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Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding

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ABSTRACT

Pusa RH10, a popular high yielding superfine grain aromatic rice hybrid and its parents Pusa6A and PRR78 are highly susceptibility to blast disease. Marker assisted backcross breeding (MABB) approach was employed to incorporate blast resistance genes *viz*., *Piz-5* and *Pi54*, from the donor lines C101A51 and Tetep into the genetic background of PRR78 to develop Pusa1602 (PRR78 + *Piz5*) and Pusa1603 (PRR78 + *Pi54*), respectively. Foreground selection for the genes *Piz-5* and *Pi54* was effected using tightly linked molecular markers, AP5930 and RM206, respectively in two independent backcross series. Further, foreground selection was coupled with stringent phenotypic selection for agronomic, grain and cooking quality traits, to accelerate recurrent parent phenome recovery. Five superior BC_2F_2 plants homozygous from each of the backcross series were selected and advanced to BC_2F_5 generation through pedigree selection to develop improved versions of PRR78 with blast resistance. Background analysis revealed the recurrent parent genome recovery up to 89.01% and 87.88% in Pusa1602 and Pusa1603 lines, respectively. The hybrids produced by crossing Pusa6A with improved lines of PRR78, were on par with original Pusa RH10 in terms of yield, grain and cooking quality traits with an added advantage of blast resistance.

1. Introduction

Rice is the staple food crop of India with an annual production of 110 mt. To meet the demand of ever increasing population, the annual production of rice needs to be enhanced to 125 mt by 2020. With the constraints imposed by static area, limited water and other resources, in addition to climate change, the sustainability of the production is a gigantic task. Exploitation of hybrid rice technology is a practically feasible approach to increase the rice yields by 15–20% over that of semi-dwarf high yielding varieties (Virmani, 1996). Among the 47 rice hybrids released for commercial cultivation in India, Pusa RH10 developed at Indian Agricultural Research Institute (IARI), New Delhi during 2001, occupies a unique status, being the first and the only hybrid released in the superfine grain aromatic category. Pusa RH10 is an early maturing (115 days) hybrid with very high per day productivity, thereby yielding an average yield of 6.5 t/ha. Despite the advantages, Pusa RH10 and its parental lines, Pusa6A (CMS line) and PRR78 (an elite Basmati rice restorer line), are highly susceptible to rice blast disease caused by the fungus *Magnaporthe oryzae*.

Rice blast being the devastating disease, is an important limiting factor for rice cultivation world-wide which causes annual losses of up to 50% (Scardaci et al., 1997) and in the Basmati belt of north India, the economic loss due to blast is high as the quality of the harvested grain is also affected in addition to actual yield loss (Variar et al., 2009). This constraint can be effectively addressed by developing cultivars with built in resistance. Till date, 85 blast resistance genes have been identified and the genes *Piz-5* and *Pi54* were reported to provide broad spectrum resistance. *Pi54* was identified and mapped to chromosome 11L in a rice variety Tetep and

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was reported to govern resistance against predominant races of the blast pathogens in India (Sharma et al., 2010). Similarly, the gene *Piz-5*, was mapped on to chromosome 6, close to the centromere in the rice genotype A5173. *Pi54* and *Piz-5* are reported to encode proteins containing NBS-LRR domain (Fjellstrom et al., 2006; Zhou et al., 2006). Therefore, we utilized these genes in marker assisted breeding program to develop blast resistant PRR78 lines. Marker-assisted selection (MAS) has been advocated as a highly efficient breeding method as it makes possible rapid and precise selection of the targeted gene (Tanksley et al., 1989). Marker-assisted backcross breeding (MABB), which involves two steps: (1) MAS for the gene of interest, known as foreground selection and (2) MAS for recovery of the recurrent parent genome, known as background selection (Hospital et al., 1992), is the most effective way of transferring specific gene(s) to an otherwise agronomically superior variety or parental lines. In rice, the feasibility of MABB to pyramid bacterial blight resistance genes has been well demonstrated (Joseph et al., 2004; Luo et al., 2005; Gopalakrishnan et al., 2008; Sundaram et al., 2008, 2009)

Earlier in our lab, we incorporated two BB resistance genes, *xa13* and *Xa21* into PRR78 through MABB (Basavaraj et al., 2010). The present study was aimed at incorporation of major blast resistance genes, *Piz-5* and *Pi54* in the genetic background of an elite Basmati rice restorer line PRR78 through MABB, and evaluation of different hybrid combinations involving the improved versions of PRR78 as a restorer lines for agronomic performance, grain and cooking quality traits and reaction to blast.

2. Materials and methods

2.1. Plant materials and breeding strategy

PRR78, a Basmati quality restorer line of Pusa RH10 carrying restorer gene *Rf1* was used as the recurrent parent for incorporation of blast resistance genes from two donors namely, C101A51 (*Piz-5*) and Tetep (*Pi54*). The gene linked SSR markers AP5930, RM206 and RM6100 were used to identify the *Piz-5*, *Pi54* and *Rf1* genes. The recurrent parent PRR78, was crossed as female parent with the donors, C101A51 and Tetep in independent backcross breeding programmes, and the lines derived thereof were named as Pusa1602 and Pusa1603. The single F_1 plant positive for the respective genes were backcrossed with PRR78 to produce the BC_1F_1 seeds. Foreground selection in the BC_1F_1 generation for the genes *Piz-5* and *Rf1* in Pusa1602; and *Pi54* and *Rf1* in Pusa1603 was performed using gene linked markers. The gene positive plants with maximum phenotypic similarity to the recurrent parent were backcrossed to PRR78 to generate BC_2F_1 seeds independently in each of the backcrosses. Foreground and phenotypic selections were carried out to select five elite plants from each backcross series to produce $BC_2F_{2:5}$ progenies following pedigree selection.

2.2. Screening for blast resistance

2.2.1. Artificial inoculation

Twenty-five isolates of *M. oryzae* obtained from infected rice blast samples collected from Basmati growing regions (Haryana, Uttar Pradesh and Uttarakhand) were used for virulence analysis on 24 monogenic blast differential lines (Sarkhel, 2010). On the basis of blast reaction pattern, four highly virulent isolates (Table 1) compatible with maximum number of *R*-genes were selected for screening the improved lines, Pusa1602, Pusa1603 and the derived hybrid combinations. The details of the four virulent isolates and their virulence spectra in monogenic blast differentials are presented in Table 2.

Table 1
Four most virulent isolates of *M. oryzae* used in screening for blast resistance under artificial inoculation.

S. no.	Isolate	Place of collection	State
1.	Mo-ni-007	Kaul	Haryana
2.	Mo-ni-012	Saharnpur	Uttar Pradesh
3.	Mo-ni-018	Jhuppa, Gandhi Budh Nagar	Uttar Pradesh
4.	Mo-ni-019	Kichha, Udham Singh Nagar	Uttara Khand

Twenty-one-day-old seedlings of selected families homozygous for individual blast resistance genes were artificially screened against the four most virulent blast isolates. Stored cultures of each pathogen isolate were revived by inoculating the colonized filter discs on oat meal agar slants. The inocula were prepared following the standard procedures established by Bonman et al. (1986). About 30–40 ml of the spore suspension containing gelatin (0.1%) and Tween-20 (0.02%) was sprayed onto seedlings using a glass atomizer. Inoculated seedlings were kept in a humid chamber with the temperature maintained at $25 \pm 1\,^{\circ}C$. The distilled water was sprinkled three to four times a day to maintain high humidity. The disease reaction of each rice line was recorded 7 days after inoculation using a 0–5 disease scoring scale (Bonman et al., 1986). The plants exhibiting 0–2 reaction were considered as resistant, reaction of 3 were considered moderately resistant while those showing 4–5 reaction were categorized as susceptible. The resistant genotypes were further field tested in hot spot locations.

2.2.2. Field screening for blast resistance in Uniform Blast Nursery (UBN)

All the promising lines were evaluated for their reaction to blast under Uniform Blast Screening Nursery (UBN) at two disease hotspot locations *viz.*, Agricultural Research Station (ARS), Mugad, Karnataka and Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand. A 50 cm row of each entry was planted in an upland nursery bed with a row spacing of 10 cm. A row of susceptible check was interplanted after every five entries and also on the borders to ensure uniform spread of the disease. Data on blast reaction of the entries were recorded thrice following 0–9 scale (SES, 1996) at 10 days interval starting after 30 days of sowing. The lines with a score of 0–3 were considered as resistant, 4–5 as moderately resistant, 6 as moderately susceptible and 7–9 as susceptible.

2.3. Molecular marker analysis

2.3.1. Marker assisted foreground selection

The marker AP5930 linked to *Piz-5* (Fjellstrom et al., 2006) and RM206 linked to *Pi54* (Sharma et al., 2005) were used for foreground selection in backcrossed and selfed generations. Since the recurrent parent PRR78 is a restorer line, a microsatellite marker RM6100 linked with fertility restorer (*Rf1*) gene in PRR78 (Prakash, 2003) was employed for selection of *Rf1* gene. The markers used and their primer sequences, chromosomal locations and linkage distance between marker and respective genes are presented in Table 3.

2.3.2. Marker assisted background analysis

A set of 435 STMS markers at 5 cM interval spanning uniformly across the 12 rice chromosomes with an average of 36.25 markers per chromosome, were used for polymorphism survey between the recurrent parent, PRR78 and resistance gene donors, Tetep and C101A51. The primer sequences for STMS markers were adapted from the Gramene SSR marker resource (www.gramene.org) and custom synthesized (Sigma Technologies Inc., USA).