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Molecular diversity assessment of rice genotypes for brown planthopper resistance using microsatellite markers

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Abstract
The brown planthopper (*Nilaparvata lugens* (Stål) is a major insect pest that is primarily present in Asia causing a significant impact on rice crop. Seventy-three rice genotypes were evaluated for molecular diversity based on known BPH resistance loci. A total number of 108 alleles were detected by 39 polymorphic markers with an average of 2.37 alleles per locus. The allele frequency which is useful in estimating the frequency of alleles, ranged from 0.054 to 0.9726, while the expected heterozygosity varied between 0.054 (RM494) and 0.688 (RM231) with an average of 0.431.The Polymorphic Information Content (PIC) values provided an estimation of the marker's discriminating power by ranged from 0.066 (RM496) to 0.621 (RM231) suggesting moderate level of polymorphism for the selected BPH specific SSR markers among the germplasm lines. Accounting the major allele frequency, heterozygosity and PIC content, the marker RM231 associated with *QBph3.1* and regarded as the most informative one along with RM19291, RM335, RM469, RM518, RM8213, RM228, RH078 and RM589 for dissecting the molecular diversity of germplasm lines with respect to BPH resistance. Based on the marker data, the genotypes were classified into seven clusters that distinguished the lines clustering as resistant and susceptible separately. The information about the genetic diversity of these lines will be extremely useful for proper selection of parents related to BPH reaction, especially for gene mapping and for marker assisted selection.

Key words
Rice, Brown planthopper resistance, Molecular diversity, Microsatellite markers

INTRODUCTION

Being the Asia's most economical and culturally important food crop, Rice (*Oryza sativa* L.) is regarded as one of the most important crops in the world. More than three billion people around the world depend on rice for 20-80 per cent of their dietary intake. Approximately 52 per cent of the global production of rice is lost annually owing to the damage caused by biotic stress factors, of which 25 per cent is attributed to the attack of insect pests (Brookes and Barfoot, 2003). Among the biotic stresses, Brown Planthopper (BPH), *Nilaparvata lugens* (Stål)is considered as the most destructive pest,causing a significant yield loss in most of the rice cultivating areas of Asia. The pest is a monophagousand non-traditional insect that has advanced from non significance to prominence, posing a threat to rice production. BPH sucks the phloem sap with its piercing-sucking mouthparts and affects the growth and vigor of rice plants leading to hopperburn symptoms (Backus *et al.*,2005). The pest also serves as a vector for

viral diseases including Grassy stunt and Ragged stunt in most of the rice cultivars of India as well as Asia (Jena and Kim, 2010; Sarao *et al.,* 2016). Chemical method of BPH control is not only costly, but also harmful to one's health, polluting the ecosystem, and disrupting the natural balance of BPH predators that hold the pest population (Jhansi Lakshmi *et al*., 2010a and b; Mohanapriya *et al*., 2019). This can also lead to the development of insecticide-resistance in the pest (Mohan *et al*., 2019). Host plant resistance as a part of integrated pest management is the most cost-effective and reliable way to control BPH (Soundararajan and Jeyaprakash, 2019).

The genetic studies of BPH resistance in rice are increasing since the discovery of molecular markers. Till date, 38 genetic loci for BPH resistance have been identified in rice species using markers assigned to different rice chromosomes (Balachiranjeevi*et al*., 2019). Because of their high polymorphic nature and transferability, SSR markers have proven to be very effective tools in the study of BPH genes, genetic diversity, and relatedness of most crop species among the PCR-based markers (Ishii *et al.,*1994). The present study was undertaken to assess the genetic variability among different rice germplasm lines using microsatellite (SSR) markers linked to BPH resistance genes in order to unravel the molecular diversity among the lines.

MATERIALS AND METHODS

The materials used in the study comprised of seventy-three genotypes of rice (**Table 1**) which includes MAGIC *indica* lines (27), BPH gene differentials (30), popular rice varieties (3), wild species (5), germplasm lines (7) and landraces (1). The materials were collected from ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad and Agricultural Research Institute (ARI), Rajendranagar, Hyderabad.

Genomic DNA was extracted from young and healthy leaves of 73 genotypes using the CTAB method given by Murray and Thompson (1980). A total of 53 microsatellite markers reportedly linked to BPH resistance (**Table 2**) on chromosomes 1, 2, 3, 4, 6, 10 and 12 were used to characterize and assess genetic diversity among the germplasm lines having varied response to brown planthopper stress. Polymerase chain reaction (PCR)

Table 1. List of 73 genotypes used in the study

S.No.	Genotype	S.No.	Genotype	S.No.	Genotype
1	Mudgo	26	Sinasivappu	51	M1
2	IR 64	27	Balamwee	52	M131
3	ASD 7	28	IR 62	53	M189
4	Milyang 63	29	RathuHeenati accession	54	M190
5	RathuHeenati	30	IR 65482-136-2-2	55	M227
6	Babawee	31	M4	56	M240
7	ARC 10550	32	M201	57	RPV1160
8	Swarnalatha	33	M229	58	RPV1355
9	T12	34	M284	59	RPV1189
10	Chinsaba	35	M286	60	IET23993
11	Pokkali	36	M312	61	CG171
12	IR 65482-7-216	37	M344	62	PH190
13	IR 71033-121-15	38	M359	63	CG180
14	MUT NS1	39	M61	64	CG156
15	OM 4498	40	M80	65	CG211
16	RP 2068-18-3-5	41	M88	66	BM71
17	MO1	42	M123	67	KNM118
18	MTU 1010	43	M179	68	BPT5204
19	RP BIO 4918-230S	44	M182	69	PTB33
20	IR 26	45	M187	70	TN1
21	IR 40	46	M276	71	IM6
22	IR 66	47	M278	72	RNR 15048
23	IR 72	48	M293	73	10-3
24	Utrirajappan	49	M362		
25	Ndiang Marie	50	M289		

Table 2. SSR markers specific for BPH resistance genes used for molecular diversity analysis

S.No.	Gene	Marker	Chromosome	Forward	Reverse
1	*Bph33(t)*	RM488	1	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC
2	*Bph33(t)*	RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
3	*Bph33(t)*	RM11522	1	TAACTGCAGTGCTCAACAAAGG	CTAGGTACCGGATTAAGATTCACC
4	*Bph13(t)*	RM250	2	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
5	*Bph13(t)*	RM240	2	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC
6	*Qbph3*	RM313	3	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC
7	*Qbph3*	RM7	3	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT
8	*QBph3*	RM2453	3	TAGGTGTTCAGGAGTAAAGA	AAACCAGTATTGCTTACAAG
9	*Qbph3.1*	RM231	3	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
10	*Bph3*	RM307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
11	*Bph3*	RH784	4	TTCTTGGCTGATTGGGGAGTAT	ATTTGGAATGGAGCAGAGTGGA
12	*Bph3*	RH078	4	GCCGATTTGGTCTAACTTCATT	CAACCAGCAAGGAAACAATAAA
13	*Bph3*	RHD9	4	GTCCATCCGAAGGTGAAAGT	CCGAACATCGAGGAATACAA
14	*Bph3*	WH2	4	CCCACCACACCAGAGATAAA	ACACAACACCCGCATACAA
15	*Bph3*	RHC10	4	CAATACGGGAGATTTGGAGT	TTGGGAAGCATACGAGTGA
16	*Bph3*	W1	4	TCCTAATCAGCCAATAAATCA	GCAATCTAGTGCACGAACATA
17	*Bph3, Bph17(t)*	RM5953	4	AAACTTTCTGTGATGGTATC	ATCCTTGTCTAGAATTGACA
18	*Bph17(t)*	RM518	4	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
19	*Bph17(t)*	RM206	4	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
20	*Bph17(t)*	RM8213	4	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG
21	*Bph17(t)*	MS10	4	CAATACGAGAAGCCCCTCAC	CTGAAGGAACACGCGGTAGT
22	*Qbph4, Bph17(t)*	RM335	4	GTACACACCCACATCGAGAAGC	TCCATGGATATACGAGGAGATGC
23	*Qbph4*	RM551	4	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
24	*Bph3*	RM584	6	TATGTGGTTGGCTTGCCTAGTGG	TGCCCATATGGTCTGGATGTGC
25	*Bph3*	RM225	6	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
26	*Bph3*	RM508	6	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC
27	*Bph3, Bph32*	RM588	6	TCTTGCTGTGCTGTTAGTGTACG	GCAGGACATAAATACTAGGCATGG
28	*Bph3, Qbph6*	RM8101	6	GTGTAGTTACGACCAATGATACGC	TATAATGAGTTCGAGCCGATCC
29	*Bph3, Bph32*	RM589	6	GTGGCTTAACCACATGAGAAACTACC	TCACATCATTAGGTGGCAATCG
30	*Bph3, Bph17(t)*	RM190	6	GCTACAAATAGCCACCCACACC	CAACACAAGCAGAGAAGTGAAGC
31	*Bph3, bph4, Qbph4, Bph17(t)*	RM401	6	TGGAACAGATAGGGTGTAAGGG	CCGTTCACAACACTATACAAGC
32	*Bph3, bph4, Bph32*	RM19291	6	CACTTGCACGTGTCCTCTGTACG	GTGTTTCAGTTCACCTTGCATCG
33	*Bph3, Qbph6*	RM469	6	AGCTGAACAAGCCCTGAAAG	GACTTGGGCAGTGTGACATG
34	*bph4*	RM217	6	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
35	*Bph32*	RM8072	6	GATCACTCAGGTCATCCATTC	AATCAGAGAGGCTAAAGACAATAAT
36	*Bph32*	RM6775	6	GCAGATCAAGTATGCCTGCC	TCGCTAGATAGGGGATGTGG
37	*Bph20 (t), Bph29*	RM435	6	CTGGTTAATTACGTGCATGTCTGG	GGCATGTCATGTCTTGGTCTCC
38	*Qbph6*	RM510	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
39	*Qbph6*	RM6818	6	GTCGCATTCGTCTCCACC	ACCATTTCCAGATGACTCGG
40	*Qbph6*	RM8215	6	GTTCTCCCTTCATGACACAG	TAGAGACTTTATATTTGGTGTGC
41	*Qbph6*	RM587	6	ACGCGAACAAATTAACAGCC	CTTTGCTACCAGTAGATCCAGC
42	*Qbph6*	RM314	6	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC
43	*Qbph10*	RM484	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
44	*Qbph10*	RM496	10	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC
45	*Qbph10*	RM216	10	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
46	*Qbph10*	RM333	10	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACTCGC
47	*Qbph10*	RM228	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
48	*Qbph10*	RM406	10	GAGGGAGAAAGGTGGACATG	TGTGCTCCTTGGGAAGAAAG
49	*Qbph10*	RM494	10	GGGAGGGGATCGAGATAGAC	TTTAACCTTCCTTCCGCTCC
50	*bph7*	RM3448	12	CTTCCTCCTTCCTCCTCCTC	CACGTGACACGTACACCCTC
51	*bph18*	RM273	12	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC
52	*bph18*	RM6506	12	GTCCTTCAGGTGATTCGCC	GTCGCTCGAAGCCAATTAAG
53	*bph18*	RM3331	12	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC

was performed in 10 µl volume using Eppendorf gradient thermocycler. The reaction mixture contained 2 µl template DNA, 0.5 µl each forward and reverse primers, 4 µl of TAKARA master mix and 3 µl of double distilled water. Thermal cycler was programmed to 1 cycle of 5 min at 94°C, 35 cycles of denaturation at 95°C for 1 min, 45s for annealing temperature (55 to 60°C depending on the marker used) and 40s at 72°C for primer elongation. Finally, 1 cycle of 7 min at 72°C was used for final extension. The reproducibility of the amplification products was checked twice for each primer. SSR analysis was carried out by 3% Agarose gel electrophoresis and the DNA fragments were visualized under UV-trans illuminator and documented using a documentation system (GELSTAN) which was stored for further scoring and permanent records.

The sizes of the amplified fragments were estimated with the help of Alpha image software by Gel documentation system using 100 bp DNA ladder (XcelGEN) as size standard. Only clear and unambiguous SSR markers were scored for the presence or absence of the corresponding band among the germplasm lines. The score '1' and '0' indicates the presence and absence of the bands, respectively. The binary data matrix of SSR markers from the 73 germplasm lines was subjected to cluster analysis and a Neighbor joining tree with bootstrap values was constructed using the un-weighted pair group method with arithmetic mean (UPGMA) using DARwin 6.0.21 software (Perrier *et al.,* 2003). To estimate the discriminatory power of a marker, the Polymorphic Information Content (PIC) for each SSR marker along with allelic frequency and observed heterozygosity were calculated using CERVUS 3.0.7 software (Kalinowski*et al.,* 2007).

RESULTS AND DISCUSSION

Assessing the genetic diversity of genotypes is essential for efficiently preserving, characterizing and exploiting biodiversity. Fifty-three microsatellite markers that were linked with BPH resistance having co-dominant and high repeatability were selected to evaluate the diversity of the rice germplasm resources (**Table 2**). Out of the 53 markers used, 39 SSR markers showed polymorphism by revealing 108 alleles (**Table 3**). The number of alleles per locus varied from a minimum of 2 (RM190, RM435, RM250, RM508, RM518, RM216, RM206, RM314, RM3331, RM494, RM212, RM488, RM7, RM510, RM8215, RM508 and RM11522) to 5 (RM8213), with an average of 2.76 alleles per locus. Significant differences in allelic diversity among BPH specific microsatellite loci with a mean of 2.26 alleles was reported by Supari *et al.*(2019), while characterizing Malaysian rice cultivars using SSR markers and 2.37 alleles by VijayaLakshmi *et al.*(2010), while assessing rice genotypes for brown planthopper resistance.

Gene diversity or expected heterozygosity (*H*), is a common statistic for assessing genetic variation within the population. In the present study, the expected heterozygosity (**Table 3 and Fig. 1**) differed among the markers ranging from 0.054 (RM494) to 0.688 (RM231) with an average of 0.431. The lesser mean value of the heterozygosity indicated that majority of the germplasm lines showed a single allele in their respective microsatellite profiles. Allele frequency, also known as gene frequency, is the relative frequency of an allele at a particular locus in a population which is expressed as fraction or per cent (Gillespie *et al.,* 2004).The mean major allele frequency was 0.64 with the marker RM494 exhibiting the highest major allele frequency of 0.9726 followed by RM496 (0.965). The lowest values for major allele frequency were observed for the markers RM307 (0.054) and RM231 (0.373).

To determine the information of each marker and its discriminatory potential, the Polymorphic Information Content (PIC) was estimated for each locus. The

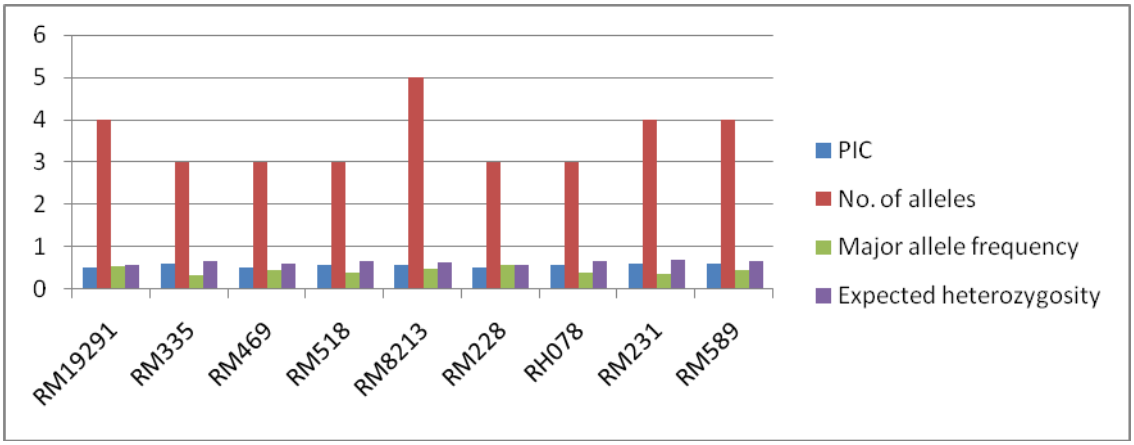

Fig. 1. Histogram representing the PIC, Number of alleles, allele frequency and expected heterozygosity of the most informative markers identified in the study

Table 3. Total number of alleles, Major allele frequency, heterozygosity and polymorphism information content (PIC) value of polymorphic SSR markers linked with BPH resistance assayed in 73 rice genotypes

S.No	Marker	Number of alleles	Expected heterozygosity	PIC	Allele frequency
1	RM190	2	0.495	0.37	0.569
2	RM435	2	0.197	0.176	0.8904
3	RM250	2	0.253	0.219	0.8529
4	RM508	2	0.424	0.332	0.6985
5	RM584	2	0.479	0.362	0.6119
6	RM216	2	0.479	0.362	0.6119
7	RM206	2	0.498	0.372	0.553
8	RM314	2	0.277	0.237	0.8356
9	RM3331	2	0.492	0.369	0.5764
10	RM494	2	0.054	0.052	0.9726
11	RM212	2	0.503	0.375	0.5205
12	RM488	2	0.487	0.367	0.589
13	RM7	2	0.465	0.355	0.6389
14	RM510	2	0.482	0.364	0.6027
15	RM8215	2	0.258	0.223	0.8493
16	RM508	2	0.388	0.311	0.7397
17	RM11522	2	0.283	0.242	0.8308
18	RM588	3	0.247	0.231	0.863
19	RM335	3	0.672	0.593	0.3361
20	RM225	3	0.367	0.333	0.7823
21	RM469	3	0.611	0.524	0.4571
22	RM518	3	0.665	0.586	0.4
23	RM496	3	0.067	0.066	0.9658
24	RM3448	3	0.486	0.43	0.6781
25	RM484	3	0.386	0.328	0.7534
26	RM6775	3	0.106	0.102	0.9452
27	RM217	3	0.531	0.47	0.6357
28	RM587	3	0.508	0.39	0.5616
29	RM228	3	0.585	0.512	0.5616
30	RM240	3	0.217	0.202	0.8803
31	MS10	3	0.542	0.453	0.5822
32	RH078	3	0.666	0.587	0.3904
33	RM401	4	0.341	0.316	0.8
34	RM19291	4	0.585	0.509	0.558
35	RM589	4	0.66	0.591	0.4615
36	RM231	4	0.688	0.621	0.3732
37	RM307	4	0.502	0.46	0.0542
38	RM333	4	0.239	0.226	0.8696
39	RM8213	5	0.636	0.566	0.4922

PIC value of a marker for detecting polymorphism is determined by the number of observable alleles and the frequency distribution of those alleles, thereby providing an estimation of the marker's discriminating power (Nagy *et al.,* 2012). Usually, markers with PIC value of more than 0.5 are considered to be informative markers for genetic studies for measuring the polymorphism for a marker locus (DeWoody*et al*., 1995). In the present study, the polymorphism information content (PIC) values ranged from 0.066 (RM496) to 0.621 (RM231) suggesting moderate level of polymorphism for the selected SSR markers specific to BPH among the rice genotypes

which was comparable to previous estimates of Joshi *et al.*(2020). The mean PIC value of the microsatellite markers used to assess the diversity of rice genetic resources was 0.3, which was much higher than the PIC values of microsatellite markers used in other studies (Aljumaili*et al.,* 2018; Suvi*et al.,* 2020). Marker RM231 linked to *QBph3.1* was considered as best for the tested germplasm lines with the highest PIC value (0.621) which was in agreement with Rashmi *et al.* (2017), who reported RM231 as the most appropriate marker to discriminate among 65 rice genotypes owing to the highest PIC value of 0.588. These results indicated that the microsatellite markers selected for use in this study are very suitable and efficient for assessing the genetic diversity. On the whole, chromosome 3 was shown to be the most divergent chromosome among all the studied rice chromosomes with the highest mean PIC value (0.621) followed by chromosome 4 (0.593). This kind of information about genetic diversity for specific chromosomes can be very valuable for gene mapping, marker assisted selection (MAS) amplification and rice breeding programs related to breeding for BPH resistance. Comparisons of all genetic parameters comprising of the major allele frequency, heterozygosity and PIC content revealed that the marker RM231 linked to *QBph3.1* to be the most informative and crucial for understanding the molecular diversity of genotypes.

Cluster analysis is very useful in revealing the complex relationships among populations of diverse origins in a more simplified manner. It is also effective in indicating accessions with useful traits belonging to different clusters for hybridization. An unrooted tree **(Fig. 2)** based on Neighbour Joining (NJ) method was constructed for the assessment of genetic diversity which revealed that the lines having similar phenotypic reaction to BPH, clustered together. The tree grouped the 73 lines into seven main clusters (**Table 4 and Fig. 2**) with cluster VI having the highest number of germplasm lines (21). Cluster 1 comprised of 16 lines which was further sub-divided into two sub-clusters with seven and nine lines, respectively. Most of the BPH gene differentials along with two MAGIC lines (M4, M201) were found in this cluster. Cluster II had seven lines, while cluster III was found to be having six germplasm lines sub-divided into two clusters (PH190, CG171 in sub-cluster I and M187, M182, M278, M276 in sub-cluster II). The fourth and fifth main clusters were also sub-divided into three minor subgroups. Cluster VI was the largest comprising of 21 germplasm lines divided into three sub-clusters with nine, four and eight lines respectively. Cluster VII was the smallest of all containing two germplasm lines, *viz.,* M123, M66. Based on the previous phenotypic reaction to BPH, it could be attributed that there was a clear-cut demarcation of the resistant germplasm lines as grouped in clusters I, II, III and VI with

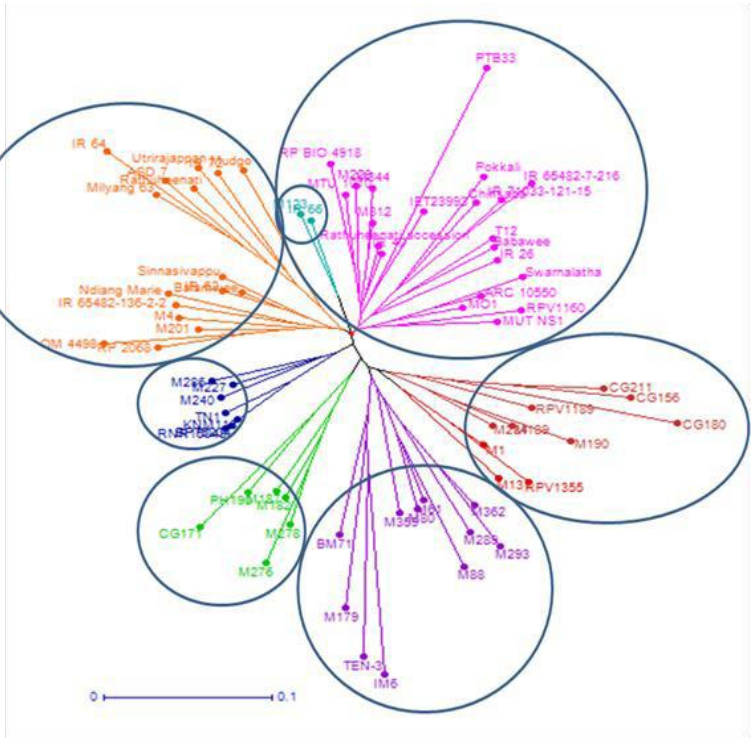

Fig. 2. Tree depicting clustering of genotypes based on Neighbour Joining (NJ) method

Table 4. Clustering of germplasm lines based on molecular diversity

Cluster	Number of genotypes	Germplasm lines
I A	7	Mudgo, IR64, IR72, ASD7, Milayang63, Rathuheenati, Utrirajappan
I B	9	Sinnasivappu, IR62, Balamwee, Ndiang Marie, OM4498, IR65482-136-2-2, RP 2068-18-3-5, M4, M201
II	7	M286, M227, M240, TN1, KNM118, BPT5204, RNR15048
III A	2	PH190, CG171
III B	4	M187, M182, M278, M276
IVA	4	BM71, M179, 10-3, IM6
IV B	4	M359, M80, M61, M88
IV C	3	M289, M362, M293
V A	3	M1, M131, RPV 1355
V B	3	M284, M189, M190
V C	4	RPV1189, CG211, CG156, CG180
VI A	9	RP BIO 4918-230S, MTU1010, M229, M344, M312, RathuHeenati accession, IR40, IET23993, PTB33
VI B	4	Pokkali, Chinsaba, IR71033, IR-65485-7-216
VI C	8	T12, Babawee, IR26, Swarnalatha, ARC10550, MO1, RPV1160, MUTNS1
VII	2	M123, IR 66

the highly resistant checks of Rathuheenati and PTB33 clustered in I and VI, respectively. Similarly, moderately resistant lines were grouped in cluster V and susceptible lines in Cluster III B and VA. Wild species PH190, CG171 alongwithCG211, CG156, and CG180 have separately formed clusters III(A) and V(C) respectively indicating the power of the chosen markers in distinguishing the wild species from the rest. On the whole, the results indicated the presence of considerable diversity in the germplasm lines studied. From the present study, the most diverse genotypes namely, PTB33, CG180, CG171 and IR64 can be selected and utilizedfor BPH resistance breeding programmes.

Having gene information for BPH target loci that are derived from markers can help breeders to use the germplasm more efficiently. The current study provided an insight of the genetic diversity of 73 rice genotypes having different reactions to the pest. The selected SSR markers were able to clearly distinguish the germplasm lines into clusters which was in agreement with the phenotypic reaction to the pest insect. Considering the major allele frequency, heterozygosity and PIC content, the marker RM231 linked to *QBph3.1* has been regarded as the most informative for dissecting the molecular diversity of germplasm lines with respect to BPH resistance.The study also identified the markers, RM19291 (*Bph3, bph4, Bph32*), RM335 (*Bph17(t)*), RM469 (*Bph3, Qbph6*), RM518 (*Bph17(t)*), RM8213 (*Bph17(t)*), RM228 (*Qbph10*), RH078 (*Bph3*), and RM589 (*Bph3*) to be useful in molecular diversity studies.

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