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IMPROVEMENT OF AN ELITE MAINTAINER LINE DRR17B FOR BACTERIAL BLIGHT AND GALL MIDGE RESISTANCE THROUGH MARKER ASSISTED SELECTION.

BALACHIRANJEEVI CH[1], BHASKAR NAIK S[1], ABHILASH V[1], MAHADEVA SWAMY HK[1], HARIKA G[1], HAJIRA SK[1], PRANATHI K, ANILA M, DILIPKUMAR T, REKHA G, HARI PRASAD AS[1], LAHA G S[1], MADAV MS[1], ARCHANA GIRI[2], SUNDARAM RM[1*].

Indian Institute of Rice Research, Hyderabad, India-500030.
Jawaharlal Nehru technological University, Hyderabad, India- 500085.

ABSTRACT

DRR17A is a stable, wild-abortive cytoplasmic male sterile line possessing fine-grains and developed by Indian Institute of Rice Research (IIRR), Hyderabad. DRR17A and its maintainer line DRR17B and highly susceptible to bacterial blight (BB) disease and the insect pest, gall midge. In order to address this problem, we have introgressed two dominant genes, Xa21 (for BB resistance) and Gm4 (for gall midge resistance) in the genetic background of DRR17B through Marker-assisted backcross breeding (MABB). A breeding line in the background of Samba Mahsuri (RPBio Ent-2) possessing Xa21 and Gm4 served as donor for both the genes. Molecular markers specific for the target genes were used at each backcross generation to identify plants possessing the target genes and backcrossing was continued till BC_3 generation. At BC_3F_2 generation, plants homozygous for both the genes and closely resembling DRR17B were identified and advanced. At BC_3F_4 generation, five elite lines possessing both Xa21 and Gm4 were screened against bacterial blight under glass house condition the lines were subjected to screening for resistance. All the five lines were observed to show high level of resistance against BB and possessed yield, grain quality and plant type similar to DRR17B and we are in the process of converting them to WA-CMS lines through marker-assisted breeding.

INTRODUCTION

Rice is the staple food crop for the large part of world's human population, especially in East, South and Southeast Asia making it the most consumed cereal grain. In upcoming decades, the productivity of rice need to improved to meet the demands of an ever-increasing population. In order to meet this demand, hybrid rice technology has been advocated as one of the most feasible options (Ahmed and Siddiq 1998). Rice hybrids generally possess a yield advantage of 15-20% over varieties (Hariprasad et al. 2014). However, hybrid rice cultivation is not spreading rapidly across India, due to various reasons like their high susceptibility to many biotic stresses, poor grain quality, problems in timely seed production and delivery etc. (Hari Prasad et al. 2014). Particularly diseases like bacterial blight (BB), blast and pests like BPH and gall midge limit not only yield of rice hybrids, but also reduce seed yields of rice hybrids and their parental lines. Indian Institute of Rice Research (IIRR) has developed an elite, stable wild-abortive cytoplasmic male sterile (WA-CMS) line, named DRR17A, having very good combining ability and hence it is being used extensively for hybrid development pipeline. However, the CMS lines and its maintainer parent (DRR17B) are highly susceptible to most of the rice pests and diseases including bacterial blight (BB) and gall midge. It is hence necessary to enhance resistance of DRR17B and DRR17A against multiple biotic stresses.

Improvement of host plant resistance is a promising strategy which is eco-friendly, yet effective and sustainable to tackle the pests and diseases in rice (Sundaram et al. 2014). Although the present day rice varieties and hybrids some level of tolerance against various biotic stresses, the chances that they possess multiple biotic stress resistance are highly unlikely (Sundaram et al. 2008). Hence there is a need to not only develop cultivars with horizontal resistance against a particular pest or disease, but also against multiple biotic stresses (Divya et al. 2015). Multiple pest resistance is not a new concept, but the advent of reliable PCR based markers has made the process of combining of genes (i.e. gene Pyramiding) easier through marker-assisted Selection (MAS). Earlier, we demonstrated the utility of marker-assisted backcross breeding (MABB) in targeted introgression of a major BB resistance gene, *Xa21* and a major blast resistance gene, *Pi54* into DRR17B (Balachiranjeevi et al. 2015). In order to enhance the spectrum of resistance against BB and to add a new dimension of resistance against gall midge in DRR17B, the present study was carried out with the objective of pyramiding another major BB resistance gene, *Xa33* and a major gall midge resistance gene, *Gm4* into DRR17B through MABB.

MATERIALS AND METHODS

Plant materials

A breeding line in the background of Samba Mahsuri RP Bio Ent-2 carrying *Xa21* and *Gm4* served as donor and DRR17B was used as the recurrent parent. Improved Samba Mahsuri and Abhaya were used as resistance check for resistance against BB and gall midge, respectively, while TN1 was used as susceptible check for both the biotic stresses.

Strategy for marker-assisted introgression of *Xa21* and *Gm4* into DRR17B:

After crossing RP Bio Ent-2 with DRR17B, the F_1s obtained were conformed for heterozygozity using the *Xa21* specific marker, pTA248 (Ronald et al. 1992). True 'F_1' plants were then backcrossed with DRR17B to obtain BC_1F_1 generation, which were then screened with pTA248 and another marker RM22562 (Himabindu et al. 2010), which is tightly linked to *Gm4*. P BC_1F_1 plants which were heterozygous for both *Xa21* and *Gm4* were then grown in pots to identify the best gene-positive' plant which closely

resembles DRR17B, based on morphological traits. The best plant thus selected was then backcrossed with DRR17B and the marker-assisted backcross breeding process (with phenotype based selection for DRR17B associated traits) was continued till BC_3 generation. A single 'gene-positive' BC_3F_1 plant, which closely resembles DRR17B was then identified and selfed to generate BC_3F_2 plants, which were then screened with the gene-specific markers to identify those which are homozygous for both the target genes (i.e. *Xa21Xa21* + *Gm4Gm4*). Such plants were then advanced through selfing for further evaluation of agro-morphological traits and resistance against BB and gall midge.

Phenotypic Screening for disease resistance:

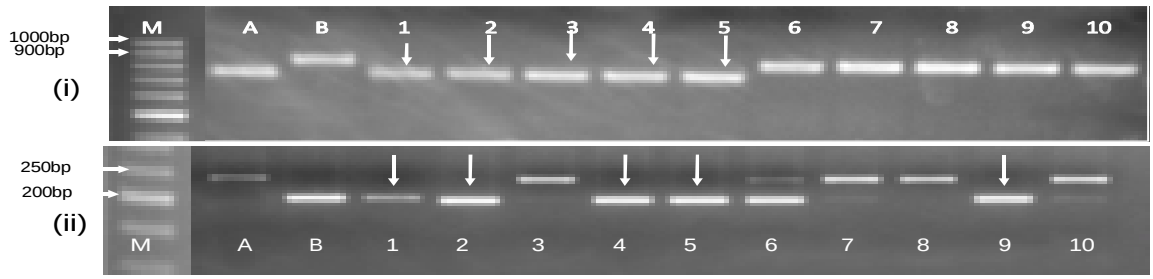
BB screening: A virulent isolate of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from BB hot-spot location in India, *viz*. DX-020 (Hyderabad, Telangana State, India) was used to screen BC_3F_4 progenies of DRR17B along with donor and recurrent parents for BB resistance under both glasshouse and field conditions. The *Xoo* strain was 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). In addition to measurement of BB lesion length, the disease score was also calculated as per IRRI standard evaluation system (IRRI-SES) scale, which is based on percent diseased leaf area (IRRI 1996).

RESULTS

Introgression of BB and gall midge resistance genes in to the background of DRR17B through MAS:

A total of 54 plants were generated from the cross RPBioEnt-2/DRR17B, of which 44 were observed to be heterozygous, i.e. 'true' F_1 plants, which were then selfed to generate BC_2F_2s. A total of 72 BC_1F_1 plants were raised and screened, 18 plants were positive (i.e. heterozygous) for both the genes. Among these a single plant (# RMSBG37), which closely resembled DRR17B was selected visually and backcrossed to generate BC_2F_1s. Among 83 BC_2F_1 plants, 17 were identified to be positive for both *Xa21* and *Gm4.* Through marker-assisted selection, coupled with visual selection, a single 'best' BC_2F_1 plant (# RMSBG37-19) was identified and backcrossed to generate BC_3F_1s. Out of total of 94 BC_3F_1 plants screened, 24 were double positive and among these, a single plant (# RMSBG37-19-8) was selected (based on visual selection as described earlier) and selfed. Out of a total of 348 BC_3F_2 plants screened, 15 plants possessed both the target resistance genes in homozygous condition (i.e. *Xa21Xa21* + *Gm4Gm4;* Figure 1). Among these, two plants (viz., RMSBG 37-19-8-102 and RMSBG 37-19-8-237) which were phenotypically closer to DRR17B were advanced for selfing up to BC_3F_4 generation. At BC_3F_4 generation five elite lines were selected (RMSBG 37-19-8-102-65-22-9, RMSBG 37-19-8-102-65-22-3, RMSBG 37-19-8-237-8-142-27, RMSBG 37-19-8-237-8-142-63 and RMSBG 37-19-8-237-8-142-69; hereafter called advanced backcross derived lines-ABLs) and advanced further.

Figure. 1 : Fore ground selection for *Xa21* and *Gm4* genes in BC_3F_2 plants through PCR based markers



(i) Gel shows the screening of *Xa21* gene with pTA248 PCR based marker in BC_3F_2 population.
(ii) Gel sows the screening of BC_3F_2 population for *Gm4* gene with RM 22562 marker.
Arrows indicates that homozygous double positive plants for both the dominant alleles. A total 348 plants were screened for their homozygous dominant alleles for both the genes.
M – Marker, A – recurrent parent(susceptible allele) and B – Donor parent (resistant allele)

Phenotypic evaluation of advanced backcross derived lines (ABLs) for BB resistance:

The recurrent parent, DRR17B showed high susceptibility to the disease with a lesion length of > 20 cm, while the donor, RPBio Ent-2 was observed to be highly resistant with a lesion length ranging from 1-3 cm. All the Five ABLs showed a high level of resistance to BB with a lesion length of < 3 cm (Table1) indicating successful introgression of *Xa21* in these lines.

Table 1: Phenotypic screening of selected backcross derived lines to check the resistance levels for BB and gall midge

S. No	Rice line	Reaction against bacterial blight#	
		DX020	
		Score	R/S
1	DRR17B	20.8. ± 0.6	S
2	ISM	1.7 ±0.3	R
4	RMSBG 37-19-8-102-65-22-9	2.7 ± 0.3	R
5	RMSBG 37-19-8-102-65-22-34	3.1 ± 0.5	R
6	RMSBG 37-19-8-237-8-142-3	1.8 ± 0.5	R
7	RMSBG 37-19-8-237-8-142-27	2.2 ± 0.3	R
8	RMSBG 37-19-8-237-8-142-69	1.8 ± 0.6	R

[#] A total of twenty plants from each of the backcross derived lines, the donor and recurrent parents were screened with the *Xoo* isolate DX 020 under glass house conditions and lesion length (cm) was calculated as an average of five leaves per plant.

DISCUSSION

The strategy of marker-assisted backcross breeding has been demonstrated to be an efficient technique for precise transfer of one or few target genes into the genetic background of an elite variety or parental line. Earlier, Sundaram et al. (2008; 2009) and Hari et al. (2011; 2013), Basasvaraj et al. (2010) developed disease resistant versions of the varieties and hybrid rice parental lines. Recently, we improved the elite, stable maintainer line, DRR17B by transferring a major BB resistance gene, *Xa21* and blast resistance gene, *Pi54* through marker-assisted selection (Balachiranjeevi et al. 2015). In the present study, following a similar approach, we have added two more resistance genes, viz., *Xa33* (for BB resistance) and *Gm4* (for gall midge resistance).

Through stringent MAS, we were able to precisely transfer the target resistance genes, while ensuring recovery of almost all the good quality traits and yield of DRR17B through careful phenotype-based selection. Five ABLs (*viz*., RMSBG 37-19-8-102-65-22-9, RMSBG 37-19-8-102-65-22-3, RMSBG 37-19-8-237-8-142-27, RMSBG 37-19-8-237-8-142-63 and RMSBG 37-19-8-237-8-142-69), which were equivalent to slightly better than DRR17B (Data not shown) and possessing strong resistance against BB have been identified (Table1). These lines will shortly be evaluated for their resistance against gall midge under controlled conditions at IIRR, Hyderabad.

As indicated earlier, through an earlier study we have developed elite near-isogenic lines of DRR17B possessing BB and blast resistance conferred by *Xa21* and *Pi54,* respectively. After confirmation of gall midge resistance of the five ABLs developed in the present study, we will be intercrossing them with the elite breeding lines of DRR17B possessing *Xa21* and *Pi54*, so that the maintainer line (i.e. DRR17B) can be made resistant against multiple biotic stresses. Such elite multiple biotic stress resistant lines of DRR17B, after conversion to WA-CMS lines will be helpful in development of high yield, disease resistant hybrids.

REFERENCES

Ahmed MI and Siddiq EA 1998: Rice. In: Banga SS and Banga SK (ed), Hybrid Cultivar Development, Narosa Publishing House, New Delhi, 221-256.

Balachiranjeevi CH, Bhaskar Naik S, Abhilash V, Akanksha S, Viraktamath BC, Madhav MS, Hariprasad AS, Laha GS, Prasad MS, Balachandran SM, Neeraja CN, Satendra Kumar M, Senguttuvel P, Kemparaju KB, Bhadana VP, Ram T, Harika G, Mahadeva swamy HK, Hajira Sk, Yugander A, Pranathi K, Anila M, Rekha G, BVN Kousik M, Dilip kumar T, Swapnil RK, Archana Giri and Sundaram RM. 2015: Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice. Mol breed. 35:15.

Basavaraj, S.H., Vikas K. Singh., Atul Singh., Ashutosh Singh., Anita Singh., Deepti Anand., Sheel Yadav., Gopalakrishnan, S., Nagarajan, M., Mohapatra, T., Prabhu K.V and Singh, A K. 2010: Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10., a super fine grain aromatic rice hybrid. Mol Breed. Vol 26: 293-305.

Divya D, Bhaskar Naik S, Sundaram RM, Laha GS, Bentur JS. 2015: Marker assisted pyramiding of bacterial blight and gall midge resistance genes in Samba Mahsuri and study of their interactions. Int Jour of Scient Resear. Volume: 4/ Issue: 6/ June 2015. ISSN no: 2277-8179.

Hari Prasad AS, Viraktamath BC & Mohapatra T. 2014: Hybrid rice India. *FAO/APSA* Expert conclusion on hybrid rice development in Asia- Assessment of limitations and potential. 2-3 July 2014. Bangkok.

Hari Y, Srinivasa Rao K, Viraktamath BC, Hariprasad AS, Laha GS, Ilyas Ahmed M, Natrajkumar P, Ramesha MS, Neeraja CN, Balachandran SM, Shobha Rani N, Balaji Suresh P, Sujatha K, Pandey M, Ashok Reddy G, Madhav MS and Sundaram RM. 2011: Marker-assisted improvement of a stable restorer line, KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain quality. Plant Breed. 130:608-616.

Hari Y, Srinivasa Rao K, Viraktamath BC, Hariprasad AS, Laha GS, Ahmed M, Natrajkumar P, Sujatha K, Srinivasprasad MS, Rani N.S, Balachandran SM, Kemparaju B, Mohan KM, Sama VSAK, Shaik H, Balachiranjeevi Ch, Pranathi K, Reddy GA, Madhav MS and Sundaram RM. 2013: Marker-assisted introgression of bacterial blight and blast resistance into IR 58025b, an elite maintainer line of rice, Journal of Plant Breed. 132: 586-594.

Himabindu K, Suneetha K, Sama VSAK, Bentur JS 2010: A new rice gall midge resistance gene in the breeding line Cr57-Mr1523, mapping with flanking markers and development of nIls. Euphyt. I74: 179–187.

International Rice Research Institute. 1996: Standard evaluation system for rice, 4th edition. Manila, Philippines: International Rice Research Institute.

Kauffman HE, Reddy APK, Hsieh SPY, and Merca SD. 1973: An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant disease repor. 57: 537-541.

Laha GS, Reddy CS, Krishnaveni D, Sundaram RM, Srinivas PM, Ram T, Muralidharan K and Viraktamath BC. 2009: Bacterial Blight of Rice and its Management. Page37 in: DRR Technical Bulletin. No. Directorate of Rice Research

(ICAR), Hyderabad.

Ronald PC, Albano B, Tabien R, Abenes L, Wu K, Mc Couch S and Tanksley S 1992: Genetic and physical analysis of the rice bacterial blight resistance locus, Xa21. Mol. Gen. Genet. 235:113-120.

Sundaram RM, Subhadeep Chatterjee, Ricardo Oliva, Laha GS, Jan,E Leach, RameshV. Sonti and Casiana Vera Cruz, 2014: Update on Bacterial Blight of Rice: Fourth International Conference on Bacterial Blight. Rice. 2014, 7:12.

Sundaram RM, Priya MRV, Laha GS, Shobha Rani N, Srinivasa Rao P, Balachandran S.M, Ashok Reddy G, Sarma NP. and Sonti RV. 2009: Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety by molecular marker assisted breeding. Biotech Journal. 4: 400-407.

Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS, Reddy A.G, Rani NS, Sharma NP and Sonti, RV 2008: Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphyt. 160: 411-422.