Screening of Rice Genotypes for Resistance to Brown Plant Hopper Biotype 4 and Detection of BPH Resistance Genes

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***Abstract*—Brown plant hopper [*Nilaparvata lugens* (Stål.)] is one of the most destructive pests of rice, which causes significant yield losses worldwide. Identification of resistant varieties is very important as the biotypes of the pest is changing its behaviour from time to time and the earlier released resistant rice varieties showing susceptibility to the pest. Identification of new sources of resistance and verification of resistance reaction of already reported donors is very important Field screening was carried out in 20 rice genotypes following standard evaluation system (IRRI, 1992). Further these genotypes were screened by standard seed box screening technique (SSST), honey dew test and nymphal survival test in the greenhouse in order to confirm the resistance and susceptibility. During screening, TN_1 and PTB33 were used as susceptible and resistant checks, respectively. The results of field screening and SSST showed low BPH damage score (3.0) in BM71, ACC5098, ACC2398, MTU1001, Rathuheenathi. In addition, honeydew excretion test was carried to know the preference or non-preference of insect. Nymphal survival, nymphal duration, % male population, wing dimorphism studies helped to know the antibiosis mechanism of resistance. Low honeydew excretion and low nymphal survival rate was observed in BM71, ACC5098 and Rathuheenathi reflecting non-preference feeding behaviour and antibiosis mechanism of resistance, respectively. Molecular studies were taken up in these lines using reported gene linked markers of major BPH resistance genes and found more than one BPH resistance genes in each resistant genotype. These lines were used as donors in rice breeding programme to develop BPH resistant varieties.**

***Index Terms*—brown plant hopper, Rice genotypes, SSR markers.**

I. Introduction

Approximately 52% of the global production of rice is lost annually owing to the damage caused by biotic stress factors, of which 25% is attributed to the attack of insect pests [1]. Rice is infested by more than hundred species of insects and about twenty of them are considered serious pests as they cause significant damage to rice crop. Among them brown plant hopper (BPH), *Nilaparvata lugens* (Homoptera: Delphacidae) is one of the most destructive insect pests causing significant yield loss in most of the rice cultivars of Asia. It is a phloem-sap-sucking insect pests of tropical and temperate rice in Asia feeds on the rice phloem sap using its piercing-sucking mouthparts, which affects the growth of rice plants and results in ‘‘hopperburn’’ [2]. BPH is also a vector, transmitting viral diseases such as Grassy stunt, Rugged stunt and associated diseases [3]. In recent years, BPH infestations have intensified across Asia, causing heavy rice yield losses [4]. Conventional measures to reduce BPH damage to rice have included the application of chemical insecticides but this is expensive, ineffective under some weather conditions and the chemicals can kill BPH predators, such as *Anagrus nilaparvatae* [5] which may lead to increased pest incidence as well as change in BPH biotypes [6]. The use of resistant rice varieties is the most economical and efficient method for controlling the BPH [7] therefore it is imperative to identify BPH resistance genes from diverse sources and incorporate them into rice cultivars. Molecular markers have demonstrated a potential to detect genetic diversity and relatedness of most crop species and to aid the management of plant genetic resources [8]. In contrast to morphological traits, molecular markers can reveal differences among genotypes at DNA level, providing a

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more direct, reliable and efficient tool for germplasm characterization, conservation and management. Among all DNA markers, microsatellites [9], [10] are codominant in nature; show high allelic diversity; are easily and economically assayed by PCR and can be automated. Many potential SSR markers have been identified in rice and over 25,000 have been developed as molecular markers [11], [12]. These molecular markers have been effectively utilized for many purposes including genome mapping, assessment of the genetic diversity and relatedness among various cultivars and marker aided breeding [13], [14].

BPH populations on rice have been categorized in to four biotypes [3]. The population in the east and Southeast Asia is reported as biotype 1, while biotype 2 originated in Indonesia and Vietnam as dominant biotype [14]. Biotype 3 was produced in the laboratory at the International Rice Research Institute IRRI [15] and in Japan, whereas biotype 4 is found only in South Asia. Till date, 26 BPH resistance genes have been identified in wild species *Oryza australiensis*, *Oryza officinalis*, *Oryza glaberima*, *Oryza eichengiri*, *Oryza rufipogon*, *Oryza minuta* and Indian cultivars [16], [17]. The objective of the present study is to identify BPH resistance donors using field as well as controlled screening methods and their genotyping by using reported gene linked markers.

II. Materials and Methods

A. Plant Material

The experimental material consists of twenty seven rice genotypes ranging from land race to improved lines viz., ACC5098, PTB33, Deepthi (MTU4870), Swarna (MTU7029), TN_1, Krishnaveni (MTU2077), Samba Mahsuri (BPT5204), Bhavapuri Sannalu (BPT2270), Akshaya (BPT2231), BM71, WGL167, Rathuheenathi, Vijetha (MTU1001), Cottondora Sannalu (MTU1010), Chandana (RNR74802), Tellahamsa (C10754), Manoharsali, Bapatla Sannalu (BPT1768), ACC2398, SRAC34997, TN_1, Sivasinapu, Vajram (MTU5249), Swarnalatha, IR65482, Prabhath (MTU3626) and MTU1064. These genotypes were obtained from the Andhra Pradesh Rice Research Institute and Regional Agricultural Research Station (APRRI & RARS) Maruteru and Directorate of Rice Research (DRR), Rajendranagar, India. Here TN_1 and PTB33 were used as susceptible and resistant checks, respectively.

B. Screening for BPH Resistance

The material was evaluated for resistance against BPH in field at APRRI, Maruteru, which is one of the BPH hot spots in India for two seasons during wet seasons of 2012 and 2013. The material was screened under controlled conditions at DRR during wet season 2013. The screening methods includes (i) standard seed box screening technique (SSST) developed at IRRI by [19], (ii) Honeydew test and (iii) Nymphal survival method.

C. Field Screening

Twenty six rice genotypes were evaluated for resistance against BPH in field. Each genotype was transplanted at 20×10 cm spacing in two rows of one meter length. All around test entries, two meters of susceptible variety TN_1 were transplanted. Number of plant hoppers on 10 plants/entry were recorded when TN_1, susceptible check showed hopper burn symptoms. Each entry was scored based on scoring system developed by the International Rice Research Institute [18] and each entry was scored as 0 = no visible damage, 1 = partial yellowing of first leaf, 3 = first and second leaves partially yellowing, 5 = pronounced yellowing or some stunting, 7 = mostly wilted plant but still alive, 9 = the plant completely wilted or dead. Interpretation of results was based on standard evaluation system where the families with a mean rating of 0 to 3, 3.1 to 6.9 and 7 to 9 are designated as resistant, moderately resistant and susceptible, respectively [18].

D. Standard Seed Box Screening Technique

The experiment was conducted at a temperature of 28 to 30 ℃ and relative humidity of 70% to 80%. The seeds were pre-soaked and sown in rows in 60 × 45 × 10 cm seed boxes along with resistant and susceptible checks. 25 to 30 seedlings per row were maintained per genotype. Ten (10) day old seedlings were infested with first instar nymphs at the rate of eight to 10 per seedling. Approximately one week after infestation "hopperburn" symptom was observed.

The genotypes were scored as scoring system developed by the International Rice Research Institute [18].

E. Honey Dew Test

The honeydew excretion is widely used to assess feeding activity and consequently a reliable index for resistance and susceptibility of a crop variety to homopteran pests. Many techniques have been developed to measure the feeding response of *Nilaparvatalugens* on resistant and susceptible rice plants. The more important were the test of filter paper dipped in a solution of bromocresol green and the test of a parafilm sachet. In the present study, the filter paper technique was used were honey dew collected on filter paper treated with ninhydrin and bromocresol green following the procedure green [20]. For each plant to be screened, five one day old adult females were kept starving for 2 h 30 min. Then, the female hoppers were released on to plants to feed for 24 h, after which the filter papers were collected. Bromocresol green indicates phloem-based honey dew as blue spots indicates resistance and susceptibility according to the amount of honey dew appear onn the filter paper. The area of each spot on the bromocresol green-filter paper was measured using a digital scanner and "Image J" software (Fig. 1).

F. Nymphal Survival Method

The nymphal survival test shows survival rate of the nymphs on different varieties of rice plants. For this, 20 first instar stage nymphs were released on 40 days old rice plant. The number of surviving nymphs was recorded for every two days until they became adults (11days).

The experiment was carried in three replications along with the resistant and susceptible checks PTB33 and TN1 respectively. The result says that high survival rate of BPH can be seen in the susceptible plants (Fig. 2).

G. Genotyping of Rice Genotypes Using BPH Resistance Gene Markers

Rice genotypes were characterized for the presence of BPH resistance genes using the reported markers.

H. DNA Isolationand PCR Amplification

Genomic DNA of 26 rice genotypes including sources for the BPH resistance was isolated. The quality of the DNA was checked on an agarose gel (0.8%, w/v). 24 markers which are reported to be closely linked for BPH resistance corresponding to biotype 4 were used for this study. SSR primers were obtained from sigma Aldrich, Bangalore. The PCR reactions were performed in 10µL volumes using peltier thermal cycler. The reaction mixture contained 3µl of template DNA, each 0.5 µl of forward and reverse primers, 1 µl dNTPs, 1 × $MgCl_2$ buffer (20 Mm Tris HCl, 15mM $MgCl_2$), and 0.1 µl Taq DNA polymerase. The amplification profile was 94℃ for 5 min followed by 35 cycles of 94℃ for 30 sec, 59℃ for 1 min and 72℃ for 1 min with a final extension of 7 min. at 72℃.Amplified PCR products were electrophoretically resolved on a 3% agarose gel using 1 ×TAE buffer. DNA banding patterns were visualized using BIO-RAD Imaging gel documentation system.

III. Results and Discussion

A. Field Screening

Twenty six rice genotypes were screened at APRRI, Maruteru under field conditions during wet seasons of 2012 and 2013. The results of screening trials showed that the genotype viz. PTB33, BM71 and Rathuheenathi genotypes were rated as resistant (R) with an average damage score of 2, 2.5 and 3.0, respectively. Eleven genotypes viz., ACC5098, Deepthi (MTU4870), Bhavapuri Sannalu (BPT1768), Akshaya (BPT2231), Vijetha (MTU1001), Cottondora Sannalu (MTU1010), ACC2398, Swarnalatha, IR65482, Prabhath (MTU3626) and MTU1064 showed moderate level of resistance with an average damage score ranging between 3.5 and 6.0. Among the eleven MR genotypes, three genotypes viz., ACC5098, Akshaya (BPT2231) and ACC2398 showed resistance as well as MR reaction during wet seasons of 2012 and 2013. Remaining genotypes were showed susceptible reaction to BPH incidence (Table I). Kumar and Tiwari, (2010) also evaluated ninety six entries of plant hopper screening trial (PHS-05 and PHS-06) were evaluated. PHS-05 entries KAUM MO 8 20 KR and PTB 33 were found highly resistant, while ARC 6650 and CB 21006 were rated as resistant and moderately resistant, respectively. PHS-06 entries CRAc 34997, 9412-13 and PTB 33 were identified as highly resistant, resistant and moderately resistant.

B. Standard Seed Box Screening Technique

Standard seed box screening technique was also conducted in order to confirm the results obtained from field screening. The results of phenotypic response of rice genotypes to brown plant hopper screening at seedling stage (10 hoppers per seedling) indicated varied genotypic responses. The rice genotypes were scored when TN_1 showed hopper burn with a damage score 9. Among the genotypes screened PTB33, BM71, Rathuheenathi, ACC5098 and ACC2398 showed resistance towards BPH (Table I).

C. Honey Dew Test

The amount of phloem in the honey dew excreted by the insect in the genotype was measured in mm^2 units (Fig. 1). Among twenty genotypes evaluated, BM71, ACC5098 and ACC2398 showed low rate off excretion indicating the low feeding activity of insect. Similar test was used by [18] to identify resistant rice genotypes (Fig. 1).

D. Nymphal Survival Method

Nymphal survival test was performed to know antibiosis mechanism of resistance. Three parameters viz., % male population, wing dimorphism and nymphal duration were observed to identify the resistant donors for BPH. The study resulted that genotypes ACC5098, ACC2398 and BM71 showed low nymphal survival rate (Fig. 2).

E. Detection of BPH Resistance Genes Using Reported Markers

A total of 24 SSR markers were used for this study which are reported linked markers to five BPH resistance genes viz., Bph3, Bph4, Bph6, Bph7 and Bph18 related to Biotype-4, of which 11 primers were polymorphic.

Analyzing the linked markers including the donor source of the particular gene with the genotypes resulted that some of the genotypes showing resistance phenotypically also carries the genes related for resistance. The genotypes BM71 showed donor allele with only one marker each, RM589 for Bph3 and RM3180 for Bph6 (Fig. 3) and ACC2398, ACC5098 (RM17008, RM3180), might be having Bph6 gene. Comparatively, the phenotypic screening studies and other reports indicated that BM71, ACC5098, ACC2398 are good resistant sources for BPH biotype 4. BM71 was hybridized with two mega rice varieties, Samba Mahsuri and Swarna to combine BPH resistance with their high yield potential and superior grain quality. The material generated would be used in future for genetic studies to identify which of these gene(s) or even new gene might be present in BM71, ACC5098, ACC2398 which confers the BPH resistance in this genotype.

Based on the results it is evident that though it was attempted to screen for seven of the genes reported for BPH biotype 4, only for Bph3+Bph4, Bph6 and Bph18, at least two markers showed the presence of donor alleles which could be considered for putative indication of presence of these genes. Though with these results, a preliminary indication as to which of these genotypes may have these genes is obtained, these need to be concluded only with more number of markers in the vicinity of these gene or functional markers.

TABLE I. REACTION OF DIFFERENT GENOTYPES TO BROWN PLANTHOPPER UNDER FIELD SCREENING AND STANDARD SEED BOX SCREENING TECHNIQUE

S. No	Name of genotypes	Field screening at APRRI				Average Damage score	Average FR Field reaction	Standard seed box screening technique at DRR, 2013	
		Wet season, 2012		Wet season, 2013				Damage score	Reaction
		DS	FR	DS	FR				
1	ACC5098	3	R	5	MR	4	MR	3	R
2	PTB33	1	R	3	R	2	R	1	R
3	Swarna (MTU7029)	7	S	7	S	7	S	9	S
4	Deepthi (MTU4870)	7	S	5	MR	6	MR	7	S
5	Krishnaveni (MTU2077)	7	S	7	S	7	S	8	S
6	Samba Mahsuri (BPT5204)	9	S	9	S	9	S	9	S
7	BhavapuriSannalu (BPT2270)	7	S	5	MR	6	MR	7	S
8	Akshaya (BPT2231)	3	R	5	MR	4	MR	5	MR
9	BM71	3	R	2	R	2.5	R	3	R
10	WGL167	7	S	5	MR	6	S	7	MR
11	Rathuheenathi	3	R	3	R	3	R	3	R
12	Vijetha (MTU1001)	5	MR	5	MR	5	MR	4	MR
13	CottondoraSannalu (MTU1010)	5	MR	5	MR	5	MR	8	S
14	Chandana (RNR74802)	7	S	7	S	7	S	9	S
15	Tellahamsa (C10754)	5	MR	7	S	6	S	9	S
16	Manoharsali	7	S	7	S	7	S	9	S
17	BapatlaSannalu (BPT1768)	7	S	7	S	7	S	7	S
18	ACC2398	3	R	5	MR	3.5	MR	3	R
19	SRAC34997	9	S	9	S	9	S	7	S
20	T N1	9	S	9	S	9	S	9	S
21	Sivasinapu	7	S	5	MR	6	S	9	S
22	Vajram (MTU5249)	9	S	7	S	7.5	S	7	S
23	Swarnalatha	5	MR	5	MR	5	MR	4.5	MR
24	IR65482	5	MR	5	MR	5	MR	5	MR
25	Prabhath (MTU3626)	5	MR	5	MR	5	MR	7	S
26	MTU1064	7	S	5	MR	6	MR	5.5	MR

Note: DS- Damage Score; FR- Field Reaction; R-Resistant; MR-Moderately Resistant and S-Susceptible.

Hence, the above results indicate in a preliminary way, the likelihood that some of the genotypes carrying the original donor allele are likely to have the corresponding BPH resistance gene(s). However, since these are not very close to the genes, the possibility of loss of the gene due to recombination cannot be ruled out. These results could be concluded in future through studies with additional markers in that region and/or by mapping to conclusively prove the presence of the genes in these new resistance sources. Once a gene is conclusively proved to be closely linked by mapping studies in these new donors, they can be deployed in marker assisted breeding

programmes which would help to develop BPH resistant cultivars and also there is a need to conclude whether the genes present in these donors are novel or similar.

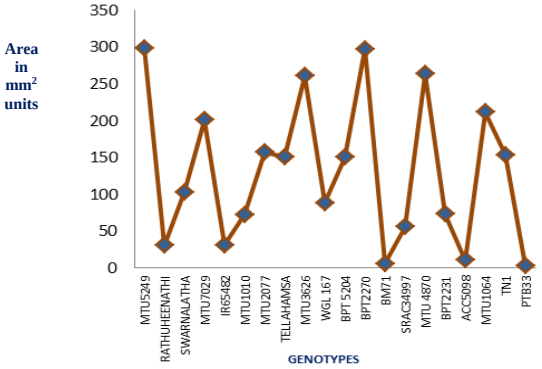


Figure 1. Honey dew chart representing the non-preferred feeding of BPH.

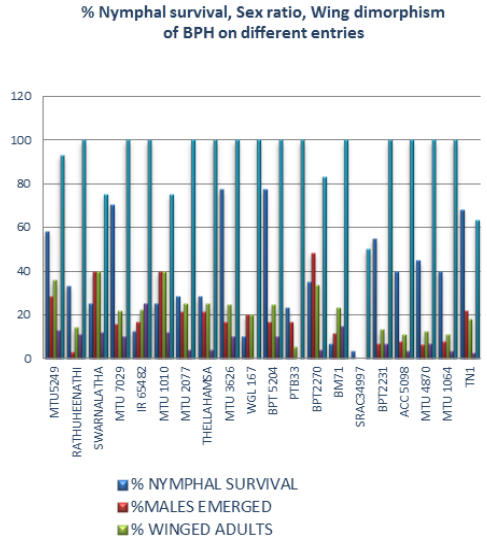
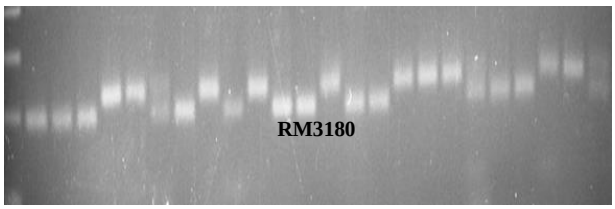


Figure 2. Histogram comparison of Nymphal survival rate.



1) Rathuheenathi 2) PTB33 3) ARC10550 4) T12
5)Swarnalatha 6) IR65482 7) Chandan 8) BPT1768
9) HR12 10) MTU2077 11) BPT2270 12) Manoharsali 13) Sivasinapu
14) MTU 5249 15) BPT 2231
16) MTU1010 17) WGL 167 18) MTU1001 19) BM71 20) ACC2398
21) ACC5098 22) TN1
23) BPT5204 24) Swarna

Figure 3. Genetic variation using RM3180 marker, linked to BPH6 resistance gene.

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