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Genetic diversity and molecular characterization of brown planthopper resistant rice cultivars

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ABSTRACT

Eight rice genotypes were used for the molecular characterization and evaluation of genetic diversity using 62 SSR primers. Forty four primers revealed polymorphism among the genotypes. The number of alleles varied from 1-3 with a mean of 1.89. Cluster analysis based on SSR data revealed that four brown plant hopper (BPH) resistant genotypes viz., BM 71, PTB 33, Sivasinapu and SRAC 34997 formed one cluster while four susceptible genotypes viz., BPT 5204, MTU 7029, MTU 4870 and PLA 1100 formed another cluster. The susceptible (BPT 5204) and resistant (SRAC 34997) genotypes were clustered at two extremes based on similarity coefficient indices. The SSR primer, RM 223 co-localized with QBphr 8 on chromosome 8, RM 190 with QBphr5b on chromosome 6 and RM 6869 with Bph 18(t) on chromosome 12 amplified resistant specific allele in four resistant cultivars giving scope for utilization of these markers in marker-assisted selection after confirming linkage of these markers with resistance and susceptibility to BPH.

Key words: *rice, genetic diversity, molecular characterization, markers, brown planthopper, resistance*

Brown planthopper (BPH) is one of the most devastating pests of rice and yield loss can be up to 60% (Panda and Khush, 1995). The use of resistant varieties is an economical and effective way to control BPH. Consistent efforts have been made to identify genes for BPH resistance from various sources to develop resistant varieties. Information regarding genetic diversity at molecular level using DNA based molecular markers could be used to identify and characterize genetically unique germplasm that compliment existing cultivars.

Twenty one BPH resistance genes (Brar *et al.,* 2009, Rahman *et al.,* 2009, Shantalakshmi *et al.,* 2010) have been reported of which *Bph1*, *Bph 2*, *Bph 9*, *Bph 10(t)*, *Bph 18(t)* and *Bph 21(t)* were mapped on chromosome 12. Four additional resistance genes *Bph 4*, *Bph 11(t), Bph 12(t)* and *Bph 13(t)* were assigned to chromosome 6, 3, 4 and 2 (Chag-Chao *et al.*, 2006) that were identified in *indica* rice cultivars and two of its wild relatives. Microsatellite markers are cost effective and abundant in rice (Chen *et al.,* 1997). These are used for evaluating genetic diversity among closely related cultivars (Akagi *et al.,* 1997). Hence, in the present investigation eight genotypes were characterized using SSR markers to study the level of genetic diversity and to establish genetic relationship among the genotypes.

MATERIALS AND METHODS

Eight rice genotypes screened for BPH resistance based on standard evaluation system (SES) (IRRI, 1986) at Maruteru, Andhra Pradesh were used for the molecular characterization and diversity analysis during 2009. The genotypes were sown in pots under sterile conditions in green house. Healthy leaf samples were collected from 18 days old seedlings and used for genomic DNA isolation.

Genomic DNA was extracted following the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The quality and quantity of DNA was estimated using eight channel spectrophotometer. Sixty two SSR primers distributed

among different chromosomes were selected randomly and used for PCR amplification. PCR amplifications were performed in 10 µl of reaction mixture containing 1 µl of 10X buffer with $MgCl_2$, 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/micro liter), 3 µl of template DNA (10 ng/ µl) and 2.5 µl of sterile distilled water. The polymerase chain reaction was performed by using Eppendorf thermo cycler with following temperature profiles:the initial denaturation was 94°C for 5 min followed by 35 cycles of denaturing at 94°C for 0.5 min annealing at 55° C for 0.5 min, extension at 72°C for 1.0 min and final extension of 7 minutes at 72°C. The PCR products were electrophoresed with ethidium bromide (10mg/ml) at 100 volts for 2 hrs in 1X TBE buffer. A 100 bp ladder (Banglore Genei Pvt. Ltd.)) was used for appropriate sizing of the products. The gel was photographed under UV light using Ingenius gel doc system.

SSR alleles were scored sequentially from the largest to the smallest size based on their position relative to the ladder. Each allele/fragment was scored as 1 or 0 depending on its presence or absence. Polymorphic information content (PIC) values were calculated as follows (Chattopadhyay *et al*., 2008)

$$PIC = 1/n \text{ s } 2f(1-f)$$

Where, f = proportion of a particular allele among the genotypes and n = number of alleles recorded for a particular primer.

The genetic associations among 8 genotypes of rice were evaluated using Jaccard's similarity co-efficient and clustered with unweighted pair-group method of arithmetical averages (UPGMA) analysis (NTSYS 2.0). Power marker 3.25 package was used for the cluster analysis.

RESULTS AND DISCUSSION

The reactions of different entries based on SES were as follows, BPT 5204, MTU 7029, PLA 1100 susceptible (all with score-9); MTU 4870 moderately resistant (score 5) and BM 71, PTB 33, Sivasinapu, SRAC 34997 resistant (all with score 1). Out of 62 SSR amplified markers, 45 markers were polymorphic among the eight genotypes. These 62 markers produced alleles ranging from 1 to 3 with an average of 1.89 and used to analyze the variation among the genotypes. A high degree of polymorphism was obtained with the primers RM 230, RM 252, RM 6615, RM 1246, RM 6953 with 3 alleles (Table 1) and 39 primers with two alleles. RM 190, RM 223, RM 6869 showed distinct polymorphism between resistant and susceptible genotypes (Fig. 1). Chakravarthi and Rambabu (2006) and Pratyusha *et al.* (2009) also reported significant difference in allelic diversity among various microsatellite loci in rice. All these markers showed an average PIC value of 0.5921 with a range of 0.3011 – 0.8750 (Table 2).

The similarity index values varied from 0.000 to 1.000, indicating the presence of wide range of genetic variability at molecular level among the 8 genotypes (Table 2). Chakravarthi and Rambabu (2006) and Pratyusha *et al.* (2009) also reported a wide range of genetic diversity using SSR markers at molecular level in germplasm lines of rice.

The dendrogram (Fig. 2) generated using pooled data divided the 8 genotypes into two major clusters at 47% level of genetic similarity. Four resistant genotypes *viz.,* BM 71, PTB 33, Sivasinappu and SRAC 34997 which are closely related formed one cluster, while four susceptible genotypes *viz.,* BPT 5204, MTU 7029, MTU 4870 and PLA 1100 formed another cluster. The susceptible (BPT 5204) and resistant (SRAC 34997) genotypes were clustered at two extremes with genetic similarity coefficient of 0.33 (Table 2).

Major allele frequency among 8 genotypes ranged from 0.25 to 0.75 with mean of 0.51 and genetic diversity mean of 0.49 (Table 2). The two polymorphic SSR markers RM 223 and RM 190 were found to be co-localized with QTL Q*Bphr8* on chromosome 8 while RM 190 with Q*Bphr5b* on chromosome 6 (Xu *et al.,* 2002). RM 6869 SSR marker was co-localized with *Bph 18(t)* gene on chromosome 12 (Jena *et al*., 2005) among the 4 resistant cultivars giving scope for utilization of these markers for foreground selection in marker assisted selection after confirming linkage of these markers with resistance and susceptibility to BPH.

The threat of BPH to rice has resulted in search of genes in the available germplasm showing resistance. The PCR based SSRs which are technically efficient, time and money saving used in this study would

Table 1. Details of polymorphic SSR markers

Marker	Chromosome number	Position CM	Allele No.	MajorAllele Frquency	Genetic Diversity	PIC
RM223	8	80.50	2	0.50	0.38	0.6047
RM182	7	61.00	2	0.50	0.38	0.6047
RM190	6	7.40	2	0.50	0.38	0.6047
RM225	6	26.20	2	0.35	0.30	0.3011
RM230	8	112.20	3	0.75	0.51	0.7706
RM252	4	99.00	3	0.75	0.63	0.8750
RM263	2	127.50	2	0.59	0.43	0.5374
RM6309	6	167.80	2	0.53	0.47	0.5589
RM490	12	104.30	2	0.53	0.30	0.5750
RM3226	1	51.00	2	0.59	0.43	0.6374
RM5479	12	74.10	2	0.50	0.30	0.6583
RM6615	12	109.20	3	0.64	0.52	0.7637
RM278	9	77.50	2	0.50	0.47	0.6589
RM7558	12	108.20	2	0.50	0.47	0.6589
RM185	4	50.80	2	0.50	0.47	0.6589
RM295	5	86.50	2	0.50	0.48	0.6047
RM316	9	1.80	2	0.30	0.30	0.3583
RM6953	12	104.30	3	0.73	0.53	0.8750
RM6396	12	97.30	2	0.50	0.30	0.3583
RM512	12	43.20	2	0.50	0.43	0.6374
RM502	8	121.80	2	0.50	0.43	0.6374
RM7003	12	41.80	2	0.50	0.43	0.6374
RM6296	12	26.70	2	0.66	0.51	0.5711
RM2529	12	47.60	2	0.50	0.38	0.5047
RM11	7	47.00	2	0.53	0.49	0.5711
RM336	7	61.00	2	0.50	0.49	0.5711
RM3246	12	48.20	2	0.70	0.38	0.6047
RM1337	12	51.50	2	0.25	0.30	0.3583
RM1246	12	65.30	3	0.63	0.51	0.8277
RM200	3	112.35	2	0.55	0.49	0.5711
RM8278	1	143.70	2	0.57	0.49	0.8698
RM3326	12	73.30	2	0.75	0.38	0.6047
RM6869	12	75.80	2	0.25	0.30	0.3583
RM229	11	77.80	2	0.25	0.47	0.3589
RM3331	12	89.50	2	0.65	0.38	0.5047
RM202	11	54.00	2	0.55	0.49	0.5711
RM2854	12	95.40	2	0.25	0.30	0.3583
RM1264	12	104.20	2	0.25	0.34	0.4800
RM228	10	110.70	2	0.50	0.43	0.5374
RM258	10	70.80	2	0.50	0.48	0.6047
RM4552	12	107.40	2	0.50	0.47	0.6589
RM1227	12	109.20	2	0.50	0.47	0.6589
RM216	8	17.60	2	0.50	0.30	0.6583
RM17	12	109.10	2	0.25	0.49	0.6711
Mean				0.51	0.49	0.5921

PIC - polymerphic information content

Table 2. Similarity Coefficients of rice genotypes

Rice Genotypes	BPT 5204	MTU 7029	BM 71	PTB 33	SIVASINAPU	SRAC 34997	MTU 4870	PLA 1100
BPT 5204	0.00							
MTU 7029	0.06	0.00						
BM 71	0.21	0.23	0.00					
PTB 33	0.22	0.24	0.12	0.00				
SIVASINAPU	0.29	0.31	0.14	0.14	0.00			
SRAC 34997	0.33	0.34	0.19	0.19	0.12	0.00		
MTU 4870	0.13	0.13	0.22	0.24	0.26	0.31	0.00	
PLA 1100	0.16	0.13	0.19	0.22	0.25	0.29	0.13	0.00

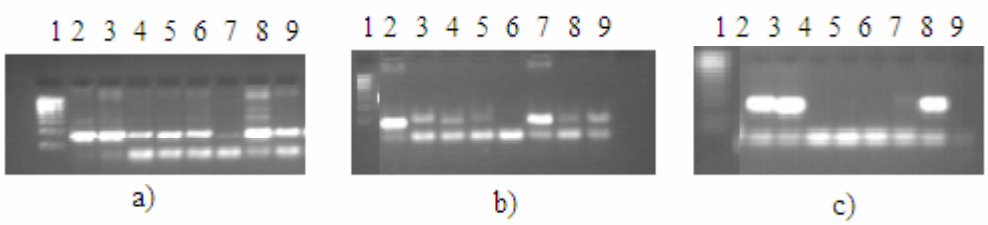

Fig 1. DNA profiles of eight rice genotypes with SSR primers a) RM 223, b) RM 190 and c) RM 6869
Lane : 1. DNA ladder, 2. BPT 5204, 3. MTU 7029, 4.BM 71, 5.PTB 33, 6.SIVA, SINAPU, 7.SRAC 34997, 8. MTU 4870 9. PLA 1100. (Resistant genotypes : Lane no. 4,5,6,7 Susceptible genotypes lane no. 2,3,8,9)

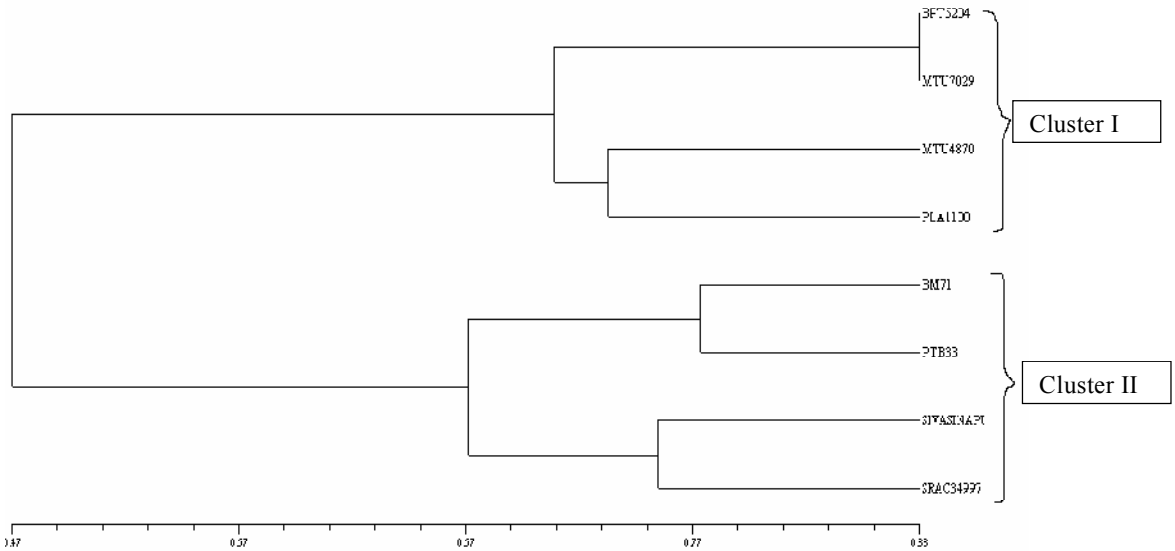

Fig. 2. Dendrogram showing genetic relationship between rice cultivars resistant/susceptible to BPH

promote the use of these resistance genes in marker-assisted selection programme for breeding new BPH resistant cultivars. Hybridization programme may be initiated between the genotypes in the two clusters for getting transgressive segregants since these genotypes showed maximum diversity.

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