Development of host plant resistance in an elite maintainer line DRR17B for Bacterial Blight and Blast through marker assisted backcross selection

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ABSTRACT

Marker assisted backcross breeding (MABB) is a promising strategy for improvement of elite crop varieties and hybrids for one or more agronomical traits with minimal linkage drag. DRR17A is a medium duration, wild-aborutive cytoplasmic male sterile (WA-CMS) line, possessing desirable, medium slender grain type and is being used for developing a few promising pipeline hybrids at Indian Institute of Rice Research (IIRR), Hyderabad, India. As DRR17A and its maintainer line, DRR17B are highly susceptible for bacterial blight (BB) and blast diseases, we attempted improve disease resistance of DRR17B by adopting MABB strategy. A breeding line in the genetic background of Samba Mahsuri, FBR1-15 possessing the bacterial blight resistance gene, Xa33 and C101A51 possessing the blast resistance gene, Pi2 served as donor lines in two separate backcrosses in which DRR17B served as the recurrent parent. At each backcross generation, plants possessing Xa33 or Pi2 in heterozygous condition were identified with help of gene-specific markers and backcrossing was continued till BC2 generation. At BC2F2, a promising backcross plant each from the two sets of backcrosses, possessing either Xa33 or Pi2 in homozygous condition were intercrossed to combine the two genes in the background of DRR17B. Homozygous lines possessing both Xa33 and Pi2 were identified with the help of gene specific markers at ICF2 generation and advanced by pedigree method till ICF5 generation. The gene pyramid lines of DRR17B possessing BB and blast resistance are being evaluated for disease resistance, yield and agro-morphological parameters.

Introduction:

Hybrid rice technology is one of the most feasible options to increase the rice yield in the coming decades. Hybrids are known to give 15-20 % higher yield over inbreds (Hari Prasad et al., 2014). Even though about 2.5 Mha are under cultivation with hybrid rice in India, the technology is not spreading rapidly due to various reasons like susceptibility of hybrids to many biotic stresses, poor grain quality, problems in timely seed production and delivery etc. (Guohui et al., 2014, Hari Prasad et al., 2014). Among these biotic stresses like bacterial blight (BB) and blast reduce yield of Indian rice hybrids significantly. Deploying host plant resistance is one of the most effective strategies for management of BB and blast (Yoshimura et al., 1995).

BB disease caused by Xanthomonas oryzae pv. oryzae (Xoo), is one of the most destructive diseases in rice (Mew 1987), in its severe form, is known to cause yield losses ranging from 74 to 81% (Srinivasan and Gnanamanickam, 2005). Till date at least 38 genes conferring resistance to BB have been identified (Sundaram et al., 2014), many of them have been tagged and mapped with closely linked markers (Divya et al., 2015). Xa33 gene was mapped on Chromosome 7 with two tightly linked markers WR7.1 and WR 7.6 (Natrajkumar et al., 2012), where it is showing the broad spectrum resistance most of the BB isolates. Similar to BB, rice blast disease caused by the fungus Magnaporthe oryzae (anamorph Pyricularia oryzae), is also one of the major threats for rice production, leads to significantly high as 70–80% yield lose during an epidemic (Khush and Jena 2009). Nearly, 100 genes conferring resistance against blast disease and 347 quantitative trait loci (QTL) associated with blast resistance have been identified and 19 resistance genes have been cloned and characterized (Ballini et al., 2008, Koide et al., 2009). A major blast resistance gene Pi2 was mapped on chromosome 6 (Yu et al., 1991) and very closely linked markers are available for the gene for use in marker-assisted selection (Fijelstorm et al., 2006).

DRR17A is an elite and wild-aborutive cytoplasmic male sterile line (WA-CMS) with fine grain type developed by India Institute of Rice Research (IIRR), Hyderabad, India. This WA-CMS line where using to develop a new hybrids with fine grain quality. But the DRR17A and its maintainer line DRR17B are highly susceptible for BB and blast diseases. The present study was initiated to improve DRR17B for BB and blast resistance by introducing Xa33 and P2 genes through MABB strategy by using gene linked markers.

Materials and Methods:

Parent material: FBR1-15, a breeding line of Samba Mahsuri served as a donor for Xa33 (Natraj et al., 2012) gene and....
C101A51 served as the donor for P2 (Yu et al., 1991) gene. DRR17B, a maintainer line of wild-abortive cytoplasmic male sterile (WA-CMS) line DRR17A served as the recurrent parent. The cultivar, TN1 was used as susceptible check for BB resistance screening, while HR-12 was used as susceptible check for blast resistance screening.

**Crossing program:** Two separate crosses were made between FBR1-15 X DRR17B and C101A51 X DRR17B. The heterozygosity of the F2s derived from the two crosses was confirmed by using the PCR-based gene linked marker for P2, AP5659-5 (Fiellstorm et al., 2006) and another two-PCR based flanking marker for Xa33, WR7.1 and WR7.6 (Natrajkumar et al., 2012). PCR protocols prescribed in Fiellstorm et al. (2006) and Natrajkumar et al. (2012) were adopted for the gene-linked markers for P2 and Xa33, respectively (Table 1). The true F2s were used for backcrossing to obtain BC1F1s, which were then genotyped using gene-linked markers specific for either Xa33 or P2 and a single positive plant, which was similar to DRR17B in phenotype was then backcrossed to the recurrent parent generate BC2F1 plants. They were then subjected for marker-assisted and phenotype based selection as described earlier and the process was continued up to BC5 generation. The confirmed BC5F2 plants possessing either Xa33 or P2 derived from the two crosses were crossed to generate intercross ICFF plants (i.e. ICF1), which were then selfed to generate ICF2 plants. Homozygous ICF2 plants possessing Xa33 and P2 were then identified using gene-linked markers and further selections were done based on morphological characters through pedigree method.

**Table 1 should come here**

**Phenotypic screening for BB resistance:** Two virulent isolates of the bacterial blight pathogen, Xanthomonas oryzae pv. oryzae (Xoo) collected from BB hot-spot locations in India, viz. DX-020 (Hyderabad, Telangana State, India) and DX-066 (Raipur, Chhattisgarh State, India) were used to screen ICF2F2 progenies of DRR17B along with donor and recurrent parents 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 10⁵ cfu/ml at maximum tillering stage (45–55 days after transplanting) through the methodology of Kauffman et al. (1973).

**Phenotypic screening for blast resistance:** A local isolate of Magnaporthe oryzae (SPI-40) from Indian Institute of Rice Research (IIRR), Hyderabad, Telangana State, India; Madhan Mohan (2011), was used to screen the donor and recurrent parents along with inter cross derived lines of DRR17B for blast resistance under in vivo conditions following uniform blast nursery (UBN) method at Indian Institute of Rice Research (IIRR), Hyderabad, India. The pathogen strains were cultured and stored as described by Srinivas Prasad et al. (2011). 1 x 10⁶ conidia/ml of the fungal conidial suspension at a concentration were used for inoculation to young seedlings at four-leaf stage and maintained high relative humidity for disease development. One week later the inoculated seedlings were monitored for the development of blast lesions. The plants were scored and evaluated on a 0–9 scale as per IRRI-SES scale (IRRI 1996).

**Results:** Deploying the BB and blast genes in to DRR17B background by MABB:

Total of 12 & 9, 32 &45 and 27& 22 positive plants were identified in F2, BC1F1, and BC2F1 generation respectively by using gene linked markers of Xa33 and P2 genes separately (Fig.1). The positive BC2F1 plants were allowed to selfing to get homozygous plants for target genes. The two positive homozygous plants (RMS-A- 4-18-7-187: for Xa33 and RMS-B-7-25-9: for P2) were selected, both for Xa33 and P2 genes with gene linked markers and which were phenotypically almost similar to DRR17B parent. Those two plants were used for inter crossing to get both genes in single plant. 25 inter cross (IC) F1 plants were raised and 12 plants were identified positive for both genes and were allowed to self to get ICF1 plants. 273 ICF1 plants raised and genotyped, 11 homozygous double positive (i.e. Xa33Xa33 + P2P2) plants were identified (Figure 1). These 11 plants seeds were subjected to phenotypic BB and blast screening in next season.

**Figure 1 should come here**

**Phenotypic screening of BB and blast disease resistance in inter cross derived lines:** Inter cross derived lines was phenotypically screened for their resistance to BB and blast disease in glass house condition. The blast resistance check C101A51 having P2 gene showed disease score of 1, and the susceptible checks DRR17B and HR-12 showed score of 9. Whereas inter cross derived lined showed the score of 1 equal to C101A51 (Table 2). With respect to BB screening the resistance check FBR1-15 having Xa33 gene showed immune level of resistance against to DX-066 isolate whereas 3 score against to DX-020 isolate(data not shown) and the susceptible checks DRR17B and TN1 showed score of 9 (Table 2). The inter cross derived lines showed immune level of resistance and 3 score with respective to DX-066 and DX-020 isolates, where these score are equal to resistance check.

**Table 2 should come here**

**Discussion:** Breeding through conventional means for disease resistance is time consuming, laborious and mostly dependent on environment condition, as compared to marker-assisted breeding. Among the strategies, available for MABB, marker assisted backcross breeding (MABB), which is simple, highly efficient and precise strategy is very helpful in targeted improved 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 utilised (Sundaram et al., 2008, 2009, Basavaraj et al., 2010, Zhou et al., Singh et al., 2012, Hari et al., 2011, 2013). DRR17B is highly susceptible for BB and blast disease. Incorporating BB and blast resistance genes through MABB in to DRR17B provides disease resistance. Earlier, Sundaram et al., (2008 and 2009), successfully introduced three BB resistance genes (i.e. Xa21, xa13 and xa5) into the genetic background of Samba Mahsuri and Triguna varieties trough MABB. Later Hari et al., (2011 and 2013) improved hybrid rice parental lines KMR3R and IR58025B for BB and blast by Xa21 and P54 genes. Singh et al. (2012) improved the restorer line named PRR78, possessing Basmati grain quality, for blast by incorporating P25 and P54 using MABB.

In the present study, we have successfully introgressed Xa33 and P2 genes in to the background of DRR17B (Figure 1) through marker assisted backcross breeding (MABB). In this process, at each backcross generation, gene-specific markers were used for foreground selection, one of the most vital processes in MABB (Hospital and Charcosset, 1997) and the best homozygous plants were identified through foreground selection coupled with phenotype based selection against local, virulent isolates of the pathogens (Table 2). The resistance levels of inter cross derived plants were observed to be identical to the donor and resistance check with respect to screening against both bacterial blight and blast confirming that the gene combination, Xa21 + Xa33 + P2 confers a high level of resistance.
against both the diseases. Further, plants possessing similar agro-morphological and grain quality characters similar or better than DRR17B were also obtained, indicating that the strategy of coupling marker-assisted foreground selection with phenotype-based background selection is highly successful in not only identifying backcross plants similar to the recurrent parent, but also those which are better than DRR17B.

We are in the process of crossing the selected improved lines of DRR17B lines with elite WA-CMS line, IR58025A to assess their maintainer ability. Those improved lines of DRR17B, which show complete sterility when crossed with IR58025A (i.e. complete maintainer ability) will then be crossed with elite restorer lines like RPHR1005 to assess heterosis of the newly derived hybrids and also to assess their bacterial blight and blast resistance. Those improved lines of DRR17B possessing good maintainer ability and also show good heterosis in test crosses with selected restorer lines will be converted to CMS lines through intensive marker-assisted backcrossing using resistance gene specific markers in future for development of elite disease resistant, heterotic hybrids.

Table 1: Markers used in the foreground selection (Xa33 and Pi2) their details information. List of primers which were used in screening of target genes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Primer name</th>
<th>Target Gene</th>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WR 7.1</td>
<td>Xa33 (Natraj et al, kumar2012)</td>
<td>7</td>
<td>Natrajkumar et al. 2012</td>
</tr>
<tr>
<td>2</td>
<td>WR 7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AP 5659-5</td>
<td>Pi-2 (Yu et al. 1991)</td>
<td>6</td>
<td>Fijellstorm et al. 2006</td>
</tr>
</tbody>
</table>

Table 2: Phenotypic screening of selected intercross derived lines to check the resistance levels for BB and blast disease

<table>
<thead>
<tr>
<th>S.No</th>
<th>Cross designation</th>
<th>BB score* (DX-066)</th>
<th>Blast score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RMS IC 14-3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>RMS IC 14-27</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>RMS IC 14-29</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>RMS IC 14-54</td>
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<tr>
<td>5</td>
<td>RMS IC 14-63</td>
<td>1</td>
<td>1</td>
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<tr>
<td>6</td>
<td>RMS IC 14-87</td>
<td>1</td>
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<td>7</td>
<td>RMS IC 14-129</td>
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<td>RMS IC 14-144</td>
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<td>11</td>
<td>RMS IC 14-245</td>
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<tr>
<td>12</td>
<td>FBR1-15</td>
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</tr>
<tr>
<td>13</td>
<td>C101A51</td>
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<td>1</td>
</tr>
<tr>
<td>14</td>
<td>DRR17B</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

* A total of twenty plants from each of the backcross derived lines, the donor and recurrent parents were screened with the Xoo isolate DX 066 under glass house conditions and lesion length (cm) was calculated (I:Immune) as an average of five leaves per plant.

* A total of 40-50 seedlings from each of the backcross derived lines, the donor and recurrent parents were screened in the Uniform Blast Nursery (UBN) available at DRR and disease score was calculated as per IRRI-SES (IRRI 1996).

Figure 1 Foreground selection for Xa33 and Pi2 genes in IC1F2 plants through PCR based markers

REFERENCES