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RESEARCH ARTICLE

INTROGROSSION OF BROWN PLANTHOPPER (BPH) AND GALL MIDGE (GM) RESISTANCE GENES INTO THE ELITE RICE VARIETY, AKSHAYADHAN THROUGH MARKER-ASSISTED BACKCROSS BREEDING

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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 brown plant hopper (BPH) and gall midge (GM) insects. In the present study, we attempted to improve Akshayadhan for resistance against BPH and GM through marker-assisted backcross breeding (MABB). A rice line in the Abhaya, possessing the gall midge (GM) resistance gene, *Gm4* and a rice line RP 2068 18-3-5 possessing the brown planthopper (BPH) resistance gene, *Bph33* served as donors and two sets of backcrosses were carried out to combine *Gm4* and *Bph33* 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 *Bph33* or *Gm4* 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 *Bph33* and *Gm4*.. At ICF_2 selected lines were subjected for screening against BPH and GM, all of them were observed to be resistant against the two insects and were advanced further by pedigree method for further evaluation. Further, the selected lines also were observed to closely resemble Akshayadhan with respect to agromorphological traits and possessed long-slender grains.

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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, Brown planthopper (BPH) and gall midge (GM) insects 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 BPH and GM (Yasala *et al*., 2012). Brown Planthopper (BPH), *Nilaparvatalugens* (Stal.), is a serious sap-sucking insect pest of rice. It is distributed in tropical and temperate areas covering south, south-east and East Asia (Nasu, 1967). BPH, is the most devastating pest of rice in Asia and causes significant yield loss annually. Around 37 BPH resistance genes have been identified so far. The Asian rice gall midge (GM), *Orseolia oryzae* (Wood-Mason) is economically third most important pest of rice in India causing average annual yield loss of about US $80 million (Divya *et al.,* 2014).

Host resistance against this insect is reported to be conferred by 11 GM genes of which eight have been tagged and mapped (Yasala *et al*., 2012). Here we report pyramiding of two each of major BPH and GM resistance genes (*Bph33* for Bph resistance and *Gm4* for gall midge resistance) in the genetic background of Akshayadhan through marker assisted selection. 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 BPH and GM 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 BPH and GM resistance by introducing *Bph33* and *Gm4* genes using gene linked markers through MABB strategy.

MATERIALS AND METHODS

Parent material: BPH resistant rice breeding line RP2068-18-3-5 (RP2068) derived from the cross between Swarnadhan X Velluthacheera was identified to have *Bph33* gene resistance against BPH (Naik *et.al.,* 2018) served as a donor, a rice line Abhaya served

as a donor for *Gm4* (Divya *et al*., 2015) gene. Akshayadhan is used as the recurrent parent. The cultivar, TN1 was used as susceptible check for BPH and RP2068 is resistant check for BPH screening, whileTN1 was used as susceptible check and Abhaya for GM resistance screening.

Breeding strategy: Akshayadhan was used as a recurrent parent and crossing was done separately with the donor lines RP2068 for *Bph33* and Abhaya for *Gm4* genes respectively. The F_1s thus generated were conformed for their heterozygosity by using PCR-based gene linked markers RM11522 (*Bph33*) (Naik *et.al.,* 2018) and *Gm4* with the functional marker LRR-del (Divya *et al*., 2015) (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 *Bph33* or *Gm4* 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 *Bph33* and *gm4* 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 BPH resistance: Phenotypic test, Damage score was done using standard seed box technique (SST) to screen ICF_2 progenies of Akshayadhan along with donor and recurrent parents for BPH resistance was done at IIRR greenhouse against BPH under controlled conditions. Phenotypic readings were noted and the plants were scored and evaluated on a 0–9 scale as per IRRI-SES scale (IRRI 1996). at the end of the experiments and were tabulated.

Phenotypic screening for gall midge resistance: Selected IF_2 plants were grown in earthen pots under glass house condition, One each of freshly emerged male and female gall midge (biotype 1) adult insects were released and eggs were collected on the fourth day. Such eggs were used for infesting the isolated tillers of the test plants (Bentur *et al*., unpublished). After 10 days from day of infestation, the tillers were dissected under microscope to check maggot survival and for HR reaction. Plants with dead maggot and with expression of HR or without HR were rated as resistant HR+ or HR- plants. Plants with live maggots were rated susceptible.

RESULTS

Marker-assisted backcross breeding (MABB) to transfer of *Bph33* and *Gm4* in to Akshayadhan: A total of 30 and 25 positive (i.e. heterozygous) F_1 plants were produced by crossing Akshayadhan and RP2068 (Cross I) and Akshayadhan and Abhaya (Cross II). crossing Akshayadhan with F_1 plants generated from Crosses I and II, respectively. In BC_2F_1 generation, a total of 39 and 43 positive plants were identified through marker analysis after screening 100 and 92 BC_2F_1 plants generated from Crosses I and II, respectively. The positive BC_2F_1 plants were selfed to obtain 302 and 315 BC_2F_2 plants. Among these, a total of 66 and 70 were identified to be homozygous from Crosses I and II, respectively, when screened with markers specific for *Bph33* and *Gm4*. Among the homozygous BC_2F_2s, two plants [viz., AR-4-22-4-134 (possessing *Bph33*; from Cross I) and AA-8-16-33-98 (possessing *Gm4;* 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 248 ICF_2 plants were raised and genotyped, among which 12 homozygous double positive plants (i.e. homozygous for *Bph33* and *Gm4*) were identified (Figure 1). These 12 homozygous ICF_2 plants were advanced further for evaluation of their progeny for resistance against BPH and GM.

Phenotypic screening: All the 12 double homozygous breeding lines (i.e. possessing both *Bph33* and *Gm4*) at ICF_3 generation were subjected to phenotypic screening for BPH and GM resistance under glass house conditions along with the donor and recurrent parents and the respective checks. With respect to screening against BPH, the resistance parent RP2068 (possessing *Bph33*) showed high level of resistance (i.e. DS 2.0) and the susceptible checks TN1 and Akshayadhan showed a score of 9.0 (Table 1). Most of the 12 ICF_2 lines showed high level of resistance (with a score of 2), while only two lines showed a score of 3. With respect to GM screening, the resistance check Abhaya having *Gm4* gene showed resistance to GM, and the susceptible checks TN1 and the recurrent Akshayadhan were susceptible. All the 13 ICF_2 lines derived lines were resistant to GM (Table 1).

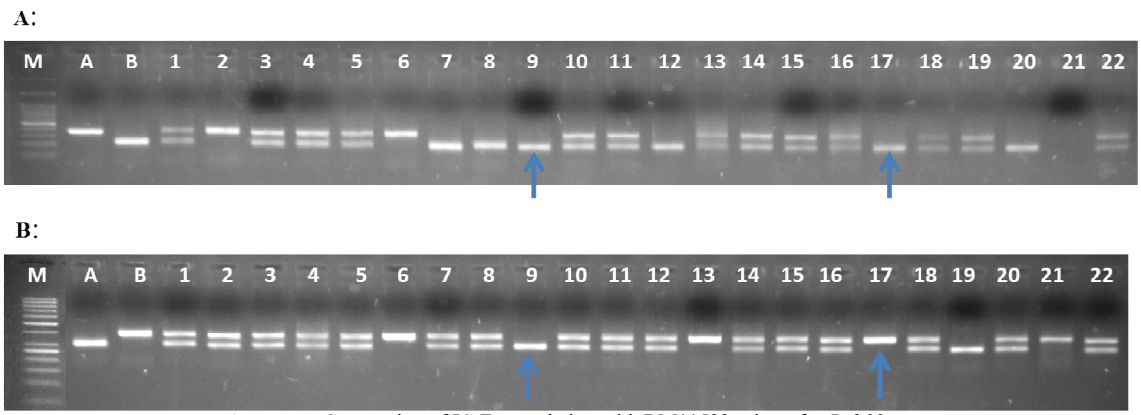
DISCUSSION

The elite rice variety, Akshayadhan, despite its high yield is highly susceptible to BPH and GM, 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), has been widely deployed for improvement of several elite varieties like Pusa Basmati 1, Samba Mahsuri (Sundaram et al. 2009), Triguna (Sundaram et al. 2009), Lalat and Tapaswini (Dokku 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).

Table 1. Phenotypic screening of selected inter-cross derived lines to check the resistance levels for BPH and GM

S.No	Designation	Genotyping[@]		BPH score[#]	GM score[#]
		RM11522 (*Bph33)*	RR Del (*Gm4)*		
1	ICF_2-21-28	++	++	2	R
2	ICF_2-21-41	++	++	2	R
3	ICF_2-21-77	++	++	3	R
4	ICF_2-21-99	++	++	2	R
5	ICF_2-21-115	++	++	2	R
6	ICF_2-21-133	++	++	2	R
7	ICF_2-21-172	++	++	3	R
8	ICF_2-21-179	++	++	2	R
9	ICF_2-21-193	++	++	3	R
10	ICF_2-21-208	++	++	2	R
11	ICF_2-21-220	++	++	3	R
12	ICF_2-21-245	++	++	2	R
13	Abhaya	++	--	9	R
14	RP2068-18-3-5	--	++	2	-
15	Akshayadhan	--	--	9	S
16	TN1	--	--	9	S

[@] ++ 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 phenotyped for GM resistance under glass house conditions. * A total of 20-25 seedlings from each of the backcross derived lines, the donor and recurrent parents were screened for Damage score (SBT) at DRR and score was calculated as per IRRI-SES (IRRI 1996).



A. Genotyping of IC_1F_2 population with RM11522 primer for *Bph33* gene.
B. Genotyping of IC_1F_2 population with LRR del primer for *Gm4* 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

Figure 1. Fore ground selection for *Bph33* and *Gm4* genes in IC_1F_2 plants through PCR based markers

In the present study, we have successfully introgressed a novel BPH resistance gene, *Bph33* (Naik *et al.,* 2018), which has not been deployed so far and a widely deployed GM resistance gene, *Gm4* into the background of Akshayadhan through MABB. Foreground selection was done at each backcross and intercross generations using the gene-specific markers to identify plants positive for either *Bph33* or *Gm4* 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 *Bph33* or *Gm4*), 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 *Bph33* and *Gm4* 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 BPH and GM (Table 1). All the homozygous ICF_2 lines were resistant for both BPH and GM and the resistant levels were similar to that of the donor and the resistance checks, showing the effective introgression of both *Bph33* and *Gm4* 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 BPH and GM through MABB into Akshayadhan. The elite lines possessing BPH and GM 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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