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International Journal of Current Research
Vol. 7, Issue, 08, pp.18943-18946, August, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INTROGROSSION OF BACTERIAL BLIGHT AND BLAST RESISTANCE INTO THE ELITE RICE VARIETY, AKSHAYADHAN THROUGH MARKER-ASSISTED BACKCROS BREEDING

Bhaskar Naik, S., Balachiranjeevi, C. H., Abhilash, V., Harika, G., Laha, G. S., Prasad, M. S. and *Sundaram, R. M.

Indian Institute of Rice Research, Hyderabad, India-500030

ARTICLE INFO

Article History:
Received 07th May, 2015
Received in revised form
10th June, 2015
Accepted 16th July, 2015
Published online 21st August, 2015

Key words:

MABB,
Blast,
Bacterial blight and Rice.

ABSTRACT

Marker assisted backcross breeding (MABB) is a promising strategy for improvement of elite crop varieties for one or more agronomical traits with minimal linkage drag. Akshayadhan is a high-yielding medium duration variety (135 days), whose yield is significantly limited by bacterial blight (BB) and blast diseases. In the present study, we attempted to improve Akshayadhan for resistance against BB and blast through marker-assisted backcross breeding (MABB). 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 donors and two sets of backcrosses were carried out to combine *Xa33* and *Pi2* into Akshayadhan separately. Backcrossing was continued till BC_2 generation, and gene-specific markers for the two resistance genes were used for marker-assisted selection at each stage of backcrossing in addition to phenotype-based selection for identification of plants closely resembling Akshayadhan. A single BC_2F_2 plant from each backcross possessing either *Xa33* or *Pi2* in homozygous condition and closely resembling Akshayadhan were intercrossed to generate inter-cross F_1s (ICF_1s) to combine the two traits. 'True'ICF_1s were identified using the gene-specific marker(s) and selfed to generate ICF_2s, which were then subjected for marker-assisted selection to identify plants which are homozygous for both *Xa33* and *Pi2*. Homozygous ICF_2 plants were advanced further by pedigree method for further evaluation. At ICF_4 selected lines were subjected for screening against BB and blast pathogens and all of them were observed to be resistant against the two diseases. Further, the selected lines also were observed to closely resemble Akshayadhan with respect to agromorphological traits and possessed long-slender grains.

Citation: Bhaskar Naik, S., Balachiranjeevi, CH., Abhilash, V., Harika, G., Laha, G. S., Prasad, M. S. and Sundaram, R. M. 2015. "Introgression of bacterial blight and blast resistance into the elite rice variety, Akshayadhan through marker-assisted Backcros breeding", *International Journal of Current Research*, 7, (8), 18943-18946.

INTRODUCTION

Rice (*Oryza sativa L.*), the largest cultivated crop with over 1.5 billion hectares and production of about 700 million metric tons, a staple food for the large part of world's making it the most consumed cereal grain. Rice crop is more prone to stress (both biotic and abiotic) Of the various biotic stresses, blast and bacterial leaf blight (BLB) diseases are considered as the major cause of severe yield loss in rice. Deploying host plant resistance is one of the most effective strategies for management of BLB and blast (Yoshimura *et al*., 1995). BLB 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% (Chiranjeevi *et al*., 2015).

Corresponding author: Sundaram, R. M.
Indian Institute of Rice Research, Hyderabad, India-500030.

Till date at least 38 genes conferring resistance to BLB have been identified (Sundaram *et al.,* 2014), many of them have been tagged and mapped with closely linked markers (Divya *et al*., 2015). In the present study we targeted *Xa33* gene, which was mapped on Chromosome 7 with two tightly linked markers WR7.1 and WR7.6 (Natrajkumar *et al*., 2012), with a broad spectrum of resistance against BLB isolates. Rice blast disease, caused by the fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), is also one of the major threats for rice production with a significant yield loose of about 70–80% during an epidemic (Khush and Jena 2009). Nearly, 100 genes 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). In the present study we targeted a major blast resistance gene *Pi2*, which was mapped on chromosome 6 (Yu *at al*., 1991). Fijellstorm *et al*. (2006) have identified closely linked markers for *Pi2* gene for its deployment in breeding programme.

Akshayadhan a medium duration variety (135 days) and an elite line, high yielding developed by crossing BR827-35 x SC5 109-2-2 and is highly susceptible to BLB and blast was used as a recurrent parent, which was developed at the Indian Institute of Rice Research, Rajendranagar, Hyderabad (http://www.rkmp.co.in). The present study was initiated to improve Akshayadhan for BLB and blast resistance by introducing *Xa33* and *Pi2* genes using gene linked markers through MABB strategy.

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. Akshayadhan is used as the recurrent parent. The cultivar, TN1 was used as susceptible check for BLB resistance screening, while HR-12 was used as susceptible check for blast resistance screening.

Breeding strategy: Akshayadhan was used as a recurrent parent and crossing was done separately with the donor lines FBR1-15 for *Xa33* and C101A51 for *Pi2* genes respectively. The F_1s thus generated were conformed for their heterozygosity by using PCR-based gene linked markers AP5659-5 (*Pi2*) (Fjellstorm *et al*., 2006) and WR7.1, WR7.6 (*Xa33*) (Natrajkumar *et al*., 2012) (Table 1). Thus identified 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* to identify positive plants. A single plant thus obtained, which was similar to Akshayadhan in phenotype was then backcrossed with the recurrent parent to generate BC_2F_1 plants. The positive BC_2F_1 plants were selfed to generate BC_2F_2 plants.

A single confirmed homozygous BC_2F_2 plant(s) derived from the two independent crosses and phenotypically similar to the recurrent parent were crossed to generate inter-cross F_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 advanced to ICF_5 and simultaneous selections were done based on morphological characters.

Phenotypic screening for BLB resistance: A virulent isolate of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from BLB hot-spot locations in India, DX-066 (Raipur, Chhattisgarh State, India) was used to screen ICF_5 progenies of Akshayadhan along with donor and recurrent parents for BLB 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). The plants were scored and evaluated on a 0–9 scale as per IRRI-SES scale (IRRI 1996).

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 Akshayadhan for blast resistance under *in-vivo* conditions following uniform blast nursery (UBN) method at 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

Marker-assisted backcross breeding (MABB) to transfer of *Xa33* and *Pi2* in to Akshayadhan

A total of 30 and 25 positive (i.e. heterozygous) F_1 plants were produced by crossing Akshayadhan and FBR1-15 (Cross I) and Akshayadhan and C101A51 (Cross II). At BC_1F_1, 38 and 32 positive plants were identified based on marker analysis after screening 89 and 82 BC_1F_1 plants produced from by crossing Akshayadhan with F_1 plants generated from Crosses I and II, respectively. In BC_2F_1 generation, a total of 41 and 40 positive plants were identified through marker analysis after screening 93 and 88 BC_2F_1 plants generated from Crosses I and II, respectively. The positive BC_2F_1 plants were selfed to obtain 292 and 323 BC_2F_2 plants. Among these, a total of 68 and 72 were identified to be homozygous from Crosses I and II, respectively, when screened with markers specific for *Xa33* and *Pi2*.. Among the homozygous BC_2F_2s, two plants [viz., AF-6-12-5-125 (possessing *Xa33*; from Cross I) and AC-9-18-30-99 (possessing *Pi2;* from Cross II)], which looked most similar to Akshayadhan phenotypically were identified and intercrossed to get intercross F_1 (ICF_1) plants. Eight such plants were identified to be 'true' hetrozygotes for both the target genes and were selfed to get intercross F_2 (i.e. ICF_2) plants. From these a total of 243 ICF_2 plants were raised and genotyped, among which 13 homozygous double positive plants (i.e. homozygous for *Xa33* and *Pi2*) were identified (Figure 1). These 13 homozygous ICF_2 plants were advanced further for evaluation of their progeny for resistance against BB and blast.

Phenotypic screening

All the 13 double homozygous breeding lines (i.e. possessing both *Xa33* and *Pi2*) at ICF_3 generation were subjected to phenotypic screening for BB and blast resistance under glass house conditions along with the donor and recurrent parents and the respective checks. With respect to screening against BB pathogen (i.e. DXO66), the resistance parent FBR1-15 (possessing *Xa33*) showed immune level of resistance (i.e. lesion length < 1 cm) and the susceptible checks TN1 and Akshayadhan showed a score of 9 (Table 1). Most of the 13 ICF_2 lines showed immune level of resistance (with a score of 1), while only two lines showed a score of 3. With respect to blast screening, the resistance check C101A51 having *Pi2* gene showed disease score of 1, and the susceptible checks HR-12 and the recurrent Akshayadhan showed score of 9. All the 13

ICF_2 lines derived lines were resistant showing a score of 1 or 3 (Table 1).

Table 1. Phenotypic screening of selected inter-cross derived lines to check the resistance levels for BLB and blast disease

S.No	Designation	Genotyping[@]		BLB score[#] (DX-066)	Blast score*
		WR7.6 (*Xa33*)	AP 5659-5 (*Pi2*)		
1	ICF_2-25-22	++	++	1	1
2	ICF_2-25-43	++	++	1	3
3	ICF_2-25-72	++	++	1	1
4	ICF_2-25-96	++	++	1	1
5	ICF_2-25-125	++	++	1	1
6	ICF_2-25-163	++	++	1	1
7	ICF_2-25-190	++	++	3	1
8	ICF_2-25-211	++	++	1	3
9	ICF_2-25-239	++	++	1	1
10	ICF_2-25-260	++	++	1	1
11	ICF_2-25-285	++	++	1	1
12	ICF_2-25-310	++	++	3	1
13	ICF_2-25-345	++	++	1	1
14	FBR1-15	++	--	1	-
15	C101A51	--	++	-	1
16	Akshayadhan	--	--	9	9

[@] ++ homozygous resistant allele at the particular gene based on screening with gene-specific marker, -- homozygous susceptible allele at the particular gene based on screening with gene-specific marker

[#] 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 and the score was recorded as an average of five leaves per plant as per IRRI-SES (IRRI 1996).

* 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).

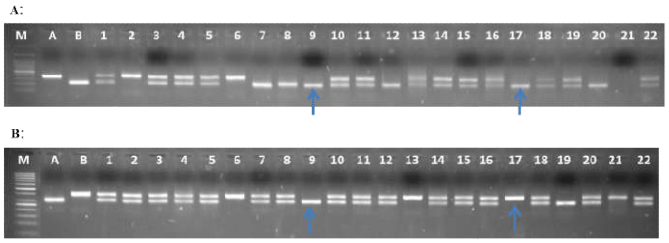

Figure 1. Fore ground selection for *Xa21* and *Pi2* genes in IC_1F_2 plants through PCR based markers

A. Genotyping of IC_1F_2 population with WR7.6 primer for *Xa33* gene.
B. Genotyping of IC_1F_2 population with AP 5659-5 primer for *Pi2* gene.
M – Marker, A – recurrent parent (susceptible allele) and B – Donor parent (resistant allele) Arrows indicates that homozygous double positive plants for both the dominant alleles

DISCUSSION

The elite rice variety, Akshayadhan, despite its high yield is highly susceptible to bacterial blight and blast diseases, which have limited its spread across India. Targeted improvement of such elite varieties for one or few target traits is possible through marker-assisted selection (MAS) and among the strategies of MAS, marker-assisted backcross breeding (MABB) (Hospital *et al*.,1997) has been widely deployed for improvement of several elite varieties like Pusa Basmati 1 (Joseph *et al*., 2004), Samba Mahsuri (Sundaram *et al*., 2008), Triguna (Sundaram*et al*., 2009), Lalat and Tapaswini (Prasad *et al*., 2013) and hybrid rice parental lines (Hari *et al*., 2011; Singh *et al*., 2012; Hari *et al*., 2013; Balachiranjeevi *et al*., 2015) for disease resistance. MABB can significantly reduce the number of generations needed to arrive at the desired population with the desired combination of genes when compared to conventional breeding which depends on phenotype-based selection (Sundaram *et al*., 2014).

In the present study, we have successfully introgressed a wild-rice derived novel BB resistance gene, *Xa33* (Natrajkumar *et al*., 2012), which has not been deployed so far and a widely deployed blast resistance gene, *Pi2* into the background of Akshayadhan through MABB. *Xa33* Foreground selection was done at each backcross and intercross generations using the gene-specific markers to identify plants positive for either *Xa33* or *Pi2* or both. Additionally, at each generation of backcrossing, positive plants which resembled Akshayadhan most closely (based on morphological traits) were advanced for backcrossing ensuring recovery of genetic background of Akshayadhan in just two backcrosses. The homozygous BC_2F_2 plants (possessing either *Xa33* or *Pi2*), which were the most similar to Akshayadhan in two sets of crosses were intercrossed to generate ICF_1 plants. Stable, inter-cross derived lines possessing *Xa33* and *Pi2* were identified in a homozygous condition at ICF_2 generation (Figure 1). All the ICF_2 lines, donor and recurrent parents along with the checks were then phenotyped and confirmed for their resistance against local, virulent isolates of the BB and blast pathogens (Table 1). All the homozygous ICF_2 lines were resistant for both BB and blast and the resistant levels were similar to that of the donor and the resistance checks showing the effective introgression of both *Xa33* and *Pi2* in the homozygous ICF_2 plants. The homozygous lines were advanced by pedigree method till ICF_5 generation and those lines possessing agro-morphological and grain quality characters similar to or better than Akshayadhan have been identified (data not shown). This indicates 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 Akshayadhan. In conclusion, through the present study, we have successfully introgressed a major gene each conferring resistance against BB and blast through MABB into Akshayadhan. The elite lines possessing BB and blast resistance, grain quality similar to Akshayadhan and yield levels equivalent to or better than the recurrent parent will be nominated for All India trials.

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