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Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods

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Twenty eight (28) rice genotypes were used to evaluate the genetic variability based on known BPH resistant loci spread through most of the genome (chromosomes 2, 3, 4, 5, 6, 8, 10, 11 and 12), using closely linked simple sequence repeat (SSR) markers and by different phenotypic screening methods. A total number of 155 alleles were detected by 30 polymorphic markers with an average of 4.6 per locus. The genetic diversity, polymorphic information content (PIC) ranged from 0.15 to 0.89 and 0.13 to 0.88, respectively and the allele frequency ranged from 0.21 to 0.89. These microsatellite markers linked to BPH resistance loci classified rice genotypes into three clusters with additional sub groups and sub sub groups. Our study reveals high genetic variation and clear genotypic relationship for BPH resistance based on BPH resistance linked markers and known phenotypic screening methods such as standard seedbox screening technique, honey dew test and nymphal survival method. Phenotypic evaluation showed clear distinction between resistant and susceptible type by clearly revealing moderately resistant types as well. Combined use of phenotypic and genotypic evaluation methods can improve the efficiency of marker assisted selection and utilization of resistant genotypes for crop improvement by rice breeders.

Key words: *Nilaparvata Lugens*, microsatellite markers, polymorphism, genetic diversity.

INTRODUCTION

Rice productivity is adversely impacted by numerous biotic and abiotic factors. An approximate 52% of the total global production of rice is lost annually owing to the damage caused by biotic factors, of which nearly 21% is attributed to the attack of insect pests (Brookes and Barfoot, 2003). Among the biotic stresses, the brown plant hopper (BPH) *Nilaparvata lugens* (Stal.) is one of the most destructive monophagous insect pest, one of the main biotic constraint of rice productivity causing huge yield losses every year in rice grown throughout tropical, subtropical and temperate areas in Asia (Park et al., 2008).

These insects draw nutrients from the phloem of rice plants. High BPH populations can destroy a plant in a short period of time (Huang, 2001, Yang et al., 2002). Large number of plant hoppers causes the infested plants

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Abbreviations: BPH, Brown planthopper; **cM,** Centimorgan; **PIC,** polymorphic information content; **GD,** genetic diversity; **AF,** allele frequency.

to become brown and dry. The condition is called hopperburn. Even if the planthopper population is not high enough to kill the plants, BPH feeding may considerably reduce yields (Watanabe et al., 1997). BPH can consume more than 28% of the total dry matter of rice plants infested at reproductive stage (Sogawa et al., 1994). BPH also transmits serious viral diseases, such as grassy stunt (Rivera et al., 1996) and ragged stunt virus (Ling et al., 1978). Development of resistant rice cultivars through host plant resistance is generally considered to be the most economic and effective way for controlling BPH damage. 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 (Ford-Lloyd et al., 1997; Virk et al., 2000; Song et al., 2003; Ram et al., 2007). In contrast to morphological traits, molecular markers can reveal differences among genotypes at DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Among all DNA markers microsatellites (Wu and Tanskley, 1993; Yang et al., 1994) 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 (Temnykh et al., 2000; McCouch et al., 2002; IRGSP, 2005). 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 (McCouch et al., 2001; Yu et al., 2003; Garris et al., 2005).

BPH populations on rice have been categorized in to four biotypes (Khush et al., 1985). The population in the east and Southeast Asia is reported as biotype 1, while biotype 2 originated in Indonesia and Vietnam as dominant biotype (Khush, 1979). Biotype 3 was produced in the laboratory at the International Rice Research Institute IRRI (Pathak and Khush, 1979) and in Japan (Ikeda and Vaughan, 1991) 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 (Zhang, 2007; Fujita et al., 2008). The objective of the present effort was to evaluate the genetic variability among different rice genotypes based on known BPH resistant loci using closely linked simple sequence repeat (SSR) markers and by different phenotypic screening methods.

MATERIAL AND METHODS

Plant material

The experimental material consisted of 28 elite rice genotypes available at Barwale Foundation, viz; 1B, 2B, 7B, 8B, 9B, 14B, 16B, 18B, 21B, 22B, 24B, 25B, 28B, 30B, 36B, 40B, 41B, 44B, IR129, 1R150, IR157, IR168, Swarna, TN1, BPT5204, Pokkali, and PTB33, where TN1 and PTB33 were used as susceptible and resistant check, respectively. The genotypes ranging from land race to improved lines, showed varied response to brown plant hopper stress.

Screening for BPH resistance

Initial population of BPH was collected from Maharajpet farm, Barwale Foundation and mass rearing of BPH and screening was done by following three methods, (i) standard seed box screening technique (SSST) developed at IRRI by Heinrichs et al. (1985), (ii) Honeydew test and (iii) Nymphal survival method.

Standard seed box screening technique

The experiment was conducted at a temperature of 28 to 30°C and relative humidity of 70 to 80%. The seeds were presoaked and sown in rows in 60 x 45 x 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. When more than 90% of susceptible check shows wilting, the plants were scored individually based on scoring system proposed by the International Rice Research Institute (IRRI, 1996) and each seedling 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 (Figure 1). 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 (IRRI, 1996).

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 (Auclair, 1959; Liu et al., 1994). Many techniques have been developed to measure the feeding response of Nilaparvata lugens on resistant and susceptible rice plants (Paguia et al., 1980; Pathak et al., 1982; Begum and Wilkins, 1998). The more important were the test of filter paper dipped in a solution of bromocresol green and the test of a parafilm sache. For our work, the filter paper technique was used. The plant at first tiller (40 days old) was located through two holes of the cup (up and down of the cup (5×5: H×R). The filter was placed at the base inside of the cup with a paper protecting it from humidity of the soil. For each plant to be screened, five female gravid hoppers 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-rimmed spots (indicate susceptible plants) and xylem-based honeydew as transparent (indicate resistant plants). The area of each spot on the bromocresol green-filter paper was measured using a digital scanner and "Image J" software (Figure 2).

Nymphal survival method

The nymphal survival test shows the difference of the survival of the nymphs on different varieties of rice plants. For this, 20 newly hatched nymphs in a pot with three rice plants (40 days old) were placed inside the mylar cages (45×5: H×R). The number of surviving nymphs was recorded every two days until they became adults (15 days). The experiment was carried in three replications and control plants were also maintained (Figure 3). Plants on which insects were released as well as control plants were cut till the base

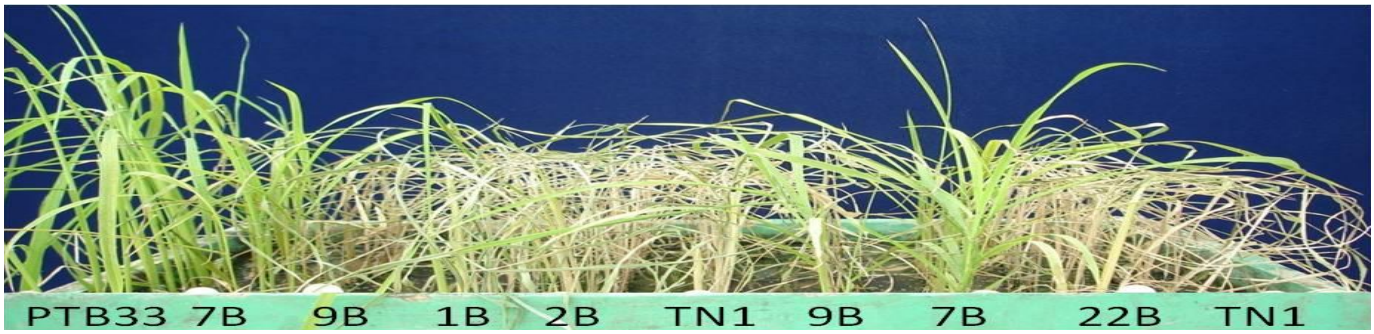

Figure 1. Rice genotypes showing varied resistance levels for brown planthopper in standard seed box screening technique

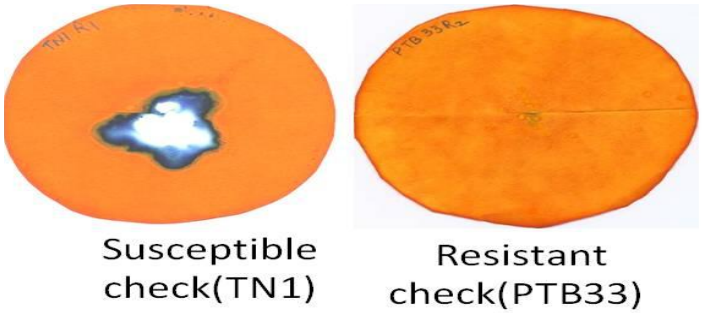

Figure 2. Amount of honeydew excreted by brown planthopper on susceptible and resistant checks.

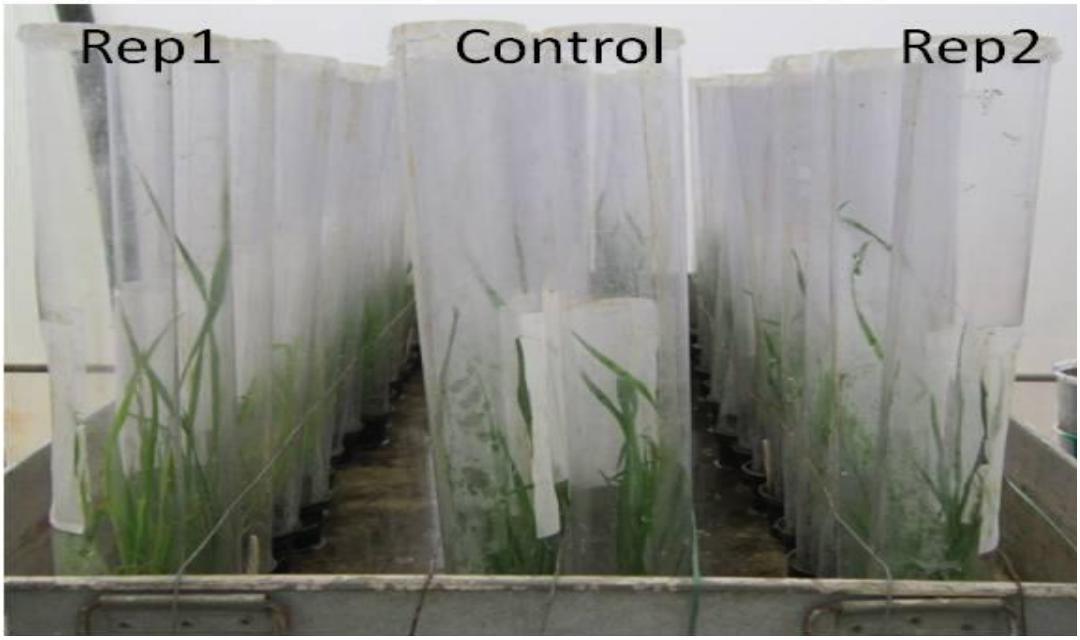

Figure 3. Experimental set up for screening rice genotypes by nymphal survival method.

of the stem and dried at 55°C for one week and biomass of infested plants and control plants were weighed. Number of insects surviving on individual genotype was counted.

DNA extraction, PCR amplification and PAGE

Total cellular DNA was isolated by rapid DNA extraction protocol

(Micklos and Freyer, 1990) and the quality of DNA was checked on agarose gel (0.8% w/v). Thirty four (34) SSR markers reported to be linked to 22 BPH resistant genes and quantitative trait locus (QTL) on chromosomes 2, 3, 4, 5, 6, 19, 11 and 12 were used for this study (Table 1). SSR primers were obtained from Sigma Aldrich, Bangalore. The PCR reaction was performed in 15 µl volume using Eppendorf gradient thermocycler. The reaction mixture contained 10 ng/µl template DNA, 5 pM each forward and reverse primers, 10 mM each dNTPs, 2 µl 10X PCR buffer (10 mM Tris HCl, 50 mM KCl, 0.01 mg/ml gelatin and 1.5 mM $MgCl_2$) and 0.5 µl of 5 U/ µl Taq polymerase. Thermal cycler was programmed to 1 cycle of 5 min at 94°C, 35 cycles of denaturation at 95°C for 1 min, 30 s for annealing temperature (55 to 60°C depending on the marker used) and 40 s at 72°C for primer elongation. Finally, 1 cycle of 7 min at 72°C was used for final extension. Amplified products were stored at -20°C until further use. The reproducibility of the amplification products was checked twice for each primer. SSR analysis was carried out by polyacrylamide gel electrophoresis following the protocol described by Paunaud et al. (1996) with some modifications.

Allele scoring and data analysis

All the genotypes were scored for the presence and absence of SSR bands and the data were entered in to a binary matrix as discrete variable, 1 for presence and 0 for the absence of the allele. Polymorphic information content (PIC) values were estimated using the formula: PIC = 1- ∑pi2, where pi is the frequency of the allele at each locus (Botstein et al., 1980). A Neighbor joining tree with bootstrap values was constructed with the help of Power marker Version 3.25 (Liu and Muse, 2005).

RESULTS

Phenotyping of rice genotypes for brown planthopper resistance

Standard seed box screening technique

The results of phenotypic response of rice genotypes to brown plant hopper screening at seedling stage (10 hoppers per seedling) indicated varied genotypic responses. Among all the 28 rice genotypes, PTB33 widely used as donor parent for BPH by rice breeders consisting of *Bph2* and *Bph3* genes (Khush, 1979) and Pokkali reported to have *Bph9* gene (Murata et al., 2001), scored as 1 and 3 respectively, and TN1 showed a score of 9 (Table 2).

Honey dew test

The amount of phloem and xylem in the honey dew excreted by the insect in the genotype was measured in mm^2 units (Figure 4). Among all the genotypes, TN1 has shown high rate of phloem consumption indicating that it is highly susceptible to brown planthopper/sucking pests; Whereas, insects on PTB33 showed least level of phloem consumption indicating its resistance. Genotypes like 1B, 2B, 9B, 21B, 22B, BPT5204, Swarna, showed high rate of phloem consumption. Genotypes like 7B, 36B, 41B, 44B, IR129, IR168, IR150, IR157, Pokkali and genotypes like 14B, 16B 18B, 28B, 24B, 25B, 40B, 29B, 30B and 8B showed high to moderate levels of xylem consumption (Figure 2). The rate of phloem and xylem consumption by the genotypes was inversely proportional based on correlation of phloem consumption versus susceptibility and xylem consumption versus resistance.

Nymphal survival method

Numbers of nymphs surviving on PTB33 were less compared to other genotypes indicating its high level of resistance against brown planthopper, whereas large number of nymphs survived on TN1, indicating its susceptibility towards brown planthopper. Genotypes like 1B, 2B, 9B, 21B, 22B, BPT5204, Swarna, showed more number of insects and 7B, 36B, 41B, 44B, IR129, IR168, IR150, IR157, Pokkali and genotypes like 14B, 16B 18B, 28B, 24B, 25B, 40B, 29B, 30B and 8B showed less to moderate count of live insects (Figure 5), including number of nymphs surviving on each genotype, biomass of control plants and biomass of infested plants was recorded. All genotypes under control conditions were showing higher biomass compared to plants infested with insects. Among the infested plant, PTB33 was showing highest biomass while TN1 was showing lowest biomass after infestation among the genotypes (Figure 6).

Genotypic scoring

Thirty-four microsatellite or SSR markers reportedly linked with genes conferring BPH resistance were used to characterize and assess genetic diversity among 28 rice genotypes having varied response to brown plant hopper stress, ranging from parental lines, introgressed lines to donors. Out of the 34 markers used, four markers produced monomorphic bands while 30 markers showed polymorphism by revealing 155 alleles. The number of alleles per locus varied from 2 (RM459, RM496, RM261, RM6308, RM185) to 12 (RM8213) with an average of 4.6. The lowest amplicon size was produced by RM459 (75 bp) while highest amplicon size belonged to RM335 (315 bp). Many studies have also reported significant differences in allelic diversity among various microsatellite loci (Thompson et al., 2009). The PIC value measures the probability that two randomly chosen alleles from a population are distinguished. The PIC averaged 0.48 ranging from 0.13 (RM261) to 0.88 (RM8213). Markers (50%) were highly informative to slightly informative (pic > 0.5, informative markers). The polymorphic pattern of RM277, RM3331 and RM510 markers in 28 rice geno-types are presented in Figure 7. The gene diversity (GD) ranged from 0.15 to 0.89. The polymorphism Information Content (PIC) ranged from 0.13 to 0.88 and Allele Frequencies (AF) ranged from 0.21 to 0.89 (Table 3).

Genetic variation using SSR markers linked to BPH resistance genes

The microsatellite markers were able to distinguish

Table 1. Details of microsatellites used in the study.

Marker	Chr	Gene/ QTL	cM	Start end	Clone	Reference(s)
RM154	2	Qbp3	1.05	1083810	AP005851	Ren et al. 2004
RM3355	2	Qbph2	95.2	25,728,952	AYO20028	Sun et al. 2007
RM177	2	Qbp3	22.35	22379610	AL662937	Ren et al. 2004
RM410	3	QTL	17.6	17642689	AP005676	Sonada et al. 2003
RM7	3	Qbph3	64	38.6	AF344009	Sun et al. 2005
RM6308	3	Bph19(t)	7.15	7160259	AC137696	Chen et al. 2006
RM185	4	Bph12(t)	18.55	18564318	AL662977	Yang et al. 2002
RM8213	4	Bph17(t)	4.4	4433168	AL662959	Sun et al. 2005
RM335	4	Bph12(t)	21.5	679883	AL606616	Yang et al. 2002
RM261	4	Bph12(t),Bph15	6.55	6558954	AL607008	Yang et al. 2002 & 2004
RM459	5	QTL	20.15	20155973	AC130607	Sonada et al. 2003
RM589	6	Bph3	1.35	1380856	AP001168	Jairipong Jairin et al. 2007
RM510	6	Qbph6	2.8	2831433	AP006533	Sun et al. 2007
RM190	6	Bph3	1.75	1764576	AP002542	Jairipong Jairin et al. 2007
RM8072	6	Bph3	1.4	1408326	AP001168	Jairipong Jairin et al. 2007
RM217	6	bph4	15	18.2	AF344038	Kawaguchi et al. 2001
RM225	6	bph4 BPH22(t)	3.4	3416523	AB023482	Kawaguchi et al. 2001 Sai Harini et al. 2010
RM588	6	Bph3	1.6	1611388	AP00391	Jairipong Jairin et al. 2007
RM19291	6	Bph3	1.2	1215874	AP003456	Jairipong Jairin et al. 2007
RM314	6	Qbph6	33.6	5413067	AF344139	Sun et al. 2007
RM496	10	Qbph10	22.1	22171961	AC087599	Sun et al. 2005
RM216	10	Qbph10	5.5	5102292	AC098566	Jairipong Jairin et al. 2005
RM209	11	Bph10	17.75	17771745	AC136150	Jena et al. 2002
RM3331	12	QTL	23.45	23460817	AL845346	Sonada et al. 2003
RM277	12	Qbph12	57.2	18100739	AF344103	Jairipong Jairin et al. 2005
RM1986	12	QTL	21.2	21212965	AL713932	Sonada et al. 2003
RM6869	12	Bph18(t)	22.2	22219502	AL731741	Jena et al. 2006
RM463	12	Bph18(t)	22	22091957	AL731742	Jena et al. 2006
RM50	6	Qbph6	21	29500000	AF343859	Jairipong Jairin et al. 2005
RM5953	4	Qbph4 Bph20(t)	9.35	9364068	AP662989	Sun et al. 2005 Rahman et al. 2009
RM5479	12	Bph21(t)	57	24378959	AL844880	Rahman et al. 2009
RM313	3	Qbph3	20	38.8	A02032481	Sun et al, 2005
RM484	10	Qbph10	20.8	20808556	AC073166	Sun et al. 2005
RM3134	3	Bph19(t)	7.25	7220514	AC134229	Chen et al. 2006

Table 2. Rice genotype ID, code and score for brown plant hopper resistance using standard seedbox screening technique.

Genotype ID	Code	Score	Genotype ID	Code	Score
IR58025B	1B	8.3	IR73793B	30B	6.9
IR62829B	2B	7.3	IR68886B	36B	5.8
IR68888B	7B	5.5	IR79156B	40B	5.6
IR68892B	8B	6.1	IR80151B	41B	5.8
IR68897B	9B	7.9	IR80156B	44B	5.6
IR69628B	14B	6	IR65482-7-216-1-2B	IR129	4.32
IR70369B	16B	6.7	IR73680-4-5-10-2-1-2	IR150	4.3
IR70959B	18B	6.6	IR71033-121-15	IR157	4.7
IR72078B	21B	8.5	IR73885-1-4-3-2-10	IR168	5.8

Table 2. Contd.

IR72080B	22B	8	MTU7029	SWARNA	9
IR72018B	24B	6.6	Thaichung native1	TN1	9
IR73320B	25B	6.8	Samba Mahsuri	BPT5204	9
IR73327B	28B	6.7	Traditional variety	PTB 33	1
IR73328B	29B	6.3	Landrace	Pokkali	3

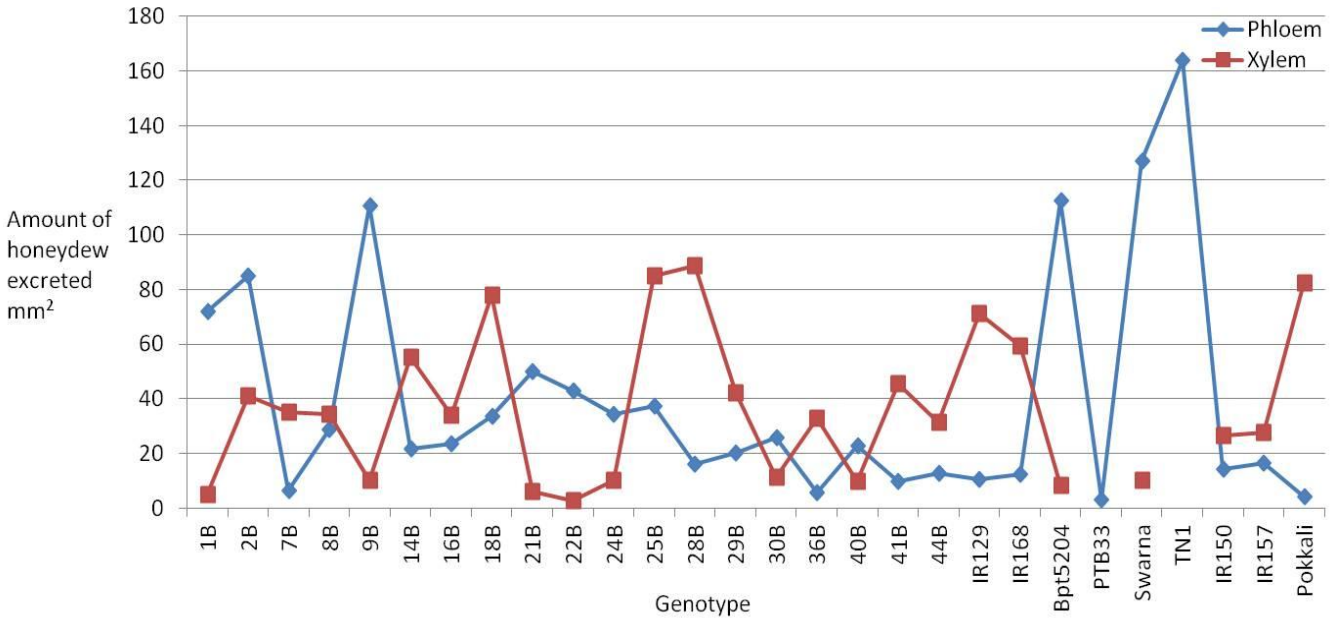

Figure 4. Amount of phloem and xylem excreted in honeydew by brown planthopper feeding on different rice genotypes.

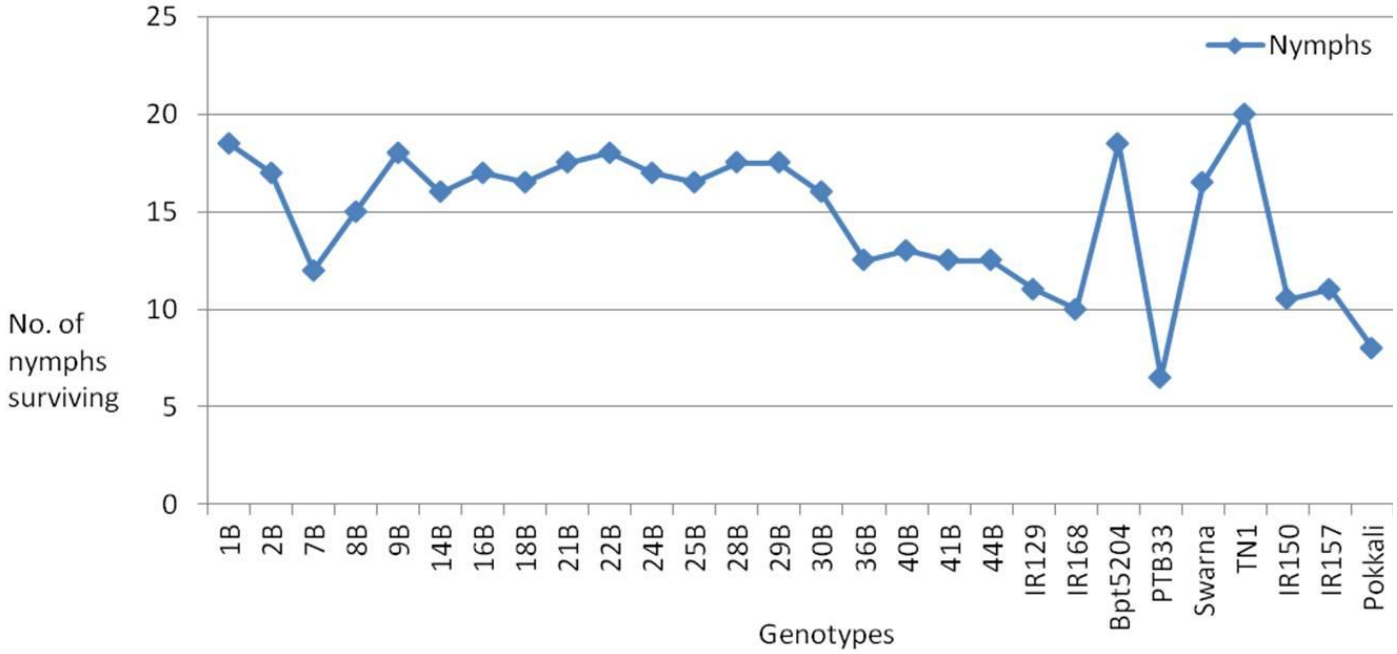

Figure 5. Number of nymphs surviving after 15 days on different rice genotypes.

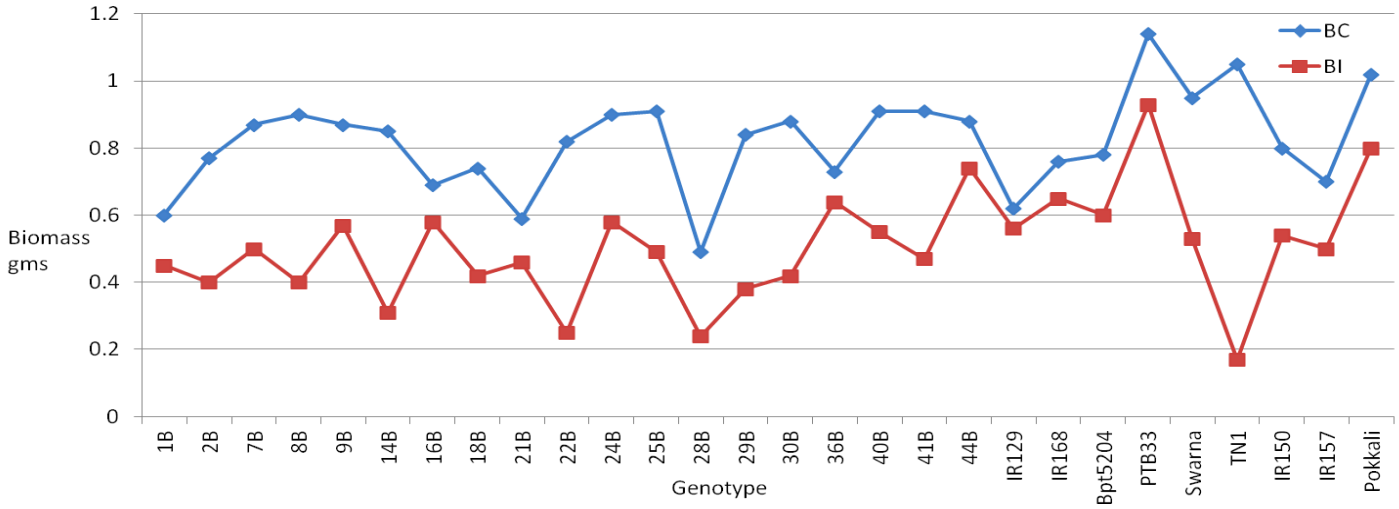

Figure 6. Amount of biomass in control and infested plants of rice genotypes in nymphal survival method.

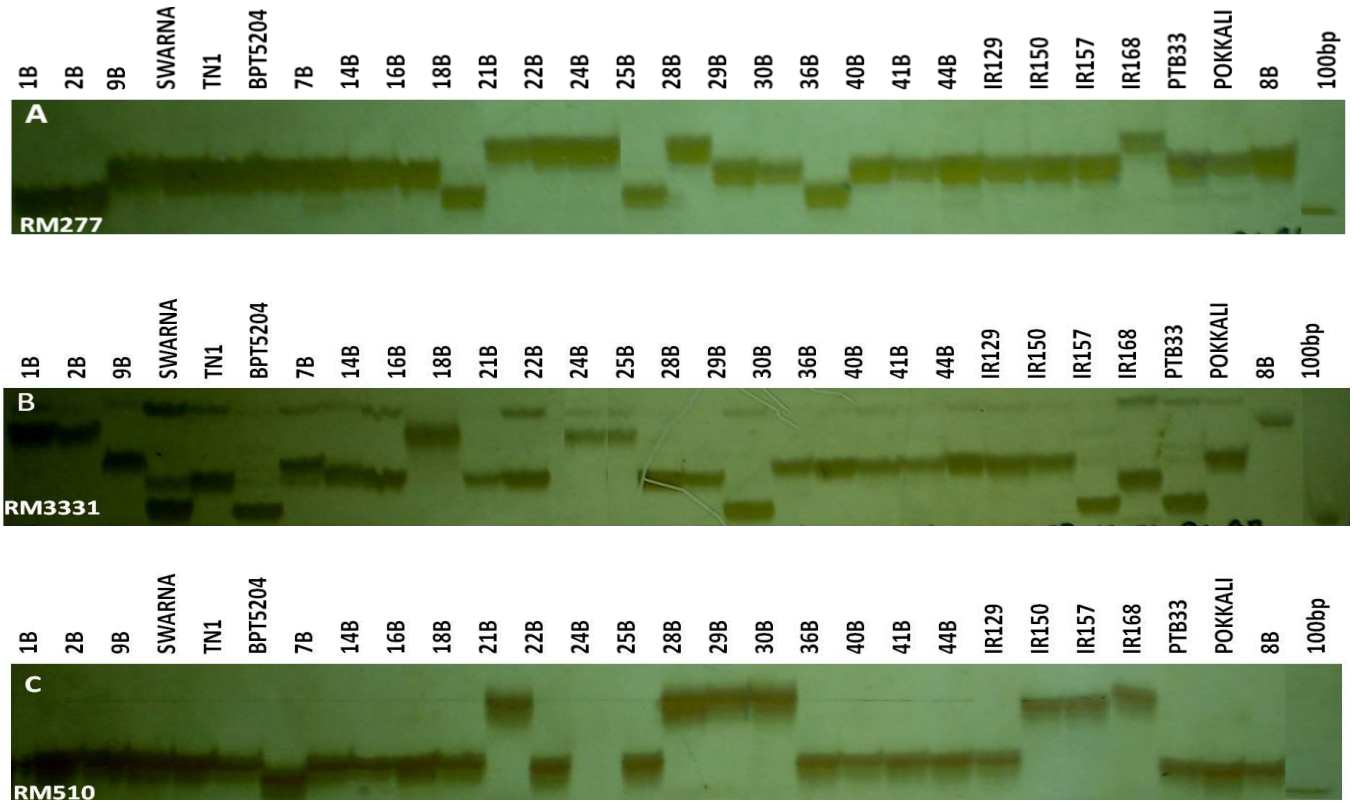

Figure 7. SSR banding patterns of 28 rice genotypes from RM277 (A), RM3331 (B), RM510(C).

between rice genotypes. The high degree of polymorphism of microsatellite allows rapid and efficient identification of rice genotypes. The microsatellite markers classified rice genotypes and the genetic relationships demonstrated among the genotypes in the neighbor joining tree (Figure 8) revealed some subgroups based on BPH resistant loci spread through most of the genome (chromosomes 2, 3, 4, 5, 6, 8, 10, 11, 12). The 28 genotypes could be easily categorized into three clusters. Moving closer in terms of similarity, sub-divisions of such groups can reveal sub-sub groups. Cluster 1 included genotypes showing moderate resistance, cluster 2 is comprised of two sub clusters. Sub cluster 2.1 included all moderately resistant genotypes and sub cluster 2.2 comprised of two sub-sub groups A and B. Sub-sub group A included moderate resistance genotypes where as sub-sub cluster

Table 3. Total number of alleles, allele frequency, allele length in bp, genetic diversity, and polymorphism information content (PIC) value of 34 SSR markers assayed in 28 rice genotypes.

Marker	Major AF	Number of alleles	Genetic diversity	PIC	AL in (bp)	AT (°C)
RM3331	0.48	6	0.70	0.69	120-150	55
RM6308	0.78	3	0.36	0.35	120-125	55
RM177	0.67	3	0.46	0.38	190-240	55
RM261	0.98	2	0.15	0.13	150-160	55
RM6869	0.51	5	0.63	0.59	120-140	55
RM314	0.76	4	0.48	0.45	118-140	55
RM209	0.53	4	0.56	0.36	145-165	55
RM589	0.25	8	0.84	0.81	170-200	55
RM19291	0.53	5	0.62	0.53	160-170	55
RM185	0.69	3	0.44	0.42	190-205	55
RM510	0.75	4	0.51	0.47	120-155	55
RM154	0.3	8	0.83	0.81	170-230	55
RM277	0.75	4	0.51	0.52	120-240	55
RM588	0.46	4	0.64	0.63	125-145	55
RM190	0.57	5	0.69	0.66	110-150	55
RM3355	0.32	8	0.84	0.77	205-230	55
RM410	0.25	8	0.87	0.86	180-295	55
RM496	0.89	2	0.19	0.17	190-300	55
RM217	0.73	4	0.52	0.47	130-145	55
RM463	0.5	3	0.53	0.48	195-200	55
RM1986	0.58	5	0.59	0.47	155-200	55
RM216	0.32	7	0.77	0.77	145-165	55
RM8213	0.21	12	0.89	0.88	155-205	58
RM8072	0.82	3	0.30	0.30	120-160	55
RM335	0.25	9	0.85	0.84	170-315	57
RM459	0.82	4	0.31	0.29	75-150	55
RM225	0.57	5	0.71	0.65	120-155	55
RM7	0.58	4	0.59	0.40	120-125	55
RM50	0.42	6	0.71	0.66	195-270	55
RM5953	0.33	3	0.67	0.62	110-115	55
RM5479	1.00	1	0.00	0.00	129-129	55
RM3134	1.00	1	0.00	0.00	175-175	55
RM313	1.00	1	0.00	0.00	111-111	55
RM484	1.00	1	0.00	0.00	299-299	55
Mean	0.61	4.6	0.52	0.48		

B comprised of resistance genotypes. All the susceptible genotypes were grouped in to cluster 3. Evidently, resistant genotypes (PTB33 and Pokkali) are undoubtedly distinct from moderately resistant genotypes (7B, 8B, 14B, 16B, 18B, 24B, 25B, 28B, 29B, 30B, 36B, 40B, 41B, 44B, IR129, IR150, IR157, IR168) and susceptible genotypes (1B, 2B, 9B, 21B, 22B, Swarna, BPT5204, TN1). The same association/relatedness is depicted by neighbor joining tree (Figure 8).

DISCUSSION

Among all the insect pests, brown planthopper, is one of the most destructive pests of rice causing severe yield losses. Identification of donors for resistance and efficient screening techniques for evaluating breeding lines plays crucial role to transfer BPH resistance genes in to high yielding popular varieties and a high level of genetic diversity reduces the risk of wide spread epidemics of pests and diseases (Zhu et al. 2000; Newton et al. 2009).

The seedling resistance and antibiosis effects (honey dew test and nymphal survival method) of 28 rice genotypes on BPH were examined in this study. High resistance (Score 1) was shown by PTB33, followed by Pokkali (Score 3) and TN1 was found to be highly susceptible (score 9) using the three methods (SSST, honeydew test, and nymphal survival method) towards brown plant hopper infestation. Genotypes like 7B, 36B,

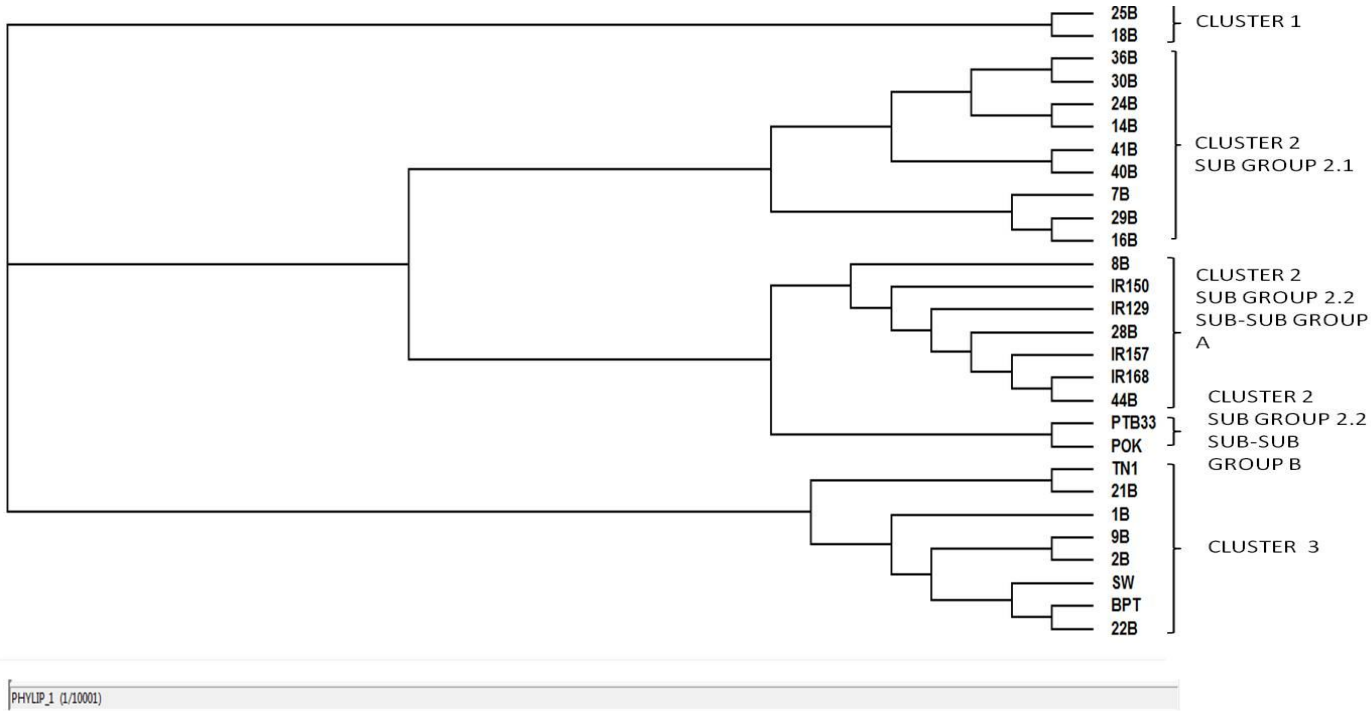

Figure 8. Cluster diagram based on similarity matrix calculated from 28 rice genotypes detected by 30 SSR markers.

41B, 44B, IR129, IR168, IR150, IR157 were showing upper grade of moderate resistance with score ranging between 3.1 to 5.8. Similarly, antibiosis methods revealed these genotypes to be moderately resistant towards brown plant hopper by consuming very low levels of phloem and high amounts of xylem as well as showing low amount of biomass consumption and low nymphal survival rate. Genotypes like 14B, 16B 18B, 28B, 24B, 25B, 40B, 29B, 30B and 8B were showing lower grade of moderate resistance with scores ranging between 6 to 6.9 and consuming phloem to some extent and later shifting to xylem consumption with moderate level of biomass consumption and nymphal survival. Rice genotypes like 1B, 2B, 9B, 21B, 22B, BPT5204, Swarna were highly susceptible (Score 7 to 9) feeding only on high amounts of phloem and high levels of biomass consumption and more number of nymphs.

Using 30 polymorphic SSR markers, total number of alleles (155) and the average number of alleles per locus (4.8) detected were higher than previously described for *Oryza sativa* genotypes (Vijaya Lakshmi et al., 2010; Narshimulu et al., 2010). Using 37 SSR markers, Vijaya Lakshmi et al. (2010) found 88 alleles with an average of 2.37, while Narshimulu et al. (2010) reported 96 alleles with an average of alleles per locus 2.67. The PIC values ranged from 0.13 to 0.88 which are similar (0.12 to 0.83) to those reported for genetic diversity assessment in rice genotypes (Pervaiz et al., 2010). Using high informative markers, indicated by their high PIC values maximized the probability of detecting high proportion of allelic variation at the individual loci. The PIC values in this study were comparable to those reported in some studies (Jain et al., 2004; Saini et al., 2004; Siwach et al., 2004; Lu et al., 2005; Jayamani et al., 2007; Thomson et al., 2007) but higher than those reported by Singh et al. (2004) and Joshi and Behera (2006). It is to be noted here that other similar studies involve whole-genome markers, while in our study, high PIC values are obtained by BPH resistance loci-specific markers, otherwise expected to reveal limited polymorphism. The alleles revealed by markers showed a high degree of polymorphism, with all markers used (excluding 2, RM496 and RM459) producing 100% polymorphic bands. This amply suggests that the selected set of SSRs used in this study were extremely informative and effective for assessing the genetic diversity at specific BPH resistance loci, where a reduced level of genetic variability is generally expected.

Genetic diversity of rice genotypes has been studied based on SSR markers linked to BPH resistance genes. The tree showed a close relationship between resistance and moderately resistant genotypes based on BPH resistance loci linked marker analysis. However, phenotyping methods could clearly differentiate rice genotypes categorized as resistant and moderately resistant genotypes. The same relatedness between resistant (R) and moderately resistant (MR) types is clearly depicted by tree as well, wherein susceptible (S) types have branched separately. Cluster analysis of the 28 rice genotypes based SSR marker data divided genotypes in to three clusters

(Figure 8) with additional sub groups within each group. The parental phenotypic evaluation is in coincidence with genotypic cultivars using SSR markers.

Conclusively, evaluation of germplasm for resistance genes plays a major role in selection of parental lines and development of new breeding material. Information on target loci obtained from markers will facilitate use of germplasm efficiently. In addition to evaluation of genetic variation at specific loci associated with BPH resistance, the genotypes were taken up for phenotypic screening for BPH resistance and the score was correlated with genotype groups for resistance reaction. Our study revealed high genetic variation and clear genotypic relationship in NJ tree generated by Power Marker. The results further indicate that since the SSR markers are neutral and co-dominant, they are powerful tools to access the genetic variability of the cultivars under study. Use of marker and phenotype information together offers an efficient tool to the breeders in selecting parents for various breeding programs *viz.* mapping and marker assisted selection (MAS).

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