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Integrating marker-assisted background analysis with foreground selection for pyramiding bacterial blight resistance genes into Basmati rice

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Bacterial leaf blight (BB), caused by the bacterium *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), is the major constraint amongst rice diseases in India. CSR-30 is a very popular high-yielding, salt-tolerant Basmati variety widely grown in Haryana, India, but highly susceptible to BB. In the present study, we have successfully introgressed three BB resistance genes (*Xa21*, *xa13* and *xa5*) from BB-resistant donor variety IRBB-60 into the BB-susceptible Basmati variety CSR-30 through marker-assisted selection (MAS) exercised with stringent phenotypic selection without compromising the Basmati traits. Background analysis using 131 polymorphic SSR markers revealed that recurrent parent genome (RPG) recovery ranged up to 97.1% among 15 BC_3F_1 three-gene-pyramided genotypes. Based on agronomic evaluation, BB reaction, aroma, percentage recovery of RPG, and grain quality evaluation, four genotypes, viz., IC-R28, IC-R68, IC-R32, and IC-R42, were found promising and advanced to BC_3F_2 generation.

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1. Introduction

Rice is an important cereal crop in the world and provides more than 21% of the food for the world population and up to 76% of the caloric intake in Southeast Asia [1,2]. Basmati is one of the premium rice groups, cultivated in the northwestern plains of India and eastern Pakistan. There is a marked increase in demand for Basmati rice worldwide for its unique aroma, cooking, and eating qualities [3]. In India, more than 60% of the total Basmati rice is grown in Haryana, and thus the latter is the major Basmati rice cultivating state. India is the leading exporter of Basmati rice and exports US$ 4.86 billion of Basmati rice annually [4]. Aroma, non-stickiness of cooked rice and elongation upon cooking are some of the important traits of Basmati rice. Bradbury et al. [5] showed that a functional BADH2 enzyme inhibits the biosynthesis of 2-acetyl-1-pyrroline, which is a major component of aroma. A fully functional copy of the gene encoding BAD2 is present in non-fragrant varieties, whereas fragrant varieties possess a copy of the gene containing an eight-base pair deletion resulting in a frame shift mutation disabling the BADH2 enzyme. Studies by Kovach et al. [6] also confirmed that BADH2 is the major genetic determinant of fragrance in rice. Amylose content and alkali spread value (ASV) are the most important constitutional indices related to cooking and processing quality of rice [7,8].

BB disease is one of the most devastating diseases effecting Basmati rice acreage in India. BB is caused by the

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Gram-negative proteobacterium, *Xanthomonas oryzae pv. Oryzae*, and causes yield losses ranging from 74% to 81% in severe conditions, depending on the stage of the crop, the cultivar's susceptibility, and the environmental conditions [9,10]. Effective control of disease is limited by the use of chemical control measures and the health issues posed by them [11,12]. The development of varieties carrying resistance genes has been considered to be the most effective way to control the BB disease [13–16]. To date, at least 38 BB resistance genes [27 dominant (*Xa1*, *Xa2*, *Xa3*, *Xa4*, *Xa7*, *Xa10*, *Xa11*, *X12*, *Xa14*, *Xa16*, *Xa17*, *Xa18*, *Xa21*, *Xa22* (t), *Xa23*, *Xa25 (t)*, *Xa26*, *Xa27*, *Xa29*, *Xa30*, *Xa30 (t)*, *Xa31 (t)*, *Xa32 (t)*, *Xa34*, *Xa35 (t)*, *Xa36 (t)*, *Xa38*), and 11 recessive (*xa5*, *xa5(t)*, *xa8*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa28 (t)*, *xa31* and *xa32)*] conferring host resistance against various strains of *Xoo* have been identified [17,18].

Molecular breeding involving MAS addresses the limitations of conventional breeding and allows pyramiding of multiple resistance genes into a single cultivar, thus providing an effective solution to resistance breakdown. Using the gene pyramiding approach, improved rice cultivars with broad spectrum and durable BB resistance have been developed by combining multiple resistance genes [13–16,19–22]. A marker-assisted background selection approach can recover up to 99% of the recurrent-parent genome in just three backcross cycles, whereas conventional breeding takes up to six backcrosses to recover 99% of the recurrent parent genome [23]. BB resistance genes *xa13* and *Xa21* have been introgressed into 'Pusa Basmati 1′ through MAS coupled with phenotypic selection for agronomic, grain, and cooking quality traits [22]. One of the improved genotypes was released as 'Improved Pusa Basmati 1′ for commercial cultivation in 2007, and is one of the first products of MAS to be released in India [24]. In the present study, we employed MAS along with stringent phenotypic selection for the introgression of three BB resistance genes, viz., *Xa21*, *xa13*, and *xa5* into Basmati variety CSR-30. This work will facilitate future efforts to transfer combinations of BB resistance genes into other preferred Basmati rice cultivars from non-Basmati rice cultivars without compromising the Basmati quality characters.

2. Material and methods

2.1. Plant material

The plant material consisted of the Basmati rice (susceptible) variety CSR-30 as a recurrent parent, the (resistant) IRBB-60 variety as a donor parent having BB resistance genes *xa5*, *xa4*, *xa13*, and *Xa21*. Crosses were made between CSR-30 and IRBB-60, and F_1 plants thus obtained were backcrossed with CSR-30. Among the BC_1F_1 population, the foreground selection was performed using polymerase chain reaction (PCR)-based sequence tagged sites (STS) markers linked to the three BB resistance genes *xa5*, *xa13*, and *Xa21*. The BC_1F_1 genotypes were advanced to BC_1F_2 and subjected to foreground selection followed by BB incidence analysis on artificial inoculation and background analysis. A similar strategy was used in the BC_2F_1 to obtain BC_3F_1 populations from which three-gene positive genotypes were selected based on molecular marker analysis and assessed for agronomic performance and disease response at the tillering stage. The BC_3F_1 plants having the three BB resistance genes were selfed and BC_3F_2 seeds thus obtained were used for grain quality evaluation.

2.2. DNA marker analysis and PCR amplification

Mini-scale genomic DNA isolation was carried out using the CTAB extraction method of Murray and Thompson [25] as modified by Saghai-Maroof et al. [26] and Xu et al. [27]. Three STS markers, viz., pTA248, RG136 and RG556 linked to the BB resistance genes, *Xa21*, *xa13* and *xa5*, respectively, were used to confirm the presence of these resistance genes at each backcross generation. The pTA248 marker is 0.2 cM from *Xa21* [28], the RG136 marker is 3.8 cM from *xa13* [29], and the RG556 marker is 1.7 cM from *xa5* [30] (Table 1). PCR was carried out in 20-μl reaction mixtures containing 50 ng of genomic DNA, 2 units of Taq DNA polymerase, 1X PCR Buffer (10 mM Tris HCL, 1.5 mM $MgCl_2$), 100 μM each of dNTPs, and 10 μM of each primer. The template DNA was initially denatured at 94 °C for 5 min, followed by 30 cycles of PCR amplification with the following conditions: 30 s of denaturation at 94 °C, 1 min of annealing at 55 °C, and 1 min of primer extension at 72 °C followed by final extension at 72 °C for 10 min. The amplified product of pTA248 was electrophoretically resolved on 1.5% agarose gel containing 0.5 μg/ml of ethidium bromide in a 1.0 X TBE buffer and visualized under UV light. For the amplified products of RG136 and RG556, 5 μl of the PCR product were used for gel electrophoresis to check DNA amplification. The remaining PCR product was used for restriction digestion. The reaction mixture used for the digestion of the PCR product with the respective restriction enzyme consisted of 0.3 μl (10 U/μl) of restriction enzyme *Hinf1* for

Table 1
Molecular markers used for marker-assisted selection of BB resistance genes and aroma gene.

Molecular marker	R genes for BB/*fgr* for aroma	Chromosome	primer sequences (5′–3′)	Reference
pTA248 (0.2cM)	*Xa21*	5	F:5′AGACGCGGAAGGGTGGTTCCCGGA3′ R:5′AGACCGGTAATCGAAAGATGAAAA3′	Yoshimura et al. [30]
RG136 (3.8cM)	*xa13*	8	F:5′TCCCAGAAAGCTACTACAGC3′ R:5′GCAGACTCCAGTTTGACTTC3′	Zhang et al. [29]
RG556 (1.7cM)	*xa5*	11	F:5′TAGCTGCTGCCGTGCTGTGC3′ R:5′AATATTTCAGTGTGCATCTC3′	Ronald et al. [28]
BAD2	*fgr*	8	F:5′TTGTTTGGAGCTTGCTGATG3′ F:5′CTGGTAAAAAGATTATGGCTTCA3′ R:5′CATAGGAGCAGCTGAAATAATACC3′ R:5′AGTGCTTTACAAAGTCCCGC3′	Bradbury et al. [5]

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