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-abortive 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 rice hybrids 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 dis-

ease, 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 (Ballini *et al.*, 2008, Koide *et al.*, 2009). A major blast resistance gene *Pi2* was mapped on chromosome 6 (Yu *at al.*, 1991) and very closely linked markers are available for the gene for use in marker-assisted selection (Fijellstorm *et al.*, 2006).

DRR17A is an elite and wild-abortive 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 *Pi2* 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 *Pi2* (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 F_1s derived from the two crosses was confirmed by using the PCR-based gene linked marker for *Pi2*, AP5659-5 (Fijellstorm *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 Fijellstorm *et al.*, (2006) and Natrajkumar *et al.*, (2012) were adopted for the gene-linked markers for *Pi2* and *Xa33*, respectively (Table.1). The true F_1s were used for backcrossing to obtain BC_1F_1s, which were then genotyped using gene-linked markers specific for either *Xa33* or *Pi2* and a single positive plant, which was similar to DRR17B in phenotype was then backcrossed to the recurrent parent generate BC_2F_1 plants. They were then subjected for marker-assisted and phenotype based selection as described earlier and the process was continued up to BC_2 generation. The confirmed BC_1F_1 plants possessing either *Xa33* or *Pi2* derived from the two crosses were crossed to generate intercross ICF_1 plants (i.e. ICF_1), which were then selfed to generate ICF_2 plants. Homozygous ICF_2 plants possessing *Xa33* and *Pi2* 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 ICF_5 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 10^{8-9} 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 10^5 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 F_1, BC_1F_1 and BC_2F_1 generation respectively by using gene linked markers of *Xa33* and *Pi2* genes separately (Fig.1). The positive BC_2F_1 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-7-25-9-83: for *Pi2*) were selected, both for *Xa33* and *Pi2* 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) F_1 plants were raised and 12 plants were identified positive for both genes and were allowed to self to get ICF_2 plants. 273 ICF_2 plants raised and genotyped, 11 homozygous double positive (i.e. *Xa33Xa33* + *Pi2Pi2*) 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 *Pi2* 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 respective 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 MAB, 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.*,.2011, 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 *Pi54* genes. Singh *et al.*, (2012) improved the restorer line named PRR78, possessing Basmati grain quality, for blast by incorporating *Piz5* and *Pi54* using MABB.

In the present study, we have successfully introgressed *Xa33* and *Pi2* 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 for agro-morphological traits and for further back crossing/selfing. When stable, inter-cross derived lines possessing *Xa33* and *Pi2* were identified in a homozygous condition in ICF_2 generation (Figure. 1), they were confirmed for their resistance through phenotype based screening 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* + *Pi2* 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.

S.No	Primer name	Target Gene	Chromosome	Reference
1	WR 7.1	*Xa33* (Natraj et al. kumar2012)	7	Natarajkumar et al. 2012
2	WR 7.6			
3	AP 5659-5	*Pi-2* (Yu et al. 1991)	6	Fijellstorm et al. 2006

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

S.No	Cross designation	BB score[#] (DX-066)	Blast score[*]
1	RMS IC 14-3	I	1
2	RMS IC 14-27	I	1
3	RMS IC 14-29	I	1
4	RMS IC 14- 54	I	1
5	RMS IC 14-63	I	1
6	RMS IC 14-87	I	1
7	RMS IC 14-129	I	1
8	RMS IC 14-144	I	1
9	RMS IC 14-193	I	1
10	RMS IC 14-227	I	1
11	RMS IC 14-245	I	1
12	FBR1-15	I	1
13	C101A51	-	1
14	DRR17B	9	9

[#] 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 Xa33and Pi2 genes in IC1F2 plants through PCR based markers
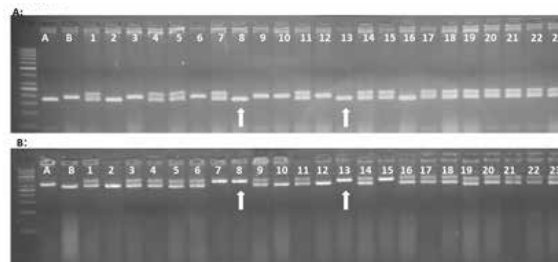
A. Gel shows the screening of *Xa33* gene with WR7.6 PCR based marker in IC_1F_2 population.
B. gel sows the screening of IC_1F_2 population for *Pi2* gene with AP 5659-5 marker.
Arrows indicates that homozygous double positive plants for both the dominant alleles. A total 273 plants were screened for their homozygous dominant alleles for both the genes.
M – Marker, A – Donor parent(resistant allele) and B – recurrent parent (susceptible allele)

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