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SHORT COMMUNICATION

Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection

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Abstract Bacterial blight (BB) is a major production threat to Basmati, the aromatic rice prized for its unique quality. In order to improve the BB resistance of two elite, traditional BB-susceptible Basmati varieties (Taraori Basmati and Basmati 386), we utilized the strategy of limited marker-assisted backcrossing for introgression of two major BB resistance genes, *Xa21* and *xa13,* coupled with phenotype-based selection for improvement of their plant type and yield. Improved Samba Mahsuri, an elite high-yielding, fine-grain-type BB-resistant rice variety served as donor for BB resistance. Backcross-derived improved Basmati lines at BC1F5 possessing a single resistance gene (i.e. either *Xa21* or *xa13*) displayed moderate resistance to BB, while lines possessing both *Xa21* and *xa13* showed significantly higher levels of resistance. Two-gene pyramid lines (*Xa21* + *xa13*) possessing good grain and cooking quality similar to their respective traditional Basmati parents, short plant stature (<110 cm plant height) and higher grain yield than the recurrent parent(s) were identified and advanced. This work demonstrates the successful application of marker-assisted selection in conjunction with phenotype-based selection for targeted introgression of multiple resistance genes into traditional Basmati varieties along with improvement of their plant stature and yield.

Keywords Gene pyramiding · Bacterial blight resistance · Basmati rice · Grain quality · Marker-assisted selection · Morphological selection

Basmati rice, a gourmet delicacy and pride of the Indian sub-continent, is well known world-wide for its long slender grains with delicate curvature, pleasant aroma, remarkable linear elongation, low breadthwise

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swelling combined with impressive flaky texture on cooking. Basmati rice ensures higher returns to farmers as it is priced three times higher than non-Basmati rice in the international as well as in the Indian domestic markets. More than half of the Basmati produced in India is exported, mainly to Saudi Arabia, UAE, UK, European Union countries, Kuwait, Bahrain etc. Among the Basmati varieties exported from India, the traditional Basmati cultivars have superiority for their unmatched grain, cooking and eating quality over the recently developed evolved Basmati cultivars (Shobha Rani et al. 2006). Bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) poses a major threat to sustainable Basmati rice production and has already caused severe epidemics in 1979 and 1980 (POS 1979, 1980). Severe disease infection can cause up to 50 % yield loss in addition to impairing the quality of the produce. All the Indian Basmati rices are susceptible to this disease and, due to the unavailability of resistance sources within the Basmati gene pool, genetic enhancement by incorporating BB resistance through recombination breeding involving only Basmati cultivars has not been possible. Combining the desirable agronomic and grain quality features of traditional Basmati with BB resistance is difficult, but is definitely an important research issue for sustaining and increasing the production and productivity of traditional Basmati cultivars. The present study was aimed at targeted introgression of two major, effective BB resistance genes *Xa21* and *xa13* from a high-yielding, medium slender (MS) grain-type semi-dwarf, BB-resistant rice variety, Improved Samba Mahsuri (ISM) (Sundaram et al. 2008), into the genetic background of two elite, low-yielding BB-susceptible traditional Basmati varieties, Taraori Basmati and Basmati 386, through the process of limited marker-assisted backcrossing (MABC) for a single generation (i.e. BC1), coupled with phenotype-based selection for short plant stature and high yield (Fig. 1).

Molecular markers linked to BB resistance, viz. *Xa21* (i.e. pTA 248; Ronald et al. 1992) and *xa13* (i.e. *xa13-prom*; Sundaram et al. 2011), were used for foreground selection and 65 simple sequence repeat (SSR) markers spanning the rice genome were used for background selection to assess the recurrent parent (RP) genome recovery in the breeding lines developed through MABC. ISM possessing the BB resistance genes *Xa21* and *xa13* was crossed with two traditional Basmati varieties (Taraori Basmati, Basmati 386) with the former used as the male parent. The F1 plants were confirmed for their heterozygosity with the 'R' gene-linked markers and were backcrossed using Basmati varieties as a female parent. The backcrosses were designated as RP4693 (Taraori Basmati//Taraori Basmati/ISM) and RP4694 (Basmati 386//Basmati 386/ISM). The resulting BC1F1 lines were subjected to foreground selection in order to select plants in heterozygous condition. Mini-scale DNA isolation for PCR analysis of the parents, F1s and backcross progenies for foreground selection was carried out among the 25-day-old seedlings following the procedure of Zheng et al. (1995), and PCR conditions described in Sundaram et al. (2008) were followed. The amplified products of the markers pTA248 and *xa13-prom* were electrophoretically resolved on a 1.2 % agarose gel containing 0.5 μg/ml of ethidium bromide in 0.5 × TBE buffer and visualized under UV light. All the plants that were heterozygous for *Xa21* were then checked for the presence of the marker linked to the *xa13* resistance allele in a heterozygous condition. 'Double-positive' BC1F1 plants possessing both the BB resistance genes in heterozygous condition were selfed to obtain BC1F2 seeds. The BC1F2 plants derived from both crosses were raised in the experimental farm of the Directorate of Rice Research, Hyderabad, India, followed by screening for BB resistance using a virulent strain of *Xoo* from Hyderabad, Andhra Pradesh, India, named DXO-020, through the leaf-clip inoculation method of Kauffman et al. (1973). Plants were scored for their BB resistance as described in Chen et al. (2000). The phenotypically resistant plants with Basmati grain features were subsequently genotyped for *Xa21* and *xa13* genes and lines homozygous for both the resistance genes were then selected for the desirable phenotypic traits such as semi-dwarf plant stature, apparent plant vigor and yield along with desirable Basmati-type grain quality features based on visual selection. The selected plants were advanced from BC1F2 to BC1F3 and then finally to BC1F4 by adopting the above-mentioned selection criteria (Fig. 1). The improved version of traditional Basmati lines so obtained were subjected to background selection using parental polymorphic SSR markers at BC1F4 and were evaluated in the field for BB resistance under field conditions using the DXO-020 isolate of *Xoo*. The resistance level of breeding lines developed in the present study was also

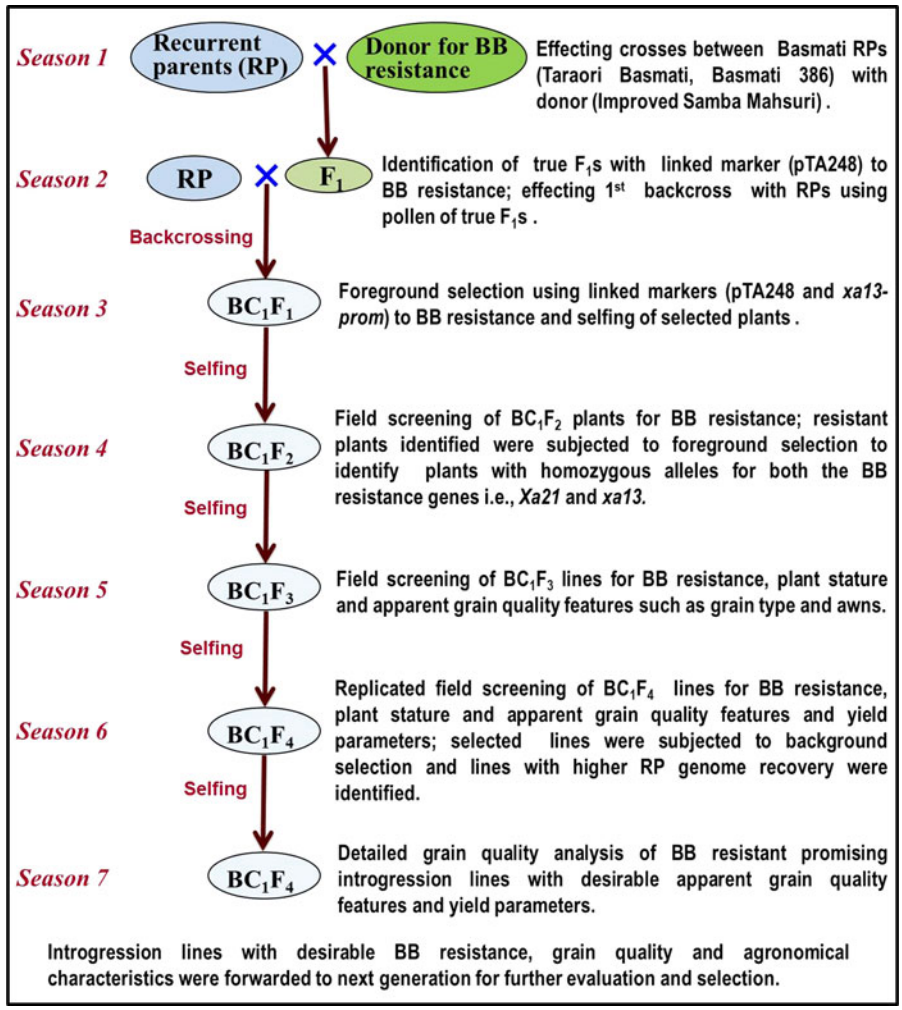

Fig. 1 Flowchart for MABC strategy to improve BB resistance showing genotypic and phenotypic selection criteria used at different generations

compared with the BB resistance gene-pyramid lines such as ISM (Sundaram et al. 2008), Improved Pusa Basmati 1 (Joseph et al. 2004) and SS1113 (Singh et al. 2001) (ESM 1). The BB resistance lines were assessed for their yield performance and were characterized for morphological and grain quality features for which a difference existed between donor and Basmati parents. The morphological features assessed included flowering duration (FD), plant height (PH), stem length (SL), panicle length (PL), flag leaf length (FLL), productive tillers, presence/absence of awns, pigmentation in lemma and grain type. The grain and cooking quality traits such as kernel length (KL), length/breadth (L/B) ratio, grain type (GT), alkali spreading value (ASV), kernel length after cooking (KLAC), elongation ratio (ER), amylose content (AC), gel consistency (GC) and aroma were critically analyzed at BC1F5.

In the cross RP4693, of the 400 BC1F2 plants, 295 plants were identified to be phenotypically resistant to BB. Among resistant plants, 64 were homozygous for *Xa21* (i.e. *Xa21Xa21*), 61 plants were homozygous for *xa13* (i.e. *xa13xa13),* while 30 were double homozygous for both the resistance genes (i.e. *Xa21Xa21 xa13xa13*) and the remaining plants were in heterozygous condition, either for *Xa21* or *xa13* (ESM 2). Similarly, in the cross RP4694, of the 361 BC1F2 plants screened, 224 BC1F2 plants were observed to be BB-resistant under field conditions. Forty-five of these exhibited amplification of *Xa21*-specific

fragments in homozygous condition, 52 were homozygous for the *xa13* gene, 25 plants were double homozygous and the remaining plants were in heterozygous condition for either *Xa21* or *xa13*. Both homozygous and heterozygous plants were forwarded to next generations; however, marker-assisted foreground selection was again performed in the BC1F3 generation only for lines selected from heterozygous BC1F2 plants. Phenotypic screening for disease reaction and Basmati grain quality features considerably reduced the number of plants selected for foreground selection based on markers, while allowing for a large number of backcross plants to be screened for their phenotypic features under field conditions, thus reducing resources required for marker-assisted selection. Selections in subsequent generations (i.e. BC1F3 to BC1F4) in the field were based on phenotype and apparent grain quality features.

A total of 96 and 16 pyramid lines in the BC1F4 generation from the crosses RP4693 and RP4694, respectively, were evaluated stringently in the field for BB resistance, agronomic and Basmati-type grain quality. In the backcross plants derived from the cross RP4693, FD ranged from 75 days (RP4693-47-4-2) to 105 days (RP4693-46-1-1) and variation in PH was observed from 106 cm (RP4693-96-5-2) to 164 cm (RP4693-101-1-1) (Table 1). Similarly, FD varied from 85 days (RP4694-126-1-2) to 108 days (RP4694-137-3-1) in the backcross plants derived from the cross RP4694, while PH was observed to be in the range from 113 cm (RP4694-171-2-1) to 168 cm (RP4694-157-2-2). Plants with semi-dwarf stature as well as tall plants similar to recurrent parents were recovered (Table 1). On screening for BB resistance, the lesion length in the cross RP4693 varied from 0.3 cm (RP4693-94-1-1) to 1.2 cm (RP4693-47-3-3, RP4693-44-4-2 and RP4693-46-1-2) when both the genes (*Xa21* and *xa13*) were present together in homozygous condition (ESM 3). The lesion length varied from 0.7 cm (RP4693-101-1-3) to 2.8 cm in plants possessing *Xa21* in homozygous condition (RP4693-73-1-5) and 1.5 cm (RP4693-96-1-3) to 5.2 cm (RP4693-53-3-1) in plants possessing the *xa13* gene in homozygous condition. Similarly, in the cross RP4694, plants possessing both the resistance genes in homozygous condition showed variation in the lesion length from 0.3 cm (RP4694-157-1-2, RP4694-171-2-1) to 1.9 cm (RP4694-137-1-4) while it was 0.8 cm (RP4694-161-1-1) to 2.6 cm (RP4694-137-3-2) in plants possessing *Xa21* in homozygous condition and 2.5 cm (RP4694-157-3-1) to 5.6 cm (RP4694-126-1-1) in plants possessing the *xa13* gene alone in homozygous condition (ESM 3). In all the generations (BC1F2, BC1F3 and BC1F4), a high degree of BB resistance was observed in both the genetic backgrounds, especially when both the genes were present together, as compared to plants possessing only a single resistance gene. Furthermore, the backcross-derived lines possessing *Xa21* were found to be more effective in terms of BB resistance than the backcross-derived lines possessing only *xa13*. Similar observations have been recorded in earlier studies (Sanchez et al. 2000; Singh et al. 2001; Joseph et al. 2004; Sundaram et al. 2008; Basavaraj et al. 2009, 2010; Rajpurohit et al. 2011).

The resistance level of improved lines of traditional Basmati rice possessing BB resistance, developed in the present study, was compared with the pyramided lines in the genetic background of Samba Mahsuri (i.e., ISM), SS1113, Pusa Basmati 1 (i.e., Improved Pusa Basmati 1) and IR 24 (i.e., IRBB 59). A total of 37 improved lines developed in the present study possessing the two-gene combination (i.e. *Xa21* and *xa13*) in homozygous condition showed equivalent resistance with ISM and SS1113 possessing *Xa21, xa13* and *xa5*. The BB resistance observed in the two-gene pyramid lines of traditional Basmati developed through the present study were comparable to the resistance level in other genetic backgrounds with two or even more genes such as IRBB59, ISM, SS1113 (*xa5* + *xa13* + *Xa21*) and Improved Pusa Basmati 1 (*Xa21* + *xa13*) (ESM 1).

The backcross lines possessing *Xa21* + *xa13* displayed equivalent resistance when compared with the gene-pyramided lines of Samba Mahsuri, PR106, Pusa Basmati 1 and IR 24 possessing *Xa21* + *xa13*, and the high level of resistance in different genetic backgrounds demonstrates that these two genes (i.e. *Xa21* and *xa13*) can provide a durable and high level of resistance in India (Sanchez et al. 2000; Singh et al. 2001; Zhang et al. 2006; Deng et al. 2006; Sundaram et al. 2008, Basavaraj et al. 2009, Rajpurohit et al. 2011).

In addition to phenotypic selection for Basmati morphological features and marker-assisted foreground and background selection, the grain and cooking quality of selected plants was also analyzed. Only those plants meeting the stringent Basmati

Table 1 Morphological, grain quality, disease reaction and recurrent parent genome recovery of improved lines of Taraori Basmati and Basmati 386 during BC1F4 generation

SN	Designation	PH (cm)	PL (cm)	FD (days)	Awns	KL	L/B ratio	GT	ASV	KLAC	ER	AC (%)	GC (mm)	Aroma	LL (cm)	% RP genome
1	RP4693-39-2-4	117	23	84	Short	7.11	3.93	LS	5	14.5	2.04	21.3	43	SS	0.6	73.8
2	RP4693-44-5-1	116	18	96	Long	7.26	4.27	LS	4	13.6	1.87	20.82	25	SS	0.5	79.2
3	RP4693-96-5-2	106	24	97	Short	7.24	4.26	LS	5	14.9	2.07	21.6	43	SS	0.8	76.1
4	RP4693-101-1-4	115	20	90	Long	7.28	4.09	LS	5	14.1	1.94	24.3	38	SS	0.6	80.3
5	RP4693-86-7-3	115	20	94	Long	7.30	4.19	LS	4	14.6	2.0	22.8	45	SS	0.5	73.8
6	RP4693-44-1-4	151	25	98	Long	7.17	4.17	LS	5	14.9	2.08	22.77	24	SS	0.4	74.6
7	RP4693-47-3-1	156	27	78	Long	7.38	4.29	LS	5	13.0	1.76	20.39	22	SS	0.6	78.7
8	RP4693-77-2-4	155	27	89	Short	7.16	4.21	LS	4	12.6	1.76	20.82	25	SS	0.8	80.3
9	RP4693-96-2-1	153	27	96	Long	7.28	4.16	LS	3	15.4	2.11	20.63	43	SS	0.4	75.4
10	RP4693-101-3-1	151	23	99	Long	7.25	4.27	LS	4	15.1	2.08	20.79	52	SS	0.4	74.6
Taraori Basmati		158	27	95	Long	7.34	4.24	LS	5	15.5	2.11	23.9	34	SS	16.7	100
11	RP4694-164-1-5	115	21	92	Long	7.09	3.91	LS	5	14.0	1.97	21.3	43	SS	0.8	79.3
12	RP4694-173-3-2	114	23	98	Short	7.11	3.93	LS	4	13.9	2.04	23.3	40	SS	0.7	81.0
13	RP4694-171-2-1	109	19	95	Short	7.09	4.01	LS	4	13.9	1.96	23.1	56	SS	0.3	75.9
14	RP4694-157-3-2	115	19	97	Long	7.11	3.93	LS	5	14.5	2.04	21.3	43	SS	0.4	82.7
15	RP4694-157-1-3	112	23	94	Short	7.13	3.92	LS	4	14.6	2.05	24.18	41	SS	3.8	81.1
16	RP4694-137-3-1	159	26	103	Long	7.03	4.13	LS	4	14.3	2.03	20.77	35	SS	1.6	81.0
17	RP4694-137-2-2	159	28	103	Long	7.09	4.07	LS	4	13.1	1.85	19.72	48	SS	0.6	79.3
18	RP4694-137-1-2	156	28	104	Long	7.03	3.95	LS	5	13.6	1.93	20.86	32	SS	0.9	79.3
19	RP4694-157-1-1	152	24	97	Short	7.13	4.10	LS	3	14.1	1.98	20.86	65	SS	0.3	75.9
20	RP4694-137-2-3	150	24	97	Long	7.04	3.98	LS	5	14.0	1.99	20.50	43	SS	0.9	81.0
Basmati 386		151	24	95	Long	7.01	3.91	LS	5	14.1	2.07	21.4	44	SS	21.6	100
Imp S. Mahsuri		104	23	110	Absent	5.01	1.82	MS	5	8.7	1.79	23.9	24	NS	1.4	0.0

SN serial number, *PH* plant height, *PL* panicle length, *FD* flowering duration, *KL* kernel length, *L/B ratio* kernel length/breadth ratio, *GT* grain type, *ASV* alkali spreading value, *KLAC* kernel length after cooking, *ER* elongation ratio, *AC* amylose content, *GC* gel consistency, *LL* lesion length, *% RP genome* percentage recurrent parent genome recovery, *LS* long slender, *MS* medium slender, *SS* strong scent, *NS* no scent

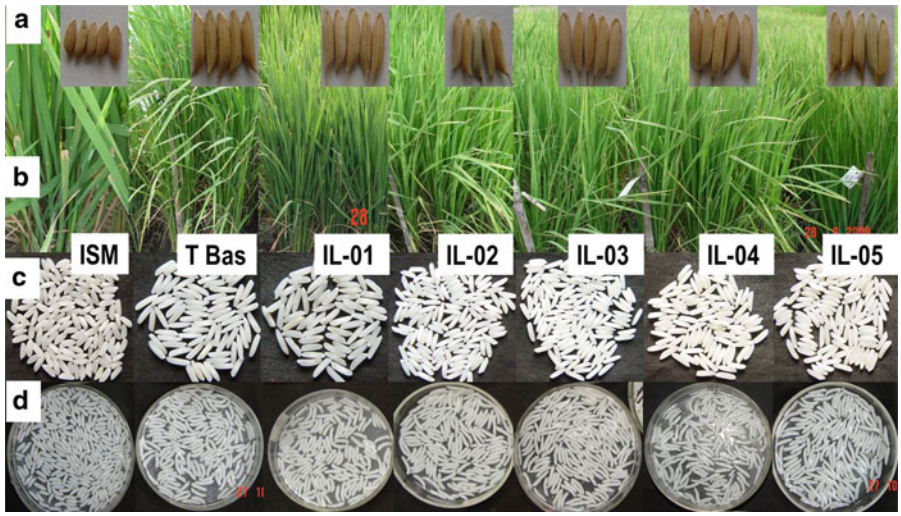

Fig. 2 Features of the backcross-derived introgression lines of Taraori Basmati **a** grain shape, **b** bacterial blight resistance, **c** grain type and **d** kernel length after cooking. *T Bas* Taraori Basmati (recurrent parent), *ISM* Improved Samba Mahsuri (donor parent)

quality standards were advanced further. This approach greatly hastened the recovery of the RP genotype and phenotype. The majority of the introgression lines selected, possessing both *Xa21* and *xa13*, displayed excellent grain cooking and eating qualities. Kernel length (KL) ranged from 7.13 (RP 4693-39-2-4) to 7.38 mm (RP 4693-47-3-1) in the genetic background of Taraori Basmati (7.34 mm) and 7.03 mm (RP 4694-137-1-2, RP 4694-137-3-1) to 7.13 mm (RP 44694-157-1-1) in Basmati 386 (7.01 mm) background (Table 1). All the selected 30 pyramid lines showed long slender (LS) grains, high KL after cooking (KLAC), high elongation ratio (ER), intermediate amylose content (AC), intermediate alkali spreading value (ASV) and low to medium gel consistency (GC). The vast majority of the lines possessed strong aroma similar to their recurrent parents. Taraori Basmati was observed to be a good combiner for grain cooking and eating quality features, as a large number of improved lines were selected from this cross. Basmati 386 was a moderate combiner in terms of grain quality traits, while introgression lines in Taraori Basmati background have shown excellent grain quality features (Table 1; Fig. 2). The utilization of an elite variety possessing excellent grain quality features, ISM as the donor parent for BB resistance facilitated the recovery of backcross-derived plants possessing the desirable grain quality features, despite the fact that the donor parent is not a Basmati.

The major outcome of this study has been the development of improved versions of Taraori Basmati and Basmati 386 possessing high level of BB resistance along with semi-dwarf plant type endowed with desirable Basmati grain and cooking quality features. Basavaraj et al. (2010) and Rajpurohit et al. (2011) recorded observations similar to our study, wherein the number of plants to be analyzed through background selection was reduced considerably from the original population. However, in earlier studies by Chen et al. (2000) and Deng et al. (2006) the identification of resistant plants was based only on marker data, and hence large numbers of plants were to be genotyped. As grain and cooking quality traits are extremely important for Basmati, emphasis was given to analyzing these traits along with other phenotypic traits in addition to BB resistance before generation advancement.

The selected backcross-derived lines with equivalent cooking and eating qualities (Fig. 2) along with other morphological features similar to their recurrent parents were subjected to background selection using 61 and 58 polymorphic SSR markers spanning all the 12 chromosomes for the breeding lines in the genetic background of Taraori Basmati and Basmati 386, respectively (ESM 4). Genome recovery ranged from 73.8 % (RP4693-47-4-4, RP4693-47-3-4) to 83.6 % (RP4693-101-3-3) and 75.9 % (RP4694-157-1-2, RP4694-157-3-2) to 82.7 % (RP4694-157-2-1) in the genetic background of Taraori Basmati and Basmati 386, respectively (Table 1). Higher recurrent parent (RP) genome recovery was achieved in both the crosses, contrary to the theoretical expected value of ~75 % at the BC1F4 generation in the present study.

Similar results have been recorded earlier by Basavaraj et al. (2010), Joseph et al. (2004) and Rajpurohit et al. (2011). Moreover, background selection for large numbers of plants with markers at each backcross generation would be an expensive proposition. Therefore, we suggest, based on the results obtained in the present study, that when resources are limited, phenotypic selection combined with marker-assisted foreground selection and selection of RP genome based on phenotype and assessment of background genome recovery in later generations through marker analysis can accelerate backcross breeding programs and make them cost-effective.

Introgression lines possessing BB resistance, good plant type and grain quality features typical of Basmati along with high RP genome recovery were forwarded to the BC1F5 generation for further selection and evaluation from both the crosses (Fig. 2). The majority of the selected improved lines possessed excellent physicochemical, cooking and eating quality traits on par with their respective recurrent parents. Some of the selected, elite breeding lines of Taraori Basmati and Basmati 386 will be nominated for the All India Coordinated Rice Improvement Project (AICRIP) trials. Furthermore, the breeding lines can also serve as an excellent source of BB resistance for development of BB-resistant Basmati varieties. Importantly, we now have BB-resistant Basmati breeding lines in the genetic background of Pusa Basmati-1 (Joseph et al. 2004), Pusa RH10 (an aromatic hybrid, Basavaraj et al. 2010) and a traditional Basmati, Type-3 (Rajpurohit et al. 2011). Advanced breeding lines/varieties derived from these BB-resistant sources will be of significant practical value in providing durable BB resistance in Basmati growing regions, widening the repertoire of Basmati quality donor lines for BB resistance breeding. The lines developed through our study will certainly contribute towards yield stability and sustainability in Basmati rice production and towards enhancing export earnings. The above results also indicate the effectiveness of the molecular marker selection strategy in hastening the process of gene pyramiding/introgression.

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