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RESEARCH ARTICLE

MARKER ASSISTED IMPROVEMENT OF THE ELITE BASMATI VARIETY, IET 18006 FOR RESISTANCE AGAINST BACTERIAL BLIGHT AND BLAST

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ABSTRACT

IET 18006 is an elite Basmati variety, with highly desirable long slender grain type and medium duration and possesses excellent aroma. However, the variety is highly susceptible two major diseases, viz., bacterial blight (BB) and blast which reduce yield of the elite Basmati variety significantly. We have improved IET 18006 through targeted introgression of the major BB resistance genes, *Xa21* and *xa13* and the major blast resistance gene, *Pi54* through marker-assisted backcross breeding (MABB). The elite variety, Improved Samba Mahsuri (ISM) possessing bacterial blight resistant genes *Xa21* and *xa13* and a Vietnamese variety, Tetep possessing blast resistant gene, *Pi54* were used as donor parents for improvement of IET 18006 through two sets of backcrosses and backcrossing was continued till BC_2 generation. At each backcross generation, plants possessing *Xa21, xa13* and *Pi54* in heterozygous condition were identified with help of gene-specific markers through foreground selection; while a set of parental polymorphic SSR markers were used for background selection. At BC_2F_2, a promising backcross plant possessing *Xa21 + xa13* was intermated with a backcross plant possessing *Pi54* to generate intercross F_1s (i.e. ICF_1s), which were then selfed. At ICF_2 generation, plants which 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 IET 18006 possessing high level of resistance against both BB and blast along with high yield and grain type similar to the recurrent parent were identified.

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INTRODUCTION

Basmati rice is pride of nation and is well known worldwide for its unique aroma, flavor, excellent cooking and eating quality. Thus, Basmati rice is characterized with unique quality features such as long slender grains with pleasant aroma. Basmati rice captures higher returns as it is priced three times higher than non-Basmati rice in the National and International market. India is major exporter of Basmati rices and earns foreign exchange of Rs. 23000 crores with 40 lakhs tones of Basmati exports (BEDF, New Delhi, June 2015). Among the diseases, Bacterial blight (BB) and blast diseases are major threat to sustainable Basmati production and export. 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 rices 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 using conventional breeding methods due to dominance and epistatic effects of genes governing disease resistance. However, 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 nearly 38 resistant genes have

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been identified for BB resistance (Sundaram *et al* 2014) and nearly 100 blast resistant genes (T R Sharma *et al* 2012) were identified. In the present study, two major genes conferring resistance against BB (*Xa21* and *xa13*) and a major gene conferring blast resistance (*Pi54*) were transferred into the genetic background of elite Indian Basmati variety (IET 18006) through marker-assisted breeding.

MATERIALS AND METHODS

Plant material

Improved Samba Mahsuri (ISM) possessing the bacterial blight (BB) resistant genes- *Xa21, xa13* and *xa5* (Sundaram *et al*., 2008) and Tetep (possessing the blast resistance gene, *Pi54*) were used as donor parents, while IET 18006 used as the Recurrent parent. In addition to these lines, Taichung Native 1 (TN1) and HR12 were used as the susceptible check for BB and blast screening, respectively.

Crossing scheme

The two donor parents, Improved Samba Mahsuri (ISM) and Tetep were crossed IET 18006 through two sets of crosses (Cross I and Cross II) during Kharif 2011 (i.e. wet season 2011). The 'true' F_1s were identified 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 intercrossed 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 mentioned above 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 IET 18006, 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) resistance

A virulent isolate of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from BB Hyderabad, India, *viz*. DX-020 (Hyderabad, Telangana State, India) was used to screen BC_2F_2 progenies of IET 18006 along with donor, ISM and the recurrent parent 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 10^{8-9} 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 on 0–9 scale as per IRRI-SES scale (IRRI 2013).

Phenotypic screening for blast resistance

A local isolate of *Magnaporthe oryzae* named, SPI-40 from Hyderabad, Telangana State, India (Madhan Mohan 2011), was used to screen the donor and recurrent parents along with intercross derived lines of IET 18006 for blast resistance through uniform blast nursery (UBN) method at ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, India. 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 on 0–9 scale as per IRRI-SES scale (IRRI 2013).

RESULTS

Marker-assisted introgression of *Xa21, xa13* and *Pi54* into IET 18006

Out of a total of 67 F_1s, which were produced by crossing ISM x IET 18006 (i.e. Cross I), 43 of these were identified to be 'true' F_1's. Similarly, from Cross II, 40 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 = 220) were screened with gene-specific markers, a total of 25 plants were heterozygous for all the three resistance genes. Among these, a single ICF_1 plant (ICF_1-10K) was observed to be highly similar to the recurrent parent (i.e. IET 18006) based on agromorphological features (i.e. based on visual observation) and was selfed to generate ICF_2s (n = 633). They were then screened with gene-specific markers to identify homozygous plants and a total of nine triple homozygous plants were identified (i.e. homozygous for *Xa21, xa13* and *Pi54*). Among these, one plant (i.e. plant # ICF2-10K-14) was identified to be similar to IET 18006 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-14-1, ICF_4-10K-14-7, ICF_4-10K-14-8, ICF_4-10K-14-11) (Table 1; Figure 1), which were similar to or better than IET 18006, were evaluated for their resistance against BB and blast.

Figure 1A, B and C displays the screening of the selected ICF_4 plants (viz., ICF_4-10K-14-1, ICF_4-10K-14-7, ICF_4-10K-14-8, ICF_4-10K-14-11) 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- IET 18006.

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.

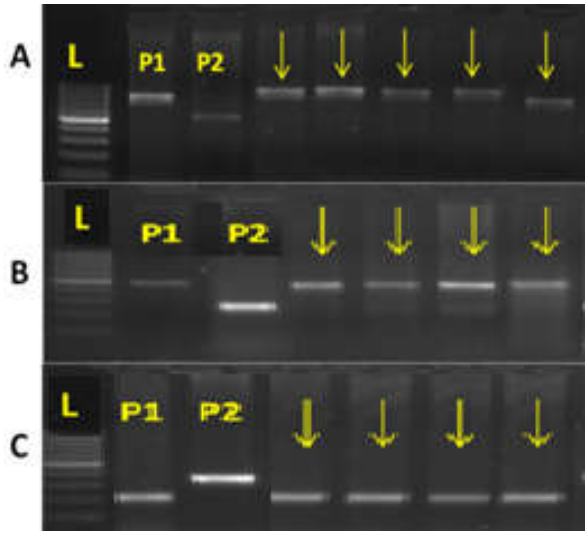

Figure 1. Confirmation of presence of *Xa21, xa13* and *Pi54* among the selected ICF_4 plants using gene-specific markers

Table 1. Phenotypic screening of selected ICF_4 lines of IET 18006 for their resistance against bacterial blight and blast diseases

S. No.	Designation	Gene possessing	Phenotypic disease screening	
			Blast Score (UBN)	BB score (Field)
1	ICF_4-10K-14-1	*Xa21+xa13+Pi54*	1	1
2	ICF_4-10K-14-7	*Xa21+xa13+Pi54*	1	1
3	ICF_4-10K-14-8	*Xa21+xa13+Pi54*	1	1
4	ICF_4-10K-14-11	*Xa21+xa13+Pi54*	1	1
5	ISM (resistant check for BB)	*Xa21+xa13*	9	1
6	Tetep (resistant check for blast)	*Pi54*	1	9
7	IET 18006 (recurrent parent)	-	9	9
8	TN1 (susceptible check for both BB and blast)	-	9	9
9	HR12 (susceptible check for both BB and blast)	-	9	9

The blast resistance check Tetep having *Pi54* gene showed a disease score of 1, and the susceptible checks IET 18006, HR-12 and TN1 showed a score of 9. All the four selected intercross derived line showed a score of 1 equal to the resistant check Tetep (Table 1). With respective to screening for BB resistance, the resistance check ISM showed immune level of resistance (score of 1), while the susceptible checks, IET 18006, 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 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 MAS has been successfully adapted (Sundaram *et al*., 2008, 2009, Basavaraj *et al*., 2010, Zhou *et al*., 2011, Singh *et al*., 2012). IET 18006 is a highly aromatic Basmati type rice variety and has excellent long slender grain type along with desirable grain, cooking and eating quality features. However, the elite variety is highly susceptible to BB and blast diseases, which limit the yields of the elite Basmati variety. Hence the present study was carried out with an objective to improved IET 18006 for its resistance against BB and blast through targeted introgression of two major BB resistance genes, *Xa21* and *xa13* and a major blast resistance gene, *Pi54*. Similar to the work done in this study, earlier studies carried out by Joseph *et al*. (2005), Gopalakrishnan *et al.* (2008), Pandey *et al*. (2013) also involved marker-assisted transfer of *Xa21* and *xa13* in the background of elite Basmati varieties. However in this study, we have transferred an additional gene, i.e. a major blast resistance gene, *Pi54* into the elite Basmati variety, IET 18006 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. Thus at ICF_2 generation, plants which were homozygous for the target resistance genes, but also equivalent to IET 18006 was identified and advanced for further selections, thus resulting in identification of four promising lines at ICF_4 generation having all the desirable features of IET 18006 along with durable disease resistance against BB and blast. The level of BB resistance in the improved versions of IET 18006, was observed to be equivalent to the donor parent, ISM (score 1; Table 1). Similarly with respect to blast screening, all the improved lines of IET 18006 showed highly resistant score like the donor parent, Tetep (score 1; Table 3 1). Thus the key objectives of the present study, i.e. introgession of two major BB and one major blast resistant

genes into IET 18006 has been achieved. We also analyzed the grain quality features of the improved lines of IET 18006 and observed that all the improved lines possess long slender grain type with good aroma indicating that the other key objective of retaining the premium grain quality features of IET 18006, while improving it for BB and blast resistance has also been achieved. The three selected improved lines of IET 18006 (ICF_4-10K-14-1, ICF_4-10K-14-7, ICF_4-10K-14-8) are presently being further evaluated for their yield and other agromorphological attributes and after their evaluation, they will be nominated for multiplication trials for possible release to farmers.

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