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INTROGRESSION OF MAJOR BACTERIAL BLIGHT AND BLAST RESISTANT GENES INTO VALLABH BASMATI 22, AN ELITE BASMATI VARIETY

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ABSTRACT

Bacterial blight and blast are two major destructive diseases in rice, particularly in basmati growing areas. For sustainable basmati rice production, there is a need to identify new resistant sources and improve the elite Basmati varieties against the two diseases. Vallabh Basmati is an elite variety, long slender grain, medium duration and possessing excellent aroma and with consumer preferable cocking and eating qualities. However, the variety is highly susceptible to BB and blast diseases, which limit its production to a significant extent. In the present study, three major resistant genes (i.e., *Xa21* and *xa13* for BB resistance and *Pi54* for blast resistance) were intogressed into Vallab Basmati 22 through the strategy of marker-assisted breeding (MAB) using Improved Samba Mahsuri (possessing *Xa21* + *xa13*) and Tetep (possessing *Pi 54*) as the donor parents through two sets of crosses. At each generation, plants possessing *Xa21* and *xa13* or *Pi54* in heterozygous condition were identified from the two crosses with help of gene-specific markers through foreground selection. At each generation, plants possessing *Xa21* + *xa13* + *Pi54* in homozygous condition were identified with the help of gene specific markers and advanced further through selfing. At ICF_4, four promising three-gene pyramid lines of Vallabh Basmati22 possessing high level of resistance against both BB and blast along with high yield and grain type similar to the recurrent parent have been identified.

INTRODUCTION

Basmati rice is pride of nation and captures higher returns as it is priced three times more than non-Basmati rice in National and International markets. India is major exporter of Basmati rice varieties and earns foreign exchange of ~ Rs. 23000 crores with 40 lakhs tones of Basmati exports (BEDF, New Delhi, June 2015). Basmati has unique quality features such as long slender grain, pleasant aroma, flavor, excellent cooking and eating qualities. The yield potential of most of the basmati varities is significantly affected by various biotic and abiotic stresses.

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Among the various biotic stresses, like bacterial blight (BB) and blast are the most important diseases and severe infection may cause yield loss up to 50%, in addition to impairing the quality of the produce. The disease is known to occur in epidemic proportions in many parts of the traditional Basmati growing areas, all Basmati rice varieties are susceptible to these devastating diseases and management through chemicals is not commercially available. Therefore, deployment of varieties with resistant genes is the only option available to contain this disease. Durable resistance can be achieved by pyramiding multiple resistant genes. But, pyramiding of multiple resistant genes is very difficult through conventional breeding methods due to dominance and epistatic effects of genes governing disease resistance and problems in screening. Marker-assisted selection (MAS) has proved its utility in several crops to overcome above problems and many genes can be pyramided either for the same trait or for different traits

along with faster recurrent parent genome recovery through intense background selection. Further more. Till date at least 40 resistant genes have been identified for BB resistance (Sundaram *et al* 2014; Kim *et al.* 2015) and nearly 100 blast resistant genes (Sharma *et al* 2012) have been identified in rice. In the present study, two major genes conferring resistance for BB (*Xa21* and *xa13*) and a major gene conferring blast resistance (*Pi54*) were transferred into the genetic background of elite Indian Basmati variety Vallabh Basmatu 22 through marker-assisted breeding.

MATERIALS AND METHODS

Plant materials

The rice varieties, Improved Samba Mahsuri (ISM) possessing the major bacterial blight (BB) resistant genes- *Xa21, xa13* and *xa5* (Sundaram et al 2008) and Tetep (possessing the major blast resistance gene, *Pi54*) were used as donor parents, while an elite basmati variety, Vallabh basmati 22 was used as the recurrent parent. In addition to these lines, TN1 and HR12 were used as the susceptible checks for BB and blast screening, respectively.

Introgression of *Xa21, xa13* and *pi54* into the background of Vallabh basmati 22

Marker assisted breeding strategy was adapted for introgression of major Bacterial blight and blast resistance genes in to the genetic background Vallabh basmati 22. The two donor parents, Improved Samba Mahsuri (ISM) and Tetep were crossed Vallabh Basmati22 through two sets of crosses (Cross I and Cross II) during Kharif 2011 (i.e. wet season 2011). The 'true' F_1s were identified from both the crosses using the molecular markers, pTA248 (specific for *Xa21;* Ronald et al. 1992), *xa13-prom* (specific for *xa13;* Sundaram et al. 2012; Hajira et al. 2016) and *Pi54-MAS* (specific for *Pi54;* Ramkumar et al. 2013; Table.1). They were then inter crossed with each other during Rabi 2011-2012 (i.e. dry season 2011-2012) to combine *Xa21, xa13* and *Pi54* in the fresh set of F_1 plants obtained (i.e. intercross F_1s or ICF_1s). 'True' ICF_1 plants possessing the three target genes in heterozygous condition were identified with the help of gene-specific markers and selfed to generate ICF_2s. Among these, plants which were homozygous for all the three target resistance genes (viz., *Xa21, xa13* and *Pi54*) were identified with the help of gene-specific markers and they were then advanced further through pedigree based morphological till ICF_4. At ICF_4 generation, promising lines, which were similar to or better than Vallabh Basmati 22, were evaluated for their resistance against BB and blast and also for their key agromorphological traits and grain quality features.

Phenotypic screening for bacterial blight (BB) and blast resistance

The introgressed lines with appropriate checks were screened for bacterial blight and blast for their disease resistance. For bacterial blight screening TN1 used as susceptible check and ISM used as resistant check. For blast screening HR12 used as susceptible check and Tetep used as resistant check, while Vallabh Basmati 22 used as recurrent parent. The score was evaluated on 0-9 scale as per IRRI-SES scale (IRRI 2013),

Bacterial blight (BB) screening: A virulent isolate of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from Hyderabad, India, *viz*. DX-020 (Hyderabad, Telangana State, India) was used to screen ICF_4 progenies of Vallabh Basmati 22 along with susceptible and resistant checks for BB resistance under both glasshouse and field conditions. The *Xoo* strains were cultured and stored as described by Laha *et al*., (2009). The rice plants were clip-inoculated with a bacterial suspension of 108-9 cfu/ml at maximum tillering stage (45 –55 days after transplanting) through the methodology of Kauffman *et al*., (1973). The plants were scored 15 days after inoculation and evaluated as per IRRI-SES scale.

Blast screening : The pathogen, *Magnaporthae oryzae*, is a local isolate of *Magnaporthe oryzae* named, SPI-40 from ICAR-Indian Institute of Rice research, Hyderabad, Telangana State, India (Madhan Mohan 2011), was used to screen the donor and recurrent parent along with intercross ICF_4derived lines of Vallabh basmati22, along with susceptible and resistant checks for blast resistance through uniform blast nursery (UBN) method. The pathogen strains were cultured and stored as described by Srinivas Prasad *et al*., (2011). A dilution was 1 x 10^5 conidia/ml of the fungal conidial suspension at a concentration was used for inoculation of young seedlings at four-leaf stage and high humidity was maintained continuously for one week for disease development. One week later, the inoculated seedlings were monitored for the development of blast lesions and were scored as per IRRI-SES scale. The lines with scores of 0-3 were considered resistant, 4-5 as moderately resistant, 6 as moderately susceptible and 7-9 as susceptible.

RESULTS

Pyramiding of BB (*Xa21, xa13)* and blast (*Pi54*) resistant genes into Vallabh Basmati 22 through Marker-assisted introgression

Out of a total of 72 F_1s, which were produced by crossing ISM x Vallabh basmati 22 (i.e. Cross I), 38 of these were identified to be 'true' F_1's. Similarly, from Cross II, 41 plants were identified to be 'true' F_1s. When the 'true' F_1s derived from Cross I and II were intercrossed with each other and the new set of F_1s (i.e. intercross F_1s; n = 178) were screened with gene-specific markers, a total of 23 plants were heterozygous for all the three resistance genes. Among these, a single ICF_1 plant (ICF_1-10K-36) was observed to be highly similar to the recurrent parent (i.e. Vallabh Basmati 22) based on agromorphological features (i.e. based on visual observation) and was selfed to generate ICF_2s (n = 528). They were then screened with gene-specific markers to identify homozygous plants and a total of seven triple homozygous plants were identified (i.e. homozygous for *Xa21, xa13* and *Pi54*). Among these, one plant (i.e. plant # ICF2-10K-36) was identified to be similar to vallabh Basmati 22 through phenotype-based morphological selection. It was then advanced by selfing through pedigree method up to ICF_4 generation.

Four promising lines (i.e. plant # ICF_4-10K-36-3, ICF_4-10K-36-7, ICF_4-10K-36-25, ICF_4-10K-36-33, ICF_4-10K-36-34) (Table 2; Figure 1), which were similar to or better than Vallabh Basmati 22, were evaluated for their resistance against BB and blast.

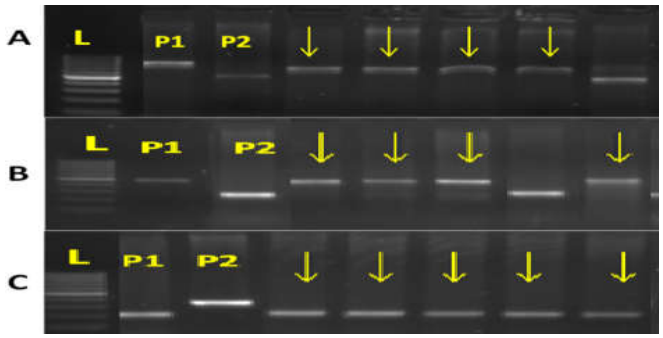

Figure 1. Foreground selection for Xa21, *xa13* and Pi54 genes in ICF_4 plants through PCR based markers

Figure 1: A, B and C displays the screening of the selected ICF_4 plants (viz., ICF_4-10K-36-3, ICF_4-10K-36-7, ICF_4-10K-36-25, ICF_4-10K-36-33, CF_4-10K-36-34) for confirmation of presence the target genes *Xa21, xa13* and *Pi4* in homozygous condition using the gene-specific markers pTA 248 and xa13 prom and Pi54MAS. All the selected ICF_4 plants were observed to be homozygous (indicated by arrow). L represents 100 bp ladder molecular weight marker, P1- Donor parent and P2- Vallabh Basmati 22

The blast resistance check Tetep having *Pi54* gene showed a disease score of 1, and the susceptible checks Vallabh Basmati 22, HR-12 and TN1 showed a score of 9. All the five selected intercross derived line showed high level of resistance with a score ranging from 1-3 (Table. 2). With respective to screening for BB resistance, the resistance check, ISM showed immune level of resistance (score of 1), while the susceptible checks, Vallabh basmati 22, HR12 and TN1 showed a score of 9 (Table.1). The intercross derived lines showed immune level of resistance with 1 score confirming that all the selected lines are indeed resistant to both BB and blast.

DISCUSSION

Breeding through conventional methods for improvement of agronomically important traits in crops require more time, is laborious and is heavily dependent on environment factors, thus limiting the progress of breeding. Marker-assisted breeding (MAB) is a highly efficient and precise strategy for targeted improvement of one or few traits of elite varieties and hybrids (Sundaram *et al.* 2014). To improve varieties and hybrid parental lines for BB and blast resistance, MAB has been successfully adapted in several earlier studies (Sundaram *et al*., 2008, 2009, Basavaraj *et al*., 2010, Zhou *et al*,.2011, Singh *et al*., 2012). Vallabh Basmati 22 is an aromatic Basmati type rice variety and has extra long slender grain (ELS) type along with desirable grain, cooking and eating quality features. However, Vallabh basmati 22 basmati variety is susceptible to BB and blast diseases, which limit its yield. Hence the present study was carried out with an objective to improved

Table 1. Details of PCR-based markers used for foreground selection

Gene	Marker	Chromosome No.	References
xa13	*xa13*promoter specific marker	8	Sanchez *et al*. (1999)
Xa21	pTA 248	11	Khush *et al*. 1990
Pi 54	Pi 54 MAS	11	(Sharma et al. 2005 a,b)

Table 2. Phenotypic screening of selected backcrossed derived lines for bacterial blight and blast resistance for their disease resistance reaction in the background of Vallabh Basmati 22

S no	Designation	Gene possessing	Phenotypic disease screening score	
			Blast (UBN)	BB (Field)
Introgressed lines (IL's)				
1	ICF_4-10K-36-3	*Xa21+xa13+Pi54*	1	1
2	ICF_4-10K-36-7	*Xa21+xa13+Pi54*	1	1
3	ICF_4-10K-36-25	*Xa21+xa13+Pi54*	1	1
4	ICF_4-10K-36-33	*Xa21+Pi54*	3	1
5	ICF_4-10K-36-34	*Xa13+Pi54*	3	1
Checks				
6	ISM (resistant check for BB)	*Xa21+xa13*	9	1
7	Tetep (resistant check for blast)	*Pi54*	1	9
8	Vallabh Basmati 22 (recurrent parent)	-	9	9
9	TN1 (susceptible check for both BB and blast)	-	9	9
10	HR12 (susceptible check for both BB and blast)	-	9	9

Phenotypic screening of BB and blast disease resistance in intercross derived lines

Selected ICF_4 lines mentioned above were phenotypically screened for their disease reaction to Blast and BB disease in UBN nursery bed and field conditions, respectively.

Vallabh Basmati 22 for its resistance against BB and blast disease through targeted introgression of two major BB resistance genes (*Xa21+xa13*) and one major blast resistance gene (*Pi54*). Similar to the work done in this present study, through an earlier study carried out by Joseph et al. (2004), the first near-isogenic line (NIL) under basmati category, Improve

Pusa basmati 1 was developed through MAB wherein two major bacterial blight resistance genes, *xa13* and *Xa21* were incorporated into the elite variety, Pusa Basmati 1. Ellur et *al* (2016) introgressed the major blast resistance genes, *Pi2* and *Pi54* and bacterial blight (BB) resistance genes *Xa38, xa13* and *Xa21* into the genetic background of two elite Basmati varieties, Pusa Basmati 1121 (PB1121) and Pusa Basmati 6, Joseph *et al*. (2005). Pandey *et al*. (2013) also utilized marker-assisted selection for transfer of *Xa21* and *xa13* in the genetic background of elite Basmati variety, Taraori Basmati. In this study, we have transferred an additional gene, i.e. a major blast resistance gene, *Pi54* into the elite Basmati variety, Vallabh Basmati 22 through marker-assisted backcross breeding. In this process, at each generation of backcrossing and intercrossing, gene-specific markers were used for foreground selection, while phenotype based selection was adopted for selecting the best plant among those which carry the desired resistance genes (i.e. background selection). Thus at ICF_2 generation, plants which were homozygous for the target resistance genes, but also equivalent to Vallabh basmati 22 were identified and advanced for further selections, resulting in identification of four promising lines at ICF_4 generation having all the desirable features of Vallabh Basmati 22 along with durable disease resistance against BB and blast.

The level of BB and blast resistance in the improved versions of Vallabh Basmati 22 in three gene combinations viz., *Xa21 + xa13 + Pi54* and (Table 2) was observed to be better than the recurrent parent and equivalent to the the donor parents ISM (BB score 1-3) and Tetep (Blast score 1) respectively at ICF_4 generation. Thus the key objectives of the present study, i.e. introgession of two major BB and one major blast resistant genes into Vallabh Basmati 22 was achieved by combining MAS and phenotype-based selection at each stage of backcrossing. Three-gene pyramids possessing two BB resistance genes *Xa21+xa13* and one blast resistance gene, i.e. *Pi54* were developed and later intercrossed and selfed to generate ICF_2 lines (adopting pedigree-based, morphological selection for key agronomic traits) in order to combine the two BB resistance genes and one blast resistance gene in different gene. Pyramid lines possessing *Xa21 + xa13+54, Xa21+Pi54* and xa*13+Pi5* were observed to show high level of resistance against BB and blast (Table 2). The promising four intercross derived lines (i.e. ICF_4 lines) of Vallabh Basmati 22 (viz., plant # ICF_4-10K-36-3, ICF_4-10K-36-7, ICF_4-10K-36-25, ICF_4-10K-36-33, CF_4-10K-36-34) are presently being further evaluated for their yield and other agromorphological attributes. They will be nominated for multiplication trials for possible release to farmers.

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