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Development of bacterial blight resistance versions of basmati rice genotypes from Jammu, Northern Himalaya using marker-assisted selection

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Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major constraint that negatively impacts rice production. Here, we explored developing potential BB resistance donors having basmati quality characteristics. The 20 BC_1F_1 cross combinations generated by randomly crossing four elite basmati cultivars with 10 bacterial blight resistant donor lines possessing resistant genes in different combinations were used for screening. Out of 20 BC_1F_1, one combination Basmati-370 x IRBB-55 was found to have basmati quality traits like intermediate amylose and high aroma content. Twenty two BC_1F_2 genotypes were selected from this cross combination for screening *Xa21* and *xa13* BB resistance genes as well as aroma gene through marker-assisted selection (MAS). Five genotypes namely, Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-13, Basmati-370 x IRBB-55-17 and Basmati-370 x IRBB-55-18 carrying both *Xa21* and *xa13* along with *fgr* gene in homozygous conditions identified phenotypically to show complete resistance to BB along with intermediate amylose and high aroma. It is suggested that these genotypes can be effectively used as basmati donors.

Keywords: BB resistance donors, Grain quality traits, MAS, *Oryza sativa*, *Xanthomonas oryzae* pv. *oryzae*

To sustain the self sufficiency as well as to meet the demand of increasing population in India the current production level of rice needs to be increased to 120 million tons by the year 2020. To achieve the targeted levels of production, breeding rice varieties for major biotic and abiotic stresses constitutes one of the important research strategies. Basmati rice a nature's gift to north-west region of Himalaya is highly susceptible to bacterial blight (BB) disease causing enormous losses. The BB disease caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major biotic constraint in the irrigated rice belts. Bacterial blight of rice caused by *Xoo* is a major factor that negatively impacts rice production, especially in irrigated and rainfed lowland ecosystems[1]. The *indica* varieties have been found to be generally more susceptible to BB than *japonica* varieties. There is no known source of BB resistance in the available basmati rice germplasm[2]. Host resistance has been shown to be the only reliable, economical and environment friendly method to control this disease; coming from non-basmati sources.

Moreover, resistance breeding is considered as the most economical and environmentally safe approach for achieving yield stability[3]. Genetic resistance is the most effective and economical control for BB and introgression of more than one resistance gene can lead to durable resistance against the disease[4]. The task of introgression of more than one gene without losing the aroma in basmati is not easy and particularly, if the genes are recessive in nature, the task of gene pyramiding becomes even more cumbersome requiring a round of progeny testing at each generation. In such cases, polymerase chain reaction (PCR) based markers linked to target genes can be gainfully used to track the introgressed genes in each segregating population. With the recent advances in tagging and mapping of major genes, the stage is set to develop new, improved varieties with pyramided genes, for durable resistance.

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Abbreviations: BB, Bacterial blight; PCR, Polymerase Chain Reaction; IRBB, International Rice Bacterial Blight; CTAB, Cetyl Trimethyl Ammonium Bromide; MAS, Marker assisted selection; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*; PCV, Phenotypic coefficient of variation; GCV, Genotypic coefficient of variation; ECV, Environmental coefficient of variation; R, Resistant; MR, Moderately resistant; S, Susceptible.

More than 32 resistance genes for BB resistance have been identified and designated in a series from *Xa1* to *xa32*[5,6]. The BB resistance genes *xa5, xa13* and *Xa21* have been identified to be the best combination since gene pyramids containing these three genes have been found to possess high levels of resistance against most of the Indian pathotypes of *Xoo* type[7,8]. Introgression of effective BB resistance genes (*Xa/xa* genes) singly or in combination (pyramiding) without losing aroma is the most economical approach to manage the disease in basmati rice varieties. Gene pyramiding is difficult to achieve using conventional breeding alone because of linkage with some undesirable traits that is difficult to break even after repeated backcrossing[9]. When two or more genes are introgressed, phenotypic evaluation is unable to distinguish the effect of individual gene precisely since each gene confers resistance and combats multiple races of the pathogen. Moreover, in the presence of a dominant and a recessive allele, the effect of the recessive gene is masked. The advent and easy availability of molecular markers closely associated with each of the resistance genes makes identification of plants with multiple genes possible at any stage and population[10]. This approach have been successfully used to introgress BB resistance genes into elite rice cultivars in earlier studies[10,11].

The basmati rice are known to have limited potential BB resistance donors[2]. Therefore, the present study was undertaken for screening of early generation's population by combining both phenotypic as well as molecular markers. We have attempted to introgress *xa13* and *Xa21* genes into elite basmati cultivars to develop pyramided lines that can be utilized as potential basmati donors with intermediate amylose and high aroma in future breeding programmes.

Materials and Methods

Plant material

The plant material comprised of four elite basmati varieties namely, Basmati-370, Saanwal basmati, Basmati 564 and Ranbir basmati and 10 bacterial blight resistance donor *viz*., IRBB-50, IRBB-51, IRBB-52, IRBB-53, IRBB-54, IRBB-55, IRBB-56, IRBB-57, IRBB-59 and IRBB-60. These donor lines possessed four BB resistant genes *viz*., *xa4, xa5, xa13* and *Xa21* in different combinations. Thirty F_1s plants obtained by randomly crossing of four elite basmati varieties with 10 donor lines were backcrossed to obtain BC_1F_1 genotypes. Twenty cross combinations of BC_1F_1's along with parents were raised in three replications at the Research Farm of Division of Plant Breeding and Genetics of Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, India during kharif 2010 for screening the morphological, basmati quality and BB resistance. Out of these the best cross combination asmati-370 x IRBB-55 was selected after doing stringent plant selection on the basis of basmati quality characteristics along with BB resistance and was self-fertilized to obtain BC_1F_2 population. Only 22 genotypes were selected from BC_1F_2 population in a stringent phenotypic selection and were screened with marker linked to BB resistance genes (*xa13* and *Xa21*) and aroma (*fgr*) gene for basmati quality traits using MAS and phenotypic selection.

DNA extraction

Individual plant was selected from BC_1F_2 generation along with parents. The total genomic DNA of the two parents and the individual progeny plants was isolated from 3-wk old seedling using the standard cetyl trimethyl ammonium bromide (CTAB) method[12]. Purity and concentration of DNA was monitored at a wavelength of 260 and 280 nm using spectro-photometer. All DNA samples were diluted to a working concentration of 50 ng/µL with distilled water. After adjusting the final DNA concentrations to 50 ng/µL, the DNA samples were stored at 4°C.

Markers analysis

Molecular markers, RG136 and pTA248 closely linked to the BB resistance genes, *xa13* and *Xa21*, respectively were used to confirm the presence of the resistance genes (Table 1). The RG136 marker for *xa13*[13] and pTA248 marker for *Xa*21[14] were used for screening of BB resistance genes in BC_1F_2 generation. For aroma marker, RM515 was used to identify the aromatic rice lines. Amplification with primer was done for identification of *fgr* gene on rice chromosome 8[15].

PCR assay

The PCR reaction was performed in a 20 µL reaction mixture containing 50 ng of template DNA, 5 pmoles of each forward and reverse primer, 10 mM dNTPs, 10X PCR buffer, 25mM $MgCl_2$, and 5 U of Taq DNA polymerase in a total volume of 20 µL. The PCR thermal cycler was programmed for 2 min at 94°C, 1 min at 52°C for primers annealing for *Xa21* and 55°C for *xa13* and *fgr*, 1 min at 72°C and a final cycle of 10 min at 72°C. Amplification product was separated on 3.5% of agarose gel in 1X TBE buffer followed by staining with ethidium bromide. A 100 bp

Table 1—Selected candidate genes, primers, annealing temperatures and amplicon lengths

Gene	Primer	Sequence	Annealing temp.(°C)[a]	Amplicon length (bp)
Xa21	pTA 248F	ATAGCAACTTTGATTGCTTGG	52°C	1370
	pTA 248R	CGATCGGTATGGCCCAGCAAAAC		
xa13	RG 136F	CATTGGATGGGTTGACACAG	55°C	700
	RG 136R	TAGCTTCGCGTCTTGGAGAT		
fgr	RM 515F	TAGGGGCGATTTCAAAGGGTGAG	55°C	210
	RM 515F	TGGCCTGCTTTTTCTCTCTCTC		

[a]As determined by gradient PCR

DNA ladder (Life Technologies-GIBCO BRL) was used to estimate the size of each band.

Grain quality characteristics

Grain quality characteristics of pyramided lines Seeds harvested from individual plants were analysed for physicochemical characters such as milled kernel dimensions, grain shape and aroma. To determine the kernel length/breadth ratio, five fully developed whole milled rice kernels were measured for their length and breadth. The grains were classified into different types based on their dimensions according to IRRI[16]. For testing aroma, 1 g milled rice kernels were soaked in 10 ml of 1.7% KOH at room temperature in covered Petri plates for 10 min[17]. Coded samples were subjectively evaluated by a panel of five experts who have rich experience in basmati rice breeding and quality evaluation. IRBLB59, the nonaromatic parent and basmati 370, a highly aromatic traditional basmati variety were used as standards. The samples were scored on 0-3 scale with 0, 1, 2 and 3 corresponding to absence of aroma, mildly aromatic, strongly aromatic and strongly aromatic, respectively. The score for a sample was recorded based on consensus among the majority of experts. The amylose content in rice grain was analyzed as per standard method[18].

Statistical analysis

The promising genotypes were identified by comparing the BB resistance pyramided genotypes with phenotypic data for various morphological, phonological, yield and its components and grain quality characters. The data was analyzed using MSTAT-C (http://www.msu.edu/'breed/mstat.htm). The promising genotypes which possess both BB resistance genes along with basmati quality traits were identified.

Results and Discussion

MAS for screening of pyramided lines for BB resistance genes

Out of 20 BC_1F_1 cross combinations, the cross Basmati-370 x IRBB-55 was found to have basmati

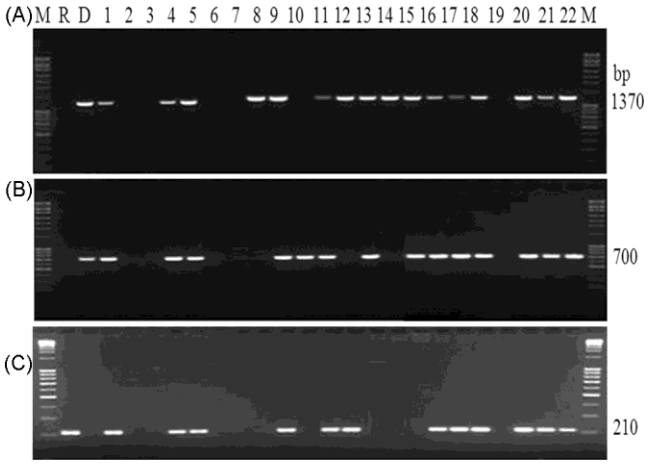

Fig. 1—PCR amplification of BC_1F_2 plants of Basmati 370 x IRBB55 cross with RM515 primer for *fgr* gene (A) *Xa21*F, *Xa21* R primers; (B) *Xa13*F, *Xa13* R primers; and (C) RM515 primer for *fgr* gene.

quality characters along with BB resistance. Twenty two genotypes of this cross combination were selected for screening of BB resistance using MAS. A perusal of Table 2 depicted the presence of BB resistance gene (s) in different genotypes of a cross Basmati-370 x IRBB-55. Out of 22 genotypes, sixteen genotypes were found to have *Xa21* gene with band size of 1370 bp (Fig. 1A), and 14 genotypes were found to possess *xa13* gene with band size of 700 bp size (Fig. 1B). Twelve genotypes were found to have aroma gene (*fgr*) with band size of 210 bp (Fig. 1C). The genotypes *viz*., Basmati-370 x IRBB-55-1, Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-9, Basmati-370 x IRBB-55-11, Basmati-370 x IRBB-55-16, Basmati-370 x IRBB-55-17, Basmati-370 x IRBB-55-18, Basmati-370 x IRBB-55-20, Basmati-370 x IRBB-55-21 and Basmati-370 x IRBB-55-22 were found to have all the three genes i.e., *Xa21*, *xa13* and *fgr* in homozygous conditions. The genotypes *viz*., Basmati-370 x IRBB-55-8, Basmati-370 x IRBB-55-14 possessed only one gene i.e., *Xa21Xa21*, whereas the genotypes Basmati-370 x IRBB-55-13 possessed *xa13xa13* gene in homozygous conditions. The genotypes *viz*., Basmati-

Table 2—Mean lesion length, distribution of *Xa/xa* and *fgr* genes and classification of potential BC_1F_2 genotypes

BC_1F_2 genotype	Mean lesion length (cm)	*Xa/xa* and *fgr* genes present	Degree of resistance
Basmati 370	2.60	*Fgrfgr*	S
IRBB 55	0.00	*Xa21Xa21 + xa13xa13*	R
Basmati-370 x IRBB-55-1	0.70	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR
Basmati-370 x IRBB-55-4	0.00	*Xa21Xa21 + xa13xa13 + fgrgfr*	R
Basmati-370 x IRBB-55-5	0.20	*Xa21Xa21 + xa13xa13 + fgrfgr*	R
Basmati-370 x IRBB-55-8	1.10	*Xa21Xa21*	MR
Basmati-370 x IRBB-55-9	1.20	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR
Basmati-370 x IRBB-55-10	1.10	*xa13xa13*	MR
Basmati-370 x IRBB-55-11	0.60	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR
Basmati-370 x IRBB-55-12	1.50	*Xa21Xa21 + fgrfgr*	MR
Basmati-370 x IRBB-55-13	0.40	*Xa21Xa21 + xa13xa13*	R
Basmati-370 x IRBB-55-14	1.90	*Xa21Xa21*	MR
Basmati-370 x IRBB-55-15	0.70	*Xa21Xa21 + xa13xa13*	MR
Basmati-370 x IRBB-55-16	0.90	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR
Basmati-370 x IRBB-55-17	0.10	*Xa21Xa21 + xa13xa13 + fgrfgr*	R
Basmati-370 x IRBB-55-18	0.00	*Xa21Xa21 + xa13xa13 + fgrfgr*	R
Basmati-370 x IRBB-55-20	0.50	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR
Basmati-370 x IRBB-55-21	0.60	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR
Basmati-370 x IRBB-55-22	1.00	*Xa21Xa21 + xa13xa13 + fgrfgr*	MR

370 x IRBB-55-13 and Basmati-370 x IRBB-55-15 possessed two resistance genes i.e. *Xa21Xa21*and *xa13xa13* in homozygous conditions. The genotype Basmati-370 x IRBB-55-12 possessed the resistance gene *xa21xa21* and aroma gene (*fgrfgr*) only. The deployment of rice cultivars that have multiple BB resistance genes is expected to lead more durable resistance.

Studies conducted to identify the best gene combinations conferring broad spectrum resistance showed that the four genes (*Xa4+xa5 + xa13 + Xa21*) combination was the most effective and did not show any sign of breakdown of resistance to various strains of the pathogen[7,8]. Pyramiding multiple resistance genes in a single rice variety is suggested as a strategy to prevent or delay the breakdown of resistance. The probability of simultaneous pathogen mutations for virulence to overcome the resistance conferred by two or more effective genes is much lower than for a single gene[19]. Availability of tightly linked molecular marker makes it possible to identify plants with multiple resistance genes. Many BB resistance genes have been mapped relative to molecular markers[20], and have been used for gene pyramiding in rice[21-23]. The main objective of this study was to combine basmati quality traits with BB resistance and development of potential basmati donors. High level of susceptibility of basmati rice to BB, caused by the bacterium *Xoo* is a serious constraint to basmati rice production, which results in major yield loss.

Screening for BB disease reactions

Out of 22 BC_1F_2, seventeen genotypes which possessed the resistance gene(s) were evaluated for disease response by leaf clipping method in the field. Mean lesion length of genotypes with different combination of resistance genes along with fragrance gene in BC_1F_2 population is presented in Table 2.The average lesion length was observed to be 0.58 cm. The five genotypes *viz*., Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-13, Basmati-370 x IRBB-55-17 and Basmati-370 x IRBB-55-18 showed lesion length of less than 0.5 cm. The genotypes Basmati-370 x IRBB-55-1, Basmati-370 x IRBB-55-8, Basmati-370 x IRBB-55-9, Basmati-370 x IRBB-55-10, Basmati-370 x IRBB-55-11, Basmati-370 x IRBB-55-12, Basmati-370 x IRBB-55-14, Basmati-370 x IRBB-55-15, Basmati-370 x IRBB-55-16, Basmati-370 x IRBB-55-20, Basmati-370 x IRBB-55-21, Basmati-370 x IRBB-55-22 showed lesion length more than 0.5 cm. The maximum lesion length (1.50 cm) was observed in Basmati-370 x IRBB-55-12 and minimum lesion length (0.10 cm) in Basmati-370 x IRBB-55-17 genotype.

As depicted in Table 2 that out of the 11 genotypes which possessed all the three genes, five genotypes *viz*., Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-13, Basmati-370 x IRBB-55-17 and Basmati-370 x IRBB-55-18 were found to show complete resistant (i.e. having lesion

length less than 0.5 cm) against bacterial blight (Table 2). Genotypes Basmati-370 x IRBB-55-1, Basmati-370 x IRBB-55-8, Basmati-370 x IRBB-55-9, Basmati-370 x IRBB-55-10, Basmati-370 x IRBB-55-11, Basmati-370 x IRBB-55-12, Basmati-370 x IRBB-55-14, Basmati-370 x IRBB-55-15, Basmati-370 x IRBB-55-16, Basmati-370 x IRBB-55-20, Basmati-370 x IRBB-55-21, Basmati-370 x IRBB-55-22 were found to have moderate resistant (having lesion length more than 0.5 cm). Most of the genotypes with two resistance genes in the homozygous condition (*Xa21Xa21/xa13xa13*) showed higher levels of resistance than genotypes with only one resistance gene with mean lesion length of around 0.50 cm.

Regarding disease reaction of pyramided lines, in some of the one gene pyramided lines, there has been an increased lesion length after 21 days of inoculation. However, there is no such expansion in the lesion length in the two genes pyramided lines. This hints on the importance of pyramiding more than two genes for evolving durable resistance against *Xoo* isolates employed[24,25]. The two genes pyramided lines developed in this study were found to show high levels of resistance against predominant *Xoo* isolates. As expected, plants with all the two resistance genes in the homozygous condition showed reduced mean lesion length ranging from 0.10 to 1.20 cm against tested *Xoo* isolates. The higher level of resistance may be the result of gene interaction or quantitative complementation between resistant genes[21,22].

Variability studies in morphological and quality characters

Analysis of variance for different morphological and quality characters revealed that all the 34 genotypes differ significantly for most of the traits with respect to plant height, days to 50 % flowering, days to maturity, panicle length, number of tillers per plant, number of effective tillers per plant, number of spikelets per panicle and grain yield per plant. Different genetic parameters that were used to explain the variability revealed that phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were high for most of morphological characters except number of days to 50 % flowering and number of days to maturity which have low PCV and GCV. However, in case of panicle length and spikelets per panicle, a moderate level of PCV and GCV was recorded. Most of the morphological characters exhibited low environ-mental coefficient of variation (ECV), expect for total tillers per plant and number of effective tillers per plant where moderate level of ECV was recorded.

Mean performance of genotypes for grain yield and quality characters

Genotypes showed significant difference in mean performance for grain yield per plant which ranged from 10.10-23.80 grams per plant (Table 3). Among the parents, Basmati 370 had the highest grain yield per plant (13.80 g), followed by IRBB-55 (10.10 g) whereas, among BC_1F_2 genotypes, Basmati-564 x IRBB-55-18 had the highest grain yield per plant (23.80 g), followed by Basmati-564 x IRBB-55-14 (23.00 g). For grain length genotype Basmati-370 x IRBB-55-1 had the longest grains (7.50 mm), followed by Basmati-370 x IRBB-55-9 (7.40 mm). L/B ratio was maximum for genotype Basmati-370 x IRBB-55-3 (3.16) followed by Basmati-370 x IRBB-55-11 and Basmati-370 x IRBB-55-12 (2.91 each).

Amylose content ranged from 28.60 to 9.20 % for genotypes Basmati-370 x IRBB-55-22 and Basmati-370 x IRBB-55-13, respectively. Most of the crosses showed strong to mild aroma but Basmati-370 x IRBB-55-7 showed strong aroma compared to recipient parent. In grain appearance, the recipient parent Basmati-370 had long slender grain appearance where as the donor resistant parent IRBB-55 had medium slender grain appearance. Estimates of heritability and genetic advance expressed as per cent of mean were high for most of the quality characters in the rice genotypes, however, estimate of genetic advance for grain breadth were recorded to be moderate, which is desirable for selection. The importance of heritability in addition to mean performance and variability was also observed[26]. High level of heritability and high genetic advance was observed for most of the quality characters. These findings are in accordance to early reports of different workers in rice breeding[23-25].

The high heritability combined with high genetic advance will be more useful than heritability alone in predicting the performance of the progenies of selected lines[27]. Accordingly, in the present investigation high heritability with high genetic advance as per cent of mean was observed with respect to number of tillers per plant, effective tillers per plant, spikelets per panicle, grain yield per plant, grain length, L/B ratio and amylose content. Similar findings were reported in earlier studies[28-30].

Table 3—Mean value of parents and pyramided basmati lines for grain yield and quality characters

Genotypes	Grain yield/ Plant (g)	Grain length (L) (mm)	Grain breadth (B) (mm)	Length/breadth (L/B) ratio	Amylose content (%)	Aroma	Grain appearance
Basmati-370	13.80	6.70	2.54	2.62	16.10	Strong	Long slender
IRBB-55	10.10	5.40	2.51	2.15	19.30	Mild	Medium slender
Basmati-370 x IRBB-55-1	14.50	7.50	2.51	3.16	18.30	Strong	Long slender
Basmati-370 x IRBB-55-2	15.50	7.10	2.70	2.62	20.00	Strong	Long slender
Basmati-370 x IRBB-55-3	14.60	7.30	2.50	2.88	21.90	Mild	Long slender
Basmati-370 x IRBB-55-4	13.40	7.20	2.61	2.75	17.40	Strong	Long slender
Basmati-370 x IRBB-55-5	12.50	7.30	2.60	2.79	10.10	Mild	Long slender
Basmati-370 x IRBB-55-6	16.70	6.50	2.60	2.48	23.20	Strong	Long slender
Basmati-370 x IRBB-55-7	20.70	7.00	2.60	2.69	24.10	Very strong	Long slender
Basmati-370 x IRBB-55-8	15.40	6.50	2.60	2.51	17.60	Strong	Long slender
Basmati-370 x IRBB-55-9	22.40	7.40	2.70	2.73	15.80	Strong	Long slender
Basmati-370 x IRBB-55-10	13.80	6.60	2.61	2.51	16.00	Mild	Long slender
Basmati-370 x IRBB-55-11	16.40	7.30	2.51	2.91	22.10	Strong	Long slender
Basmati-370 x IRBB-55-12	14.00	7.30	2.51	2.91	19.90	Strong	Long slender
Basmati-370 x IRBB-55-13	17.80	5.50	2.51	2.19	9.20	Strong	Long slender
Basmati-370 x IRBB-55-14	23.00	6.80	2.62	2.58	14.00	Strong	Long slender
Basmati-370 x IRBB-55-15	16.40	6.90	3.10	2.21	21.20	Strong	Long slender
Basmati-370 x IRBB-55-16	14.80	6.50	2.51	2.57	15.00	Strong	Long slender
Basmati-370 x IRBB-55-17	12.80	6.40	2.60	2.44	10.20	Strong	Long slender
Basmati-370 x IRBB-55-18	23.80	6.80	2.40	2.81	19.20	Strong	Long slender
Basmati-370 x IRBB-55-19	16.00	6.50	2.51	2.60	12.20	Strong	Long slender
Basmati-370 x IRBB-55-20	14.60	6.30	2.51	2.50	13.30	Strong	Long slender
Basmati-370 x IRBB-55-21	13.40	6.60	2.51	2.63	20.90	Mild	Long slender
Basmati-370 x IRBB-55-22	19.10	6.50	2.51	2.60	28.60	Mild	Long slender
CD(0.05)	1.420	0.220	0.069	0.127	0.880	-	-
CV (%)	6.062	2.028	1.596	3.059	0.880	-	-

The pyramided lines with resistance genes would show a wider spectrum and higher level of resistance at all the stages of plant growth. Although markers can be used in any stage during a typical plant breeding programme, MAS is a great advantage in early segregating generation because plants with undesirable gene combination can be eliminated. In the present study, out of 20 BC_1F_1 cross combinations, one cross Basmati-370 x IRBB-55 were found to have basmati quality traits and was selfed to obtain BC_1F_2 population. Twenty two genotypes of BC_1F_2 were used for molecular screening of bacterial blight resistant genes and aroma (*fgr*) gene through MAS. Out of 22 genotypes selected from Basmati-370 x IRBB-55 cross combination five genotypes of BC_1F_2 *viz*., Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-13, Basmati-370 x IRBB-55-17 and Basmati-370 x IRBB-55-18 showed complete resistant to bacterial leaf blight. The plants showing complete resistance possessed two resistant genes *viz., Xa21* and *xa13* along with *fgr* gene in homozygous conditions, while other plants which possessed only one resistant gene either *Xa21* or *xa13* in homozygous condition showed moderate resistance for BB. It is also concluded that BC_1F_1 population should be thoroughly screened for morphological, basmati quality and BB resistance on field-based trials. Then marker-based analysis should be done to identify closely linked markers which can save time, money and other resources. Thus pyramided lines with two BB resistance genes were observed to show a wider spectrum and a higher level of resistance than lines with only a single gene[21,22]. The genotypes Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-13, Basmati-370 x IRBB-55-17 and Basmati-370 x IRBB-55-18 which showed complete resistance to BB along with good aroma can be used as potential donors for the introgression of BB resistance genes into the elite varieties of basmati genotypes.

Conclusion

The present study was designed to identify desirable basmati quality lines with resistant genes of

bacterial blight. Twenty BC_1F_1 cross combinations generated by randomly crossing of four elite basmati cultivars with 10 bacterial blight resistant donor lines possessing resistant genes in different combinations were used for screening, and one combination Basmati-370 x IRBB-55 was found to have basmati quality traits like intermediate amylose and high aroma content. Twenty two BC_1F_2 genotypes were selected from this cross combination for screening *Xa21* and *xa13* BB resistance genes as well as aroma gene through marker assisted selection (MAS). Five genotypes namely, Basmati-370 x IRBB-55-4, Basmati-370 x IRBB-55-5, Basmati-370 x IRBB-55-13, Basmati-370 x IRBB-55-17 and Basmati-370 x IRBB-55-18 identified phenotypically to show complete resistance to BB along with intermediate amylose and high aroma, and hence can be used as potential basmati donors.

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References

1 Mew TW, Vera Curz CM & Medalla ES, Changes in race frequency of *Xanthomonas oryzae pv. oryzae* in response to rice cultivars planted in the Philippines. *Plant Dis*, 76 (1992) 1029

2 Salgotra RK, Gupta BB, Millwood RJ, Balasubramaniam M & Stewart Jr CN, Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). *Euphytica*, 187 (2012) 313.

3 Abbasi FM, Shah AH, Masood R, Mujadad R, Nawaz F, Sajid M, Afzal M, Majid A, Akhtar N & Bukhari I, Production and molecular characterization of wide cross derivatives in rice. *Afr J Biotech*, 9 (2010) 3732

4 Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E & Anandan A, Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice*, 8 (2015) 1.

5 Wang J, Wan X, Li H, Pfeiffer WH & Crouch J, Application of identified QTL-marker associations in rice quality improvement through a design-breeding approach. *Theor Appl Genet*, 115 (2007) 87.

6 Ram T, Laha GS, Gautam SK, Deen R, Madhav MS & Brar DS, Identification of new gene introgressed from *Oryza brachyantha* with broad-spectrum resistance to bacterial blight of rice in India. *RGN*, 25 (2010) 57.

7 Shanti ML & Shenoy VV, Evaluation of resistance genes and their pyramids against rice bacterial leaf blight pathogen *Xanthomonas oryzae pv. Oryzae*. *Oryza*, 42 (2005) 169.

8 Nayak D, Shanti ML, Bose LK, Singh UD & Nayak P, Pathogenicity association in *Xanthomonas oryzae pv. oryzae* the causal organism of rice bacterial blight disease. *J Phytopathol*, 3 (2008) 12.

9 Tanksley SD, Young ND, Paterson AH & Bonierbale MW, RFLP mapping in plant breeding: new tools for an old science. *Biotechnology*, 7 (1989) 257.

10 Shanti ML, Shenoy VV, Devi GL, Kumar VM, Premalatha P, Kumar GN, Shashidhar HE, Zehr UB & Freeman WH, Marker-assisted breeding for resistance to bacterial leaf blight in popular cultivar and parental lines of hybrid rice. *J Plant Pathol*, 92 (2010) 495.

11 Bhatia D, Sharma R, Vikal Y, Mangat GS, Mahajan R, Sharma N, Lore JS, Singh N, Bharaj TS & Singh K, Marker-assisted development of bacterial blight resistant, dwarf, and high yielding versions of two traditional basmati rice cultivars. *Crop Sci*, 51 (2011) 759.

12 Doley JJ & Doley JL, Isolation of plant DNA from fresh tissue. *BRL Focus*, 12 (1990) 13.

13 Chu Z, Fu B, Yang H, Xu C, Li Z, Sanchez A, Park YJ, Bennetzen JL, Zhang Q and Wang S, Targeting *xa13*, a recessive gene for bacterial blight resistance in rice. *Theor Appl Genet*, 112 (2006) 455.

14 Chen X, Temnykh S, Xu Y, Cho Y & McCouch SR, Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet*, 95 (1997) 553.

15 Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S & McCouch SR, Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. *Genome Res*, 11 (2001) 1441.

16 IRRI, Standard evaluation system for rice, 3rd edn. IRRI, Manila. (1988)

17 Sood BC & Siddiq EA, A rapid technique for scent determination in rice. *Indian J Genet Plant Breed*, 38 (1978) 2268.

18 Thimmaiah SK, Standard method of Biochemical analysis, Kalyani Publisher New Delhi, (1999) 130.

19 Mundt CC, Use of multiline cultivars and cultivar mixtures for disease management. *Phytopathology*, 80 (1990) 221.

20 Ronald PC, Albano B, Tabien R, Abenes L, Wu K, McCouch SR & Tanksley SD, Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Mol Genet*, 336 (1992) 113.

21 Huang H, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J & Khush GS, Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor Appl Genet*, 95 (1997) 313.

22 Sanchez AC, Brar DS, Huang N, Li Z & Khush GS, Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci*, 40 (2000) 792.

23 Singh SJ, Sidhu JS, Huang N, Vikal Y, Li, Z, Brar DS, Dhaliwal HS & Khush GS, Pyramiding three bacterial blight resistance genes (*xa-5*, *xa-13* and *Xa-21*) using marker-assisted selection into indica rice cultivar PR-106. *Theor Appl Genet*, 102 (2001) 1011.

24 Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS, Ashok Reddy G, Shobha Rani N, Sarma NP & Sonti RV, Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, 80 (2008) 411.

25 Suh JP, Jeung JU, Noh TH, Cho YC, Park SH, Park HS, Shin MS, Kim CK & Jena KK, Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice*, 8 (2013) 1.

26 Panse VG, Genetics of quantitative characters in relation to plant breeding. *J Genet*, 17 (1957) 318.

27 Johnson HW, Robinson HF & Comstock RE, Estimates of genetic and environmental variability in soybeans. *J Agron*, 47 (1995) 314.

28 Rathi S, Yadav RNS & Sarma RN, Variability in grain quality characters of upland rice of Assam, India. *Rice Sci*, 17 (2010) 330.

29 Vanaja T & Babu LC, Variability in grain quality attributes of high yielding rice varieties (*Oryza sativa* L.) of diverse origin. *J Trop Agric*, 44 (2006) 61.

30 Sabesan T, Suresh R & Saravanan K, Genetic variability and correlation for yield and grain quality characters of rice grown in coastal saline low land of Tamil Nadu. *Elect J Plant Breed*, 1 (2009) 56.