General Impact Factor (GIF): 0.875
e- ISSN: 2394-5532
p- ISSN: 2394-823X
International Journal of Applied And Pure Science and Agriculture
www.ijapsa.com

PHENOTYPIC AND MOLECULAR SCREENING OF ADVANCED BACK CROSS POPULATION FOR NECK BLAST RESISTANCE IN RICE

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Abstract

Rice blast is the most devastating disease causing severe yield loss due to prevalence of neck blast at reproductive stage. Location specific and wide variation in physiological races of blast pathogen results in identification of less stable resistant lines by phenotypic screening. Molecular markers are powerful tools to detect presence of blast resistant genes besides screening in blast nurseries. Present study was undertaken with an objective to detect neck blast resistant lines in advanced back cross (BC_2F_3 and BC_2F_4) population of Samba mahsuri/ WGL 167 and samba mahsuri/OR 2309-19 in neck blast nurseries and using molecular markers. Polymorphic marker RM 224 linked to pi-1 blast resistant gene identified between Samba mahsuri and WGL 167 where as RM 206 a pik-h gene linked marker between Samba mahsuri and OR 2309-19 were used to screen advanced back cross lines. Thirty three lines were found to be resistant (score 0 or 1) in Samba mahsuri/WGL 167 population and 136 lines in Samba mahsuri/OR 2309-19 under disease pressure of 9 as per SES of IRRI in BC_2F_3 population during rabi 2010. Among the resistant lines, twenty six lines co segregated with resistant allele of RM 224 in Samba mahsuri/WGL 167 mapping population where as seventeen lines co segregated with resistant allele of RM 206. These selected resistant lines advanced to BC_2F_4 generation subjected to neck blast screening in rabi 2011 and identified 19 resistant lines in Samba Mahsuri/WGL 167 mapping population and 22 resistant lines in Samba Mahsuri /OR 2309 showing disparity in disease reactions in the two seasons. But fourteen lines co segregated for resistant allele of RM 224 in samba mahsuri/ WGL 167 and seven lines cosegregated with resistant allele of RM 206 showing stable disease reaction in both the seasons. These results indicated that molecular markers are powerful in identification of resistant lines as phenotypic screening is influenced by environment in expression of virulence of pathogen against resistant genes.

Key words: neck blast resistance, rice, SSR markers.

I. INTRODUCTION

Rice blast caused by *Pyricularia grisea* Sacc. [teleomorph *Magnaporthe grisea* (Hebert) Barr.] a devastating disease because of its wide distribution and its destructiveness under favourable conditions (Ou, 1985), continues to be a serious constraint in all the rice ecosystems of the country (Muralidharan, 2006). The blast fungus can attack the aerial parts of the rice at any stage of growth which is characterized by the appearance of lesions on the leaves, nodes and panicles. (Dinakar and Muralidharan, 2007). Neck blast is the most devastating symptom causes severe losses in yield (Rajarajeswari and Muralidharan, 2006). Wide variation in physiological races of blast pathogen, host and favorable environment results in identification of less stable resistant lines by phenotypic screening. Molecular markers are powerful tools to detect presence of blast resistant genes. Nearly 100 blast resistance genes have been reported in different genotypes of rice. Cloning of nineteen of these genes

and identification of more than 350 QTLs (Quantitative Trait Loci) has been completed (Sharma *et al.*,2012). Presence or absence of eight major blast resistance genes (*Pib*, *Pia*, *Piz*, *Piz-t*, *Pi9*, *Pi5*, *Pita*, and *Pi40*) in parents were validated using gene-specific DNA markers (Suh *et al.*, 2009). . Present study was undertaken at Andhra Pradesh Rice Research Institute and Regional Agricultural Research station, Maruteru with an objective to detect neck blast resistant lines in advanced back cross population of Samba mahsuri/ WGL 167 and samba mahsuri/OR 2309-19 in neck blast nurseries and using molecular markers.

II. MATERIALS AND METHODS

Two neck blast donors WGL 167 and OR2309-19 identified from neck blast screening nursery at A.P. Rice Research Institute and Regional Agricultural Research Station (APRRI&RARS), Maruteru and widely cultivated highly susceptible variety samba mahsuri (BPT 5204) were used to detect presence of blast resistance genes. Two mapping populations were developed using susceptible parent samba Mahsuri as female and neck blast donors WGL167 and OR2309-19 as male parents from 2008-2011.Generated 400 BC_2F_3 and 43 BC_2F_4 population were subjected to phenotypic screening for neck blast resistance besides using molecular markers linked to blast resistance genes in the rabi season of 2010 and 2011.

Phenotypic screening

For neck blast screening, one month old seedlings of these genotypes were transplanted in two rows of one meter length at 20X10 cm spacing during the same crop season. Application of slightly higher dosage of nitrogenous fertilizer (150 kg/ha) was done to ensure maximum disease development. . The neck blast incidence was recorded by observing all the tillers of 10 randomly selected hills of each advanced line by experienced personnel . during flowering stage and 7 days before harvesting stage. Scoring of neck blast disease was given from 0 to 9. No disease =0, less than 5% panicles necrotic =1, 5 to 30% panicle branches necrotic=3, 31 to 50% panicles necrotic =5, 51 to 100% necrotic=9. Final neck blast scoring data was considered for the present study. The genotypes showing a disease rating of 0-3, 3.1-5, 5.1-9 were designated as resistant, intermediate and susceptible, respectively.

Molecular screening

Total genomic DNA was extracted using modified protocol of Zheng *et al* 1995 for PCR amplification. The quality and quantity of DNA was estimated using ND 8000 eight-channel spectrophotometer. Amplification of simple sequence repeats were performed in 10 µl of reaction mixture containing 1 µl of 10X buffer with MgCl2, 0.5 µl of dNTPs (25 m M. L-1), 1 µl (5 µ molar) each of forward and reverse primers, 1 µl Taq DNA polymerase (0.5 U/µl), 3 µl of template DNA (10 ng/µl) and 2.5 µl of sterilized, distilled water. Eppendorf thermo cycler was used for PCR reactions with the following temperature profiles: The initial denaturation was at 94oC for 5 min, followed by 35 cycles of denaturing at 94oC for 0.5 min, annealing at 55o C for 0.5 min, extension at 72oC for 1.0 min and 7 min at 72oC for the final extension. The PCR products were subjected to electrophoresis with ethidium bromide (10mg/ml) at 100 volts for 1 hr in 1X TBE buffer. A 100 bp ladder (Genei) was used for appropriate sizing of the products. The gel picture was captured under UV light using Ingenius gel doc system.

Girija *et al.* (2013) reported WGL 167 as new source for neck blast resistance conferring as pi-1 through bulk segregant analysis. Polymorphic marker RM 224 linked to Pi-1 blast resistant gene identified between Samba mahsuri and WGL 167 and RM 206 a Pi-kh gene linked marker which was found to be

polymorphic between Sambamahsuri and OR 2309-19 on chromosome 11 were used to screen 400 advanced back cross lines in rabi 2010 and 43 lines in rabi 2011.

III. RESULTS AND DISCUSSION

In screening trial for resistance against neck blast, thirty three lines were found to be resistant (score 0 and 1) in BC_2F_3 population of Samba mahsuri/WGL 167 population and 136 lines in Samba mahsuri/OR 2309-19 under high disease pressure of 9 as per the SES of IRRI during Rabi 2010. Twenty six lines were co segregated with resistant allele of RM 224 in Samba mahsuri/WGL 167 mapping population where as seventeen lines were co segregated with resistant allele of RM 206(Fig1 &2). These selected lines advanced to BC_2F_4further subjected to neck blast screening during rabi 2011 and identified 19 resistant lines in Samba Mahsuri/WGL 167 mapping population and 22 resistant lines in Samba Mahsuri /OR 2309-19 showing disparity in disease reaction in the two seasons. But fourteen lines co segregated for resistant allele of RM 224 in samba mahsuri/ WGL 167 and seven lines co segregated with resistant allele of RM 206 showed stable disease reaction in both the seasons(Table 1 and 2). Wide variation in phenotypic screening in detection of resistant lines for same local pathotype for two different population was observed. This indicated resistant genes are donor specific and consistency in disease reaction is influenced by conducive environment and influence of other minor genes. Use of molecular markers besides phenotypic screening helped in precise selection in stable resistant lines besides phenotypic screening. These results indicated that molecular markers are powerful tools in identification of resistant lines as phenotypic screening is influenced by environment in expression of avirulence of pathogen against resistant genes.

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Table 1:Summary of neck blast disease reaction in Sambamahsuri/WGL 167 mapping population

S.No	Designation	Score of resistant allele of RM 224	Neck blast disease score	
			BC_2F_3 lines in Rabi 2010	BC_2F_4 lines in Rabi 2011
1	2197-88	B	3	1
2	2197-96	H	1	1
3	2197-110	H	1	1
4	2197-137	B	1	1
5	2197-189	H	3	1
6	2197-216	H	5	1
7	2197-236	B	0	1
8	2197-243	H	3	1
9	2197-253	B	5	1
10	2197-254	H	0	1
11	2197-258	B	3	1
12	2197-262	B	1	1
13	2197-300	B	1	1
14	2197-303	B	1	1

(B=RESISTANT PARENT WGL 167 allele, H: Heterozygote allele)

Table 2:Summary of neck blast disease reaction in Sambamahsuri/OR2309-19 mapping population

S.No	Designation	Score of resistant allele of RM 206	Neck blast disease score	
			BC_2F_3 lines in Rabi 2010	BC_2F_4 lines in Rabi 2011
1	2198-111	B	1	1
2	2198-112	B	1	1
3	2198-119	B	1	1
4	2198-148	B	0	1
5	2198-156	B	0	1
6	2198-157	B	1	1
7	2198-165	B	1	1

(B=RESISTANT PARENT WGL 167 allele, H: Heterozygote allele)

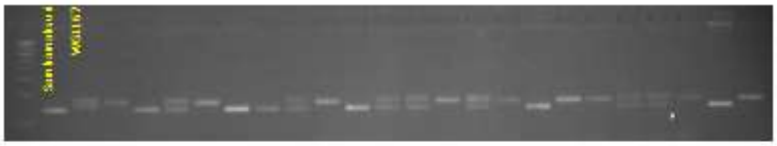

Fig 1:Screening of BC_2F_3 lines of samba mahsuri/WGL 167 with RM 224 linked to *pi-1* gene linked marker

Fig 2:Screening of BC_2F_3 lines of samba mahsuri/OR2309-19 with RM 206 linked to *Pi-kh* gene linked marker

