restoration using two gene based/linked marker namely, DRRM-RF3-10 and RM 6100 (Table 2). Screening for quality traits using linked molecular marker was also done among the improved lines by using the QTLs identified in the mapping population constituting Pusa Basmati 1121 and Pusa 1342 (Amarawathi et al. 2008).

Result

Foreground selection for BB and blast resistance genes

PB 1509 was crossed with P 1790 to produce F_1s and

Table 2. Gene based/ gene linked markers used for selection of *Rf3 and Rf4* and other grain and cooking quality traits

S.No.	Gene	Markers	Linkage group	Gene based/ Gene linked	Reference
1	*Rf3*	DRRM-RF3-10	1	Gene based	Suresh et al. 2012
2	*Rf4*	RM 6100	10	Gene linked	Prakash, 2003
3	*badh2*	nksbad2	8	Gene based	Amarawathi et al. 2008
4	*elr*11-1	(RM1812-209)	11	Gene linked	Amarawathi et al. 2008
5	*amy6-1*	RM3–RM217	6	Gene linked	Amarawathi et al. 2008
6	*asv6-1*	RM3–RM217	6	Gene linked	Amarawathi et al. 2008

heterozygous F_1s plants were identified using the gene linked/ gene based markers. Total of 4 plants found heterozygous for four genes were backcrossed with PB 1509 to produce BC_1F_1s, these plants were screened for the presence of all the target genes. The plants heterozygous for all the four genes were identified and selfed to produce the BC_1F_2s. In BC_1F_2 generation, a total of 62 plants were subjected to foreground selection, out of which only 2 plants homozygous for all the four genes were identified (Fig. 1). All the 62 plants were harvested on single plant

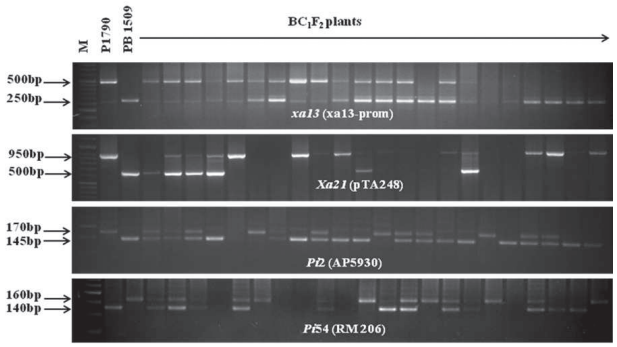

Fig. 1. Representative PCR amplification profile of the gene based/ gene linked markers for *xa13, Xa21, Pi2* and *Pi54* in BC_1F_2 generation used in foreground selection. M: 50 bp ladder

basis and subjected for grain and cooking quality analysis. Total of 29 progenies having good cooking quality were selected and advanced to produce BC_1F_3 families. Single plant selection was done in each of the BC_1F_3 families; five similar looking plants with good plants phenotype were tagged and harvested separately. Subsequent generations upto BC_1F_6 generation were advanced through panicle to progeny method.

Disease resistance, agronomic performance and cooking quality

All the 245 BC_1F_4 families were evaluated at ICAR-IARI farm in three rows for each family. 10 plants from each of the families were inoculated with Xoo in the field with the race 2, race 4 and race 6 found in the Basmati growing regions. Disease lesions were recorded 21 days after inoculation and only the families showing resistance reaction to BB inoculation were harvested and the yield per plot was recorded. Based on grain and cooking quality analysis, 48 progenies were advanced to BC_1F_5 generation and eight of these producing higher yield than PB 1509, were evaluated at two locations namely, New Delhi and Karnal in BC_1F_6. Disease inoculation and data on BB lesion length was recorded. All the progenies were resistant to three Xoo isolate. Average lesion length of the donor parent was 2 cm while recipient parent had a lesion length of 18 cm and the range of lesion length among the pyramided lines varied from 1.5 cm to 2.5 cm showing that they were highly resistant to BB. For blast disease, average score among the advance line varied from 0-2, while recipient parent has a score of 4 and donor parent had a score of 0 (Table 3). Data

Table 3. Disease scores of the selected pyramided lines for BB and blast

Pyramids	Bacterial blight lesion length (cm)			Blast score (Monwi38)
	Race 2	Race 4	Race 6	
P1847-12-62-8-3-1	2.10	1.96	1.26	0
P1847-12-62-10-4-2	2.07	2.03	2.02	2
P1847-12-62-37-8-3	2.88	2.61	1.99	0
P1847-12-62-66-12-4	1.40	1.84	1.50	0
P1847-12-62-85-16-5	3.20	3.55	1.90	2
P1847-12-62-115-20-6	2.43	2.43	2.44	2
P1847-12-62-190-39-7	1.12	1.67	1.33	0
P1847-12-62-194-40-8	2.35	2.33	1.59	0
PB 1509	20.83	18.06	15.58	4
P 1790	1.86	2.02	2.02	0

was also recorded for agro-morphological and yield traits on five plants from each of the families. Number of filled grains ranged from 77.5 to 101.60. P1847-12-62-115-20-6 was found to have the highest number of filled grains per panicle and is significantly higher than the PB 1509 which had 89 grains per panicle. Plant height ranged from 87 cm to 104.80 cm, panicle length varied from 22.60 to 29.30 cm, DFF from 85 to 92 days, yield varied from 54.66 to 68.88 q/ha, P1847-12-62-115-20-6 and P1847-12-62-190-39-7 had 68.88 and 62.44 (HRR) q/ha, respectively (Table 4). Head rice recovery for PB 1509 and P 1790 was 56.4 % and 67.37%, respectively. The variation for HRR was significant among the progenies and it ranged from 58.70 to 63.99%, as compared to low HRR in PB 1509 (56.40%) and higher HRR in the donor genotype (67.34%). The kernel elongation after cooking was significantly higher in case of PB 1509 and the four

Table 4. Agronomic performance of the four gene pyramided Basmati rice genotypes

Pyramids	Filled grain	Spikelet fertility (%)	Test weight	Plant height	Tillers/ plant	Panicle length (cm)	Yield (q/ha)	DFF
P1847-12-62-8-3-1	90.00	85.71	28.46	98.40	12.80	28.60	62.77	88
P1847-12-62-10-4-2	81.60	79.67	28.46	96.60	11.80	28.80	54.66	85
P1847-12-62-37-8-3	91.60	88.60	31.46	95.00	15.40	27.60	56.33	92
P1847-12-62-66-12-4	98.30	86.84	27.06	88.60	13.00	24.40	56.66	87
P1847-12-62-85-16-5	77.50	82.39	28.40	87.00	9.60	22.60	58.88	92
P1847-12-62-115-20-6	101.60	77.40	29.56	94.40	11.80	28.20	68.88	91
P1847-12-62-190-39-7	92.20	82.22	28.66	104.80	16.80	29.30	62.44	89
P1847-12-62-194-40-8	84.40	86.95	32.00	98.40	11.20	27.00	58.44	89
PB 1509	89.40	90.53	25.70	94.30	11.70	23.40	57.88	85
P 1790	97.40	94.50	26.34	97.80	19.80	25.32	60.23	91
CD (0.05)	5.74	2.09	1.96	7.28	1.90	1.62	2.99	3.54

gene pyramids as compared to P1790. KLAC varied from 15.33 to 17.0 mm, as compared to 14.25 mm observed in P1790. The progenies with reduced grain chalkiness compared to PB1509 were selected (Table 5).

genotypes including both the parents, whereas, *Rf3* allele was present only in P1790 while PB1509 did not possess the restorer allele of *Rf3*. Out of the 48 advanced progenies in BC_1F_4, 16 genotypes were found

Table 5. Grain and cooking quality analysis of selected pyramided lines

Pyramids	HRR (%)	KLBC (mm)	KWBC (mm)	L/B	KLAC (mm)	KWAC (mm)	KER	Non chalky (%)	Chalky (%)
P1847-12-62-8-3-1	58.70	7.93	1.73	4.58	16.33	2.75	1.88	97	3
P1847-12-62-10-4-2	63.99	7.80	1.60	4.87	15.33	2.75	1.85	94	6
P1847-12-62-37-8-3	62.03	8.27	1.67	4.95	17.00	2.67	1.89	80	20
P1847-12-62-66-12-4	59.15	7.93	1.67	4.74	16.33	2.58	1.86	98	2
P1847-12-62-85-16-5	63.83	7.93	1.60	4.95	15.92	2.58	1.90	90	10
P1847-12-62-115-20-6	59.00	8.07	1.47	5.48	16.33	2.75	1.79	97	3
P1847-12-62-190-39-7	60.68	8.27	1.73	4.78	16.25	2.50	1.92	90	10
P1847-12-62-194-40-8	63.36	8.53	1.60	5.33	16.33	2.50	1.98	95	5
PB 1509	56.40	8.13	1.40	5.80	17.00	2.25	2.03	65	35
P 1790	67.34	7.47	1.60	4.66	14.25	2.50	1.90	97	3
CD (0.05)	3.82	0.37	0.21	0.60	1.04	0.27	0.13	-	-

Molecular characterization of the four gene pyramided lines

Molecular marker analysis was done using the markers linked to the fertility restoration and quality characters. Since P 1790 is a restorer of fertility for WA cytoplasm, molecular characterization with respect to the restoration of fertility was done using *Rf3* and *Rf4* gene based marker. *Rf4* allele was present in all the

to be homozygous for both *Rf3*, as well as *Rf4*. The presence of aroma gene *BADH2* located in chromosome 8 was also confirmed in the progenies and all the progenies were found to possess the 8bp deletion corresponding to the aromatic allele for production of 2-acetyl-1-pyrroline (2-AP), which was expected as both parents were aromatic (Fig. 2). The accumulation of 2-AP in aromatic rice is explained by the loss of function mutations in the *BADH2* gene

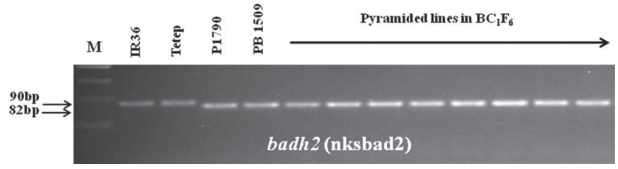

Fig. 2. Amplification profile of the marker, "nksbad2" based on 8bp deletion in *bahd2* in the pyramided lines in BC_1F_6 generation along with recurrent parent, donor parent and two non-aromatic checks, IR36 and Tetep

(Bradbury et al. 2005; Chen et al. 2008). For AC and ASV linked marker, RM 217 was screened across the selected progenies and all the progenies had the same allele but different allelic size as parents. Advanced progenies were screened for QTL *elr11-1*, associated with elongation ratio and located on chromosome 11 between the marker interval, RM1812 and RM209. Linear cooked kernel elongation is one of the essential quality traits of the Basmati rice varieties that is described as kernel elongation ratio (KER) based on the ratio of the grain length after and before cooking. All the advanced progenies carried PB 1509 alleles of RM1812 and RM209 (Table 6). The genomic contribution of the parents, PB 1509 and P 1790 was estimated using the SSR markers across the genome. Comparison was made for the contribution of PB 1509 genome in the selected progenies, it ranged from 59% in P1847-12-62-85-16-5 to 74% in P1847-12-62-37-8-3, indicating thereby that these lines still carried substantial proportion of donor genome, which had contributed to enhanced performance of the selected lines compared to recurrent parent for certain grain and cooking quality traits, yield and yield components. Adoption of MARBB approach facilitated retention to donor genome in substantial proportion leading to transgression of certain traits in desirable direction compared to PB 1509.

Discussion

Most of the Basmati varieties show susceptibility to major diseases such as BB and blast, threatening its cultivation in the Basmati growing regions of India. The current investigation was carried out to develop premium Basmati rice genotype with resistance to both BB and blast. The pyramiding strategy using backcross breeding is a most common method of improving an adapted and popular cultivar. The number of backcrosses to given, depends on the proportion of genomic contribution that one would like to retain from

Table 6. Molecular profile of the selected four gene pyramided lines used in multilocation testing

Pyramids	*Pi54* (RM206)	*Pi2* (AP5930)	*xa13* (*xa13* prom)	*Xa21* (pTA248)	*badh2* (8bp deletion)	*elr11-1* (RM1812-209)	*amy6-1* and *asv6-1*	*Rf3*	*Rf4*	PB 1509 genome contribution (%)
P1847-12-62-8-3-1	+	+	+	+	+	+	+	+	+	65.0
P1847-12-62-10-4-2	+	+	+	+	+	+	+	+	+	67.0
P1847-12-62-37-8-3	+	+	+	+	+	+	+	-	+	74.0
P1847-12-62-66-12-4	+	+	+	+	+	+	+	-	+	68.0
P1847-12-62-85-16-5	+	+	+	+	+	+	+	-	+	59.0
P1847-12-62-115-20-6	+	+	+	+	+	+	+	+	+	60.0
P1847-12-62-190-39-7	+	+	+	+	+	+	+	+	+	68.0
P1847-12-62-194-40-8	+	+	+	+	+	+	+	+	+	73.0
PB 1509	-	-	-	-	+	+	+	-	+	100.0
P 1790	+	+	+	+	+	+	+	+	+	0.0

the recurrent and donor parents, which in case of a typical backcross breeding programme is more than 90% of recurrent parent genome (RPG). The faster recovery of RPG is facilitated by the MABB. However, if the donor parent also has a desirable agronomic base and retention of a relatively higher proportion of donor genome is likely facilitate better product development than the recurrent parent, a modified MABB approach, referred as MARBB, as described earlier, is preferred. In this study, MARBB was used to combine BB and blast resistance in PB 1509 while achieving higher yield, reduced grain chalkiness along with BB and blast resistance from donor parent, P 1790. The two parents involved in the crosses were related through common ancestry, while, the recurrent parent PB1509 is premium quality Basmati rice variety, the donor parent is aromatic long slender grain rice variety with high yield, resistance to BB and blast and reduced grain chalkiness. As the donor genotype used in the present study is an elite donor genotype, restricted backcrossing followed by selfing and handling the segregating generation through pedigree selection helped in retaining the useful traits of both the parents. In this case, marker assisted foreground selection and background selection for the Basmati quality traits helped identification of useful recombinants in which desirable traits of both the donor and recipient parents were combined. Use of gene based markers for the target traits such as BB and blast facilitated foreground selection greatly. It was possible to identify four gene positive plants in the small population of 62 in BC_1F_2 generation, possibly because two target genes namely *Pi54*, and *Xa21* are located closer to each other on chromosome 11.

Pyramiding multiple genes through conventional breeding is difficult due to dominance and epistatic effects of the disease resistance genes which may lead to a similar phenotype (Suh et al. 2013). The use of modern molecular tool has enabled the development of new BB and blast resistant Basmati rice cultivars with high yield potential with considerable ease. To ensure the selection of BB and blast resistant progenies, disease screening and harvesting of only highly resistant progeny was done at early generations. The most virulent Xoo races prevalent in the Basmati growing were used for phenotypic screening, which will sustain the durability of the resistance spectrum offered by the genotypes. Recently, PB1121 NILs and PB6 NILs showing resistance against *Xoo* races of north-western of India have been developed and synergistic effect of two broad spectrum resistance genes namely, *Pi2* and *Pi54* against *Magnaporthe oryzae* isolates of prevalent in Basmati growing regions of India have been demonstrated (Ellur et al. 2016a). As the Basmati variety, PB 1509 is susceptible to both BB and blast, and shows occasional chalkiness in the grain, selection for resistance to these biotic stresses and non-chalky grain with high head rice recovery percentage was major objective of the present study. Marker assisted selection enables pyramiding of genes governing resistance to BB and blast diseases into rice variety without any linkage drag. As the parents used in the study, share a common pedigree, the possibility of linkage drag was comparatively less, and the recovery of the Basmati quality traits could be recovered with ease. Many of the MABB reported earlier were solely focused on the selection of only recurrent parent genotypes for BB (Singh et al. 2001; Joseph et al. 2004; Hari et al. 2011; Singh et al. 2012; Singh et al. 2013; Ellur et al. 2016b) and for blast (Liu et al. 2003; Yanoria et al. 2010; Zhan et al. 2012; Hari et al. 2013; Khanna et al. 2015a,b). In the present study, the objective of selection was to identify novel segregants generated through restricted backcrossing along with the Basmati quality traits. The yield of P1847-12-62-115-20-6 and P1847-12-62-190-39-7 were 68.88 and 62.44 q/ha, respectively which showed a yield advantage of 15.54% and 7.87% respectively, over PB 1509. The HRR also improved from 56% in PB 1509 to 63.83% in the pyramided line, P1847-12-62-85-16-5. Higher grain number of 101.60 in P1847-12-62-115-20-6 was recorded, showing the advantage of 12 grains per panicle over the recurrent parent. Head rice recovery after milling is one of the major factors determining the suitability of rice varieties especially Basmati rice varieties for rice processing and export industry. The minimum HRR for a Basmati rice variety to be promoted in the national Basmati trials is 45%, and development of genotypes with higher HRR is desirable for enhancing the volume of head rice thereby providing more return to millers and better income to the farmers in terms of better paddy price. As one of the parents P1790 possessed higher HRR, selection for better HRR was significantly rewarding among the progenies and it ranged from 58.70 to 63.99%, while PB1509 has low HRR of 56.40%. The advantage in HRR of 7.5% will be highly rewarding for the milling and processing industry. These selected combinations in pyramids through restricted backcross breeding provide enough evidence for the opportunity to select segregants with higher HRR, while in case of conventional backcross breeding, the maximum HRR would have been as good as recurrent parent PB 1509.

HRR is directly affected by grain filling and grain chalkiness. Chalkiness affects the grain cooking quality, as the chalky part may allow pooling of water in the free spaces leading to the higher water content after the middle stage of grain filling, thus hampering proper grain elongation (Ishimaru et al. 2009).

The four gene pyramided lines were also screened for the presence of fertility restoration genes of WA cytoplasm namely, *Rf3* and *Rf4* and the five selected entries with fertility restoration behaviour when crossed to male sterile lines can be further used as potential restorers in Basmati hybrid rice breeding programme, as till date only one Basmati quality rice restorer PRR 78 has been used for the development of first ever superfine aromatic rice hybrid PRH10. Since, grain length and elongation wise selected lines are superior to PRR78, commercial exploitation of pyramided lines with high fertility restoration as a potential rice restorer for Basmati hybrid breeding programme will be highly rewarding. The lines were also selected for the desirable combination of traits from both the parents, using QTLs linked to molecular markers for different quality traits mapped in the population derived from the cross between PB 1121 and P 1342 (Amarawathi et al. 2008). PB 1121 is one of the best premium Basmati rice varieties with longest cooked kernel length and one of the parents of the PB 1509, thus selection for PB 1121 type alleles for these QTL linked markers in the backcross derived lines helped in retaining the premium Basmati rice traits. The promising four genes pyramided lines will be tested for their suitability for commercial release as Basmati rice variety in the National Basmati trials and they can also be serve as a potential restorer in the Basmati hybrid rice breeding programme.

Authors' contribution

Conceptualization of research (AKS, SGK); Designing of the experiments (AKS, SGK); Contribution of experimental materials (AKS, SGK); Execution of field/ lab experiments and data collection (VS, PD, KKM, MN, SGK GP); Analysis of data and interpretation (VS, SGK); Preparation of manuscript (VS, SGK, AKS).

Declaration

The authors declare no conflict of interest.

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