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Assessment of Genetic Diversity of aromatic rice genotypes for BPH resistance using SSR marker

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ABSTRACT

Nilaparvata lugens L., also known as the Brown Plant Hopper (BPH), is one of the most damaging insect pests that significantly reduces the output of the majority of rice varieties in Asia and India. A normal phloem feeder, that is. Thus, the current work was conducted to molecular characterize the BPH population using SSR markers in order to provide a thorough understanding of the diversity of BPH in aromatic rice germplasm. Finding the presence of brown planthopper (BPH) resistance genes in certain rice varieties is the primary objective of the current study in order to supply starting material for the breeding program of BPH resistant rice varieties. In this study, several molecular markers were used, such as RM185, RM216, RM209, RM463, and RM588 etc, and associated with BPH resistance genes Bph4, Bph12, Bph10, QBph4.2, and Bph7. The average PIC value for all the amplified SSR marker was 0.186 and the average number of polymorphic alleles was 47.00. The resolving power range between 3.708- 1.833. All 48rice germplasm were categorized into three clusters. cluster I had maximum germplasm by UPGMA. This leads to the conclusion that molecular screenings of rice crops employing SSR markers gave sufficient knowledge on biotic tolerance traits at the molecular level. This research will be used in future strategic crop enhancement breeding programs.

INTRODUCTION

Rice (***Oryza sativa*** **L.**) is a staple food in India, many countries of Africa and other parts of the world. It is the most important staple food for about half of the human race. More over half of the world's population depends on rice (Oryza sativa L.) as their main cereal food crop. It is produced annually on 167.13 million hectares of land, generating 782.00 million tonnes of grain. A quarter of the world's population, or around 3 billion mouths, rely on rice for their sustenance, and it makes up over 75% of Asia's staple nutritional needs. Rice provides more than half of India's people with the calories they need, either directly or indirectly. (Pathak *et al.*, 2019).

China and India are the biggest producers, with an estimated 500 million metric tonnes produced worldwide. It is anticipated that the average yield worldwide would range from 3.0 to 3.5 tonnes per hectare, with notable regional and national variances. West Bengal, Uttar Pradesh, Punjab, Haryana, Andhra Pradesh, Tamil Nadu, and Odisha are the main rice-growing states in India, with an anticipated 44.5 million hectares under cultivation in 2022-2023. With West Bengal, Punjab, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Haryana, and Odisha among the major producing states, the total rice output is anticipated to be 180 million metric tonnes. Due to improved agricultural techniques, Punjab and Haryana are predicted to produce more rice than the rest of India, with an average output of 3.5 to 4 tonnes per hectare. This information is based on projections for India's rice growing season in 2022-2023. FAOSTAT, 2023 Uttar Pradesh's rice cultivation is expected to reach 2.6 million hectares by 2023-24, with an estimated production of 13.5 million metric tons. The average yield is expected to be 5 tons per hectare, higher than the national average, due to improved farming practices and the adoption of high-yielding varieties. This overview provides a concise overview of rice cultivation in Uttar Pradesh APEDA, 2023.

The production of rice is limited by a number of factors, and diseases brought on by bacteria, nematodes, viruses, and fungus cause significant financial losses in India. Sheath blight, bacterial leaf streak, brown spot, rice blast, and tungro disease are common illnesses. Insect pests have always emerged as a major constraint to agriculture, resulting in significant loss of yield as well as deterioration in grain quality. These insects, while feeding, secrete saliva into the plant tissues, which contain enzymes or effector molecules that interfere with the plant defense responses. (Guo *et al.*, 2020). Among these insect pests, brown planthopper (BPH, Nilaparvata lugens Stål) is the most devastating pest of rice, accounting for about 20% to 80% of yield loss and an overall economic loss to around $300 million in Asia annually. Subsequently, BPH evolved as a monophagous insect, which selectively feeds on rice plants (Jing *et al.*, 2017; Zhao et al., 2016). BPH causes serious damage to rice crops by sucking the sap from the xylem and phloem tissues, which ultimately leads to "hopper burn". BPH also causes indirect damage by transmitting viral diseases such as grassy stunt virus and ragged stunt virus (Cabautan *et al.*, 2009).

Genetic diversity is also helpful in the evaluating the important source genes of particular traits within the existing germplasm (Roy and Panwar, 1993). To feed the growing population we need to produce more rice from limited area through varieties or hybrids which should perform well in the different adverse conditions to produce higher yield. Rice accessions are rich source of genetic variability that can be harnessed for rice improvement programme (Rasmi *et al.*, 2017). Genetic diversity is the foremost thing to carry out any crop breeding programme which out come in to a superior recombinant (Manonmani and Fazlullah Khan 2003) and proper identification and selection of donor and recipient parents having wider adaptability and variability for different traits (Nayak *et al.*, 2004).

Materials and Methods

The DNA was isolated using the protocol described by Edwards *et al.*, (1991). The young newly leaves were taken and disinfected with 70% ethanol to avoid contamination for DNA extraction. Leaf sample (100 mg) germplasm were collected, cleaned, and stored in an icebox before being crushed in liquid nitrogen. Extraction buffer (500 µl) was added, and the leaf tissue was gently macerated for a few seconds and then kept in a hot water bath at 65 °C for 45 minutes. The sample was cooled to room temperature and centrifuged at 10000 rpm for 10 minutes. The aqueous layer was transferred to a new Eppendorf tube, and 200 µl of chloroform: iso-amyl alcohol (24:1) was added and mixed by gentle inversion for 5-6 times. After that, the mixture was centrifuged for 10 minutes at 8000 rpm. A double amount of chilled iso-propanol was added to the supernatant and incubated at -20 °C overnight. The solution was centrifuged at 8000 rpm for 10 minutes the next day, and pellets were collected. The pellet was rinsed with 100 µl of 70% ethanol and then centrifuged for 10 minutes at 8000 rpm. The pellet was dried, re-suspended in 50 µl of 1x TE buffer, incubated for 30 minutes at 37 °C in a water bath, and then kept at -20 °C for further use. Using the Agarose gel electrophoresis technique, the quality of the extracted DNA was verified.

The UPGMA method utilizing pooled SSR data was used to form a dendrogram from the generated molecular data. This approach is ideal for diversity analysis since it divides the individuals into multiple clusters, which makes it easier to distinguish between the clustered parental lines and show how the clusters are arranged.

Twenty SSR markers were used for screening 48 rice genotypes. Polymerase Chain Reaction (PCR) was performed in Thermal Cycler programmed for 35 cycles of standardized cycling conditions as described by Temnykh *et al.*, 2001. The basic profile was 5 min. at 94°C, 35 cycles of 1 min. at 94°C, 1 min. at 55°C, 61°C or 67°C (depending upon primer amplification temperature), were used to amplify specific primer sets, and primer extension at 72°C for 2 min. This was followed by a final extension step at 72°C for 7min. followed by storage at 4°C until electrophoresis. The results of the amplification process were observed using a UV transilluminator. The size of the amplified products was scored using the molecular weight markers. Both the presence (1) and absence (0) of the amplified fragments were scored for each marker system. Using NTSYS-PC version 1.70, genetic similarity values were calculated using Jaccard's similarity coefficient. Dendrograms were used to show the relationships between cultivars, and cluster analyses based on similarity matrices were carried out using UPGMA (unweighed pair group method with arithmetical averages). Polymorphism Information Content (PIC) value of each marker was also calculated.

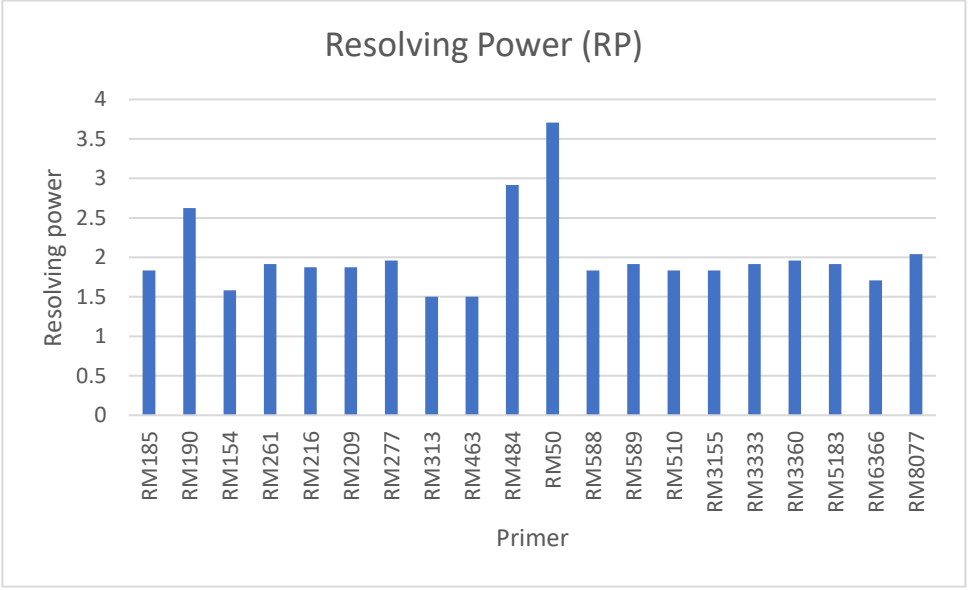
List of SSR Primers and their sequence used for Genetic Diversity analysis.

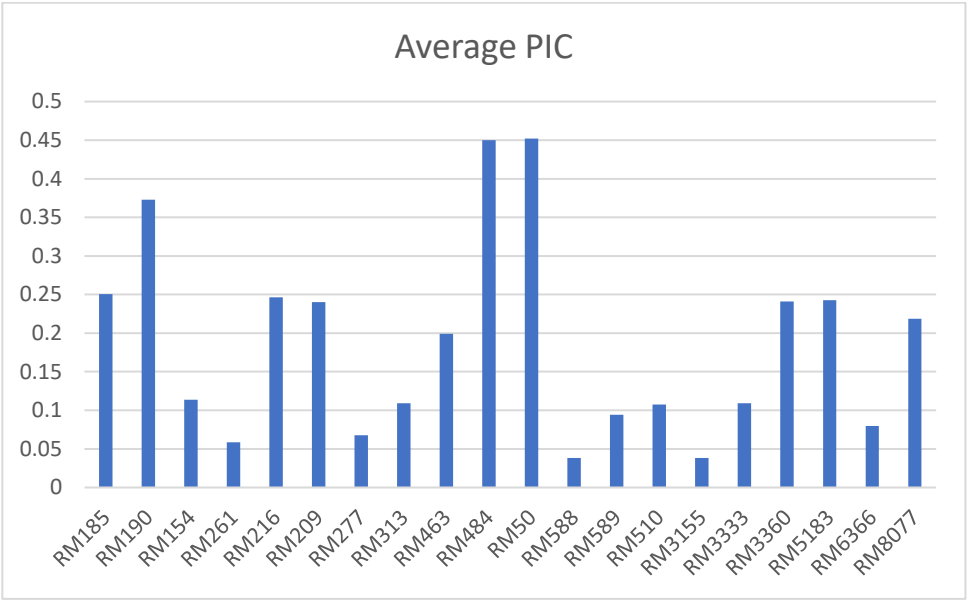
S.No	PRIMER	FORWARD PRIMER	REVERSE PRIMER	gene	Reference
1.	RM185	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC	Bph 12	Lee, K. et al., 2015
2.	RM190	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	Bph 4	Kawaguchi et al, 2001
3.	RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	QBph 3	Hu et al, 2015
4.	RM261	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	QBph4.2	Hu et al, 2015
5.	RM216	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	QBph-10	Rahman et al, 2009
6.	RM209	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC	Bph-10	Wu et al, 2014
7.	RM277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	QBph-12	Lü et al, 2014
8.	RM313	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC	bph7	Qiu et al, 2014
9.	RM463	TTCCCCTCCTTTTATGGTGC	TGTTCTCCTCAGTCACTGCG	BPH18(t)	Jena et al, 2006
10.	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	QBph-10	Rahman et al, 2009 4S

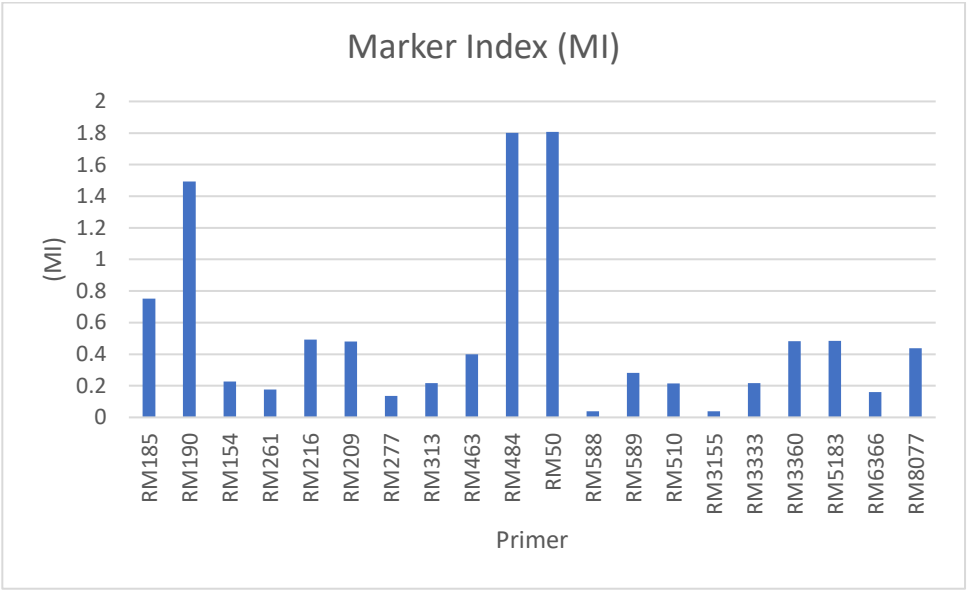
11.	RM50	ACTGTACCGGTCGAAGACG	AAATTCCACGTCAGCCTCC	Bph12	Wu et al, 2014
12.	RM588	GTTGCTCTGCCTCACTCTTG	AACGAGCCAACGAAGCAG	Bph3	Jairin et al, 2007
13.	RM589	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	Bph-3	Wu et al, 2014
14.	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	QBph-10	Lü et al, 2014
15.	RM 3155	GATCGTTCGTGTTCGTGTTG	GAAAAGGACAGGGGAAAAGC	Mg, Ca	Goncharova, J.2023
16.	RM 3333	GCACCTTACAATTTGGCACC	AAGCTATCGACACCGTGACC		
17.	RM 3360	ATTTTCGGCTGCTGGTAGTG	GTAACTTACACAAGGCCGGG		
18.	RM 5183	AGCTTGAACCTTATATATTG	AATGAGCTAATGTTTCTAAG		
19.	RM 8077	GGGGTATAGTAGACAACATCAAAA	TGTAAAGTTGTCAAGGGACTACTC		
20.	RM 6366	GTGTGTTCCAACAGTGGTGG	TGTCCTTACCCTTGTTTCGC		

Results And Discussion

All the twenty primers successfully amplified. The average PIC value for all the amplified SSR marker was 0.186 exhibiting the highest PIC value was 0.452 for RM50 SSR marker, the lowest PIC value was 0.038 for RM588 SSR marker. The average number of polymorphic alleles was 47.00, amplified by the SSR Primers. The maximum number of bands were recorded with primer RM50 (86 bands), RM484 (71 bands) and RM277 (51 bands) RM313 amplified minimum number (34) of bands. The maximum resolving power was found in primer RM50 (3.708) followed by primer RM484 (2.917), RM190 (2.625), RM190 (2.625), RM8077 (2.042), RM3360 (1.958), RM589, RM5183 (1.917) RM3155 (1.833) respectively. Out of twenty, all SSR markers were found to be polymorphic. The highest PIC value was found for RM 50 and lowest was found for RM 588 SSR markers. The highest no. of alleles was found for RM 50 and RM 190 and lowest was found for RM 588 SSR markers. The maximum no. of amplified polymorphic bands was recorded with primer RM 50, RM 484, SSR markers.







Forty-eight rice genotypes were examined for polymorphism using twenty sets of SSR primers. The presence and absence of the SSR bands were assessed for each genotype. The values in the matrix range from 0 to 1, where 0 indicates no similarity between two samples, while 1 represents complete similarity. The score obtained using all the primers in the SSR analysis were pooled to create a binary data matrix and used to construct a dendrogram using UPGMA (Unweighted Pair Group Method of Arithmetic Means). The dendrogram illustrated above represents the hierarchical clustering of various aromatic rice samples, providing insights into their genetic relationships and diversity. The scale of similarity coefficients ranges from 0.68 to 1.00, where higher values indicate greater genetic structure of aromatic rice genotypes.

This analysis investigates the relationships, diversity, and clustering patterns among the samples to infer genetic relationships and identify potential applications in breeding and conservation. The dendrogram's branching pattern depicts the genetic or phenotypic relationships among the analyzed aromatic rice samples Branch Lengths: The length of the branches reflects the genetic distance between the samples. Shorter branches indicate higher genetic similarity, while longer branches suggest greater divergence. Similarity Coefficient Axis: The horizontal axis represents the similarity coefficients. Samples with a coefficient close to 1 are highly similar, whereas those with coefficients around 0.77 are more genetically distinct.

The Jaccard similarity index is a statistical measure widely used to quantify the similarity between sets of data. All 48 cultivars are grouped into three major groups based upon the multivariant analysis of genetic similarity data. Cluster 1-High Similarity Between Certain Pair, Cluster 2- Moderate Similarity Values Indicating pair, Cluster 3- Low Similarity Values Highlighting Diversity.

Cluster 1 has very high similarity suggests that these two samples are likely derived from the same genetic lineage or have undergone minimal genetic divergence. at J-0.94 contain various genotypes pair Basmati Mehtrah-A and Basmati Mehtrah-c, Basmati 6113-C and Basmati 5836-C, Basmati 622-K and Basmati 370 A-F, Basmati sufaid-A and Basmati mehtrah-C, Basmati sufaid-B and Basmati surakh-A. At J-0.91 contains various genotypes pair such as kalanamak and Basmati 1A-G, Bsamat 5836-B and Bsamati 5836-C, Bsamati 5836-C and Bsamati 5888-C, Bsamati 5875-B and Bsamati 6141-B, Bsamati 622-K and Bsamati 6113-C, Bsamati norat-C and Bsamati 93 B-E, Bsamati sufaid-B and Bsamati mehtrah-C, Bsamati sufaid-B and Bsamati norat-C, Bsamati Tall-B and Bsamati sufaid-A. At J-0.90 contains various genotypes pair such as kaladhan and Basmati 93B-C, Basmati 370A-F and Basmati 1A-G, Basmati 5874-A and kalanamak, Basmati 5875-B and Basmati 6129-B, Basmati6113-B and Basmati 370 A-F, Basmati 6113-C and Basmati 5836-B, Basmati 6129-D and juhi bangal, Basmati 6129-A and Basmati93B-E.

Cluster 2 has moderate similarity, these values suggest that while the samples share common genetic or phenotypic traits, they also retain significant differences. For example: at J-0.78 contain various genotypes pair such as Lalmatiya and Basmati106-12-D, Lalmatiya and lalmati, Basmati 372 A-H and Basmati 1A-G, Basmati375-F and kaladhan, Basmati375-F and Basmati 106-12-D, Basmati6141-B and Basmati622-K, Basmati6113-C and Basmati6131-G.

Cluster 3- Low Similarity Values Highlighting Diversity- Some pairs of samples exhibit lower Jaccard similarity values, generally below 0.75. These values indicate significant genetic or phenotypic divergence. Cluster 3 is further divided into Three subgroup 3A,3B and 3C. Group 3A at J-0.73 contain various genotypes pair such as Juhi bangal and Basmati 375-C, Basmati372 A-H and badshsh pasand, Basmati 5875-B and Basmati 106-12-D, Basmati 5888-D and Basmati-c-622-I, Basmati 6141-B and kanikabhog, Basmati bahar and badshsh pasand, Basmati 622-K and taraori Basmati. Group 3B at J-0.69 contain various genotypes pair such as Basmati bahar and Basmati 106-12D, Basmati mehtrah-C and Basmati 6141-B, Basmati mehtrah -C and Basmati 6313-B, Basmati tall- B and Basmati 6141-B, Basmati tall-B and Basmati 6313-B. Group 3C at J-0.68 contain various genotypes pair such as Basmati bahar and Basmati 622-I, Basmati Bahar and Basmati 5836-B.

The range of similarity values observed in the matrix, from 0.68 to 1.00, underscores the presence of both closely related and genetically diverse samples. This diversity is crucial for sustainable breeding programs, as it provides a pool of traits that can be used to develop improved Basmati rice genotypes with enhanced adaptability, resistance, and yield.

Molecular markers have been used in genetic improvement programmes to study genetic diversity and to select parents for planning crossing between parents from divergent backgrounds and to know marker trait association Islam *et al.,* (2013). The results of present investigation showed that the 20 SSR markers analysed were informative and are in agreement with the results of A similar trend was reported by Jena *et al.,* (2015), in which they screened 58 rice genotypes for BPH resistance under controlled conditions and genotyped using 22 gene specific SSR markers. Simllarly Vang, P. (2020) revealed that 10 rice varieties exhibited sustained resistance to specific BPH populations using SSR molecular markers RM1103, RM204, RM217, RM545, and RM401. Dhama *et al.,* (2018) studied the genetic relationship in aromatic rice using SSR markers. Srivastava *et al.,* (2023) also reported that A total of 27 markers distributed evenly across the 12 rice chromosomes were employed for a polymorphic survey between the resistant and susceptible parents.

According to Jaccard's Coefficient values, Basmati Mehtrah-A and Basmati Mehtrah-C, Basmati 6113-C and Basmati 5836-C shows very high similarity while Basmati Bahar and Basmati 5836-B shows leastSimilarity. The result indicated significant genetic variation among the rice genotypes. Similarly, Mayabini J. *et al.,* (2015) reported that genetic diversity analysis of fifty-eight rice genotypes through 22 gene-linked markers of BPH resistance and categorized them into 4 major clusters with the 40% level of genetic similarity. Randive *et al.,* (2019) reported that the Bph25 and Bph26 genes were also searched for in rice genotypes by using the markers RM-6775, RM-309, and RM-5479. Cheng *et al.,* (2021) also reported that molecular studies were taken up in rice lines using resistance to BPH in the variant PTB33 allelic relationship between Bph6 and gene on Chromosome 4, bordered by the genetic markers RM 5757 and RM 6997. Prafulla K. Behera *et al.,* (2023), also reported the genetic diversity assessment in rice genotypes, using high informative markers, indicated by their high PIC values maximized the probability of detecting high proportion of allelic variation at the individual loci.

Mean value of polymorphism information content (PIC), marker index (MI) and resolving power (RP)and heterozygosity index (HI) were 0.326, 1.037, 1.558 and 0.420 respectively.. The PIC values in this study were reported in some studies like Sourav M. *et al.,* (2024), Shamsunnahar *et al.,* (2024). Cluster analysis was performed using the UPGMA method to group the studied varieties based on simple matching similarity coefficient group were formed at genetic similarity level of Group-I contained sixteen genotypes. This study noticed that considerable amount of molecular diversity exists in rice genotypes. Present result found similar to Lapitan *et al.,* (2007) reported that SSR markers can distinguish quality rice subspecies and classified cultivars with the same cooking and eating quality.

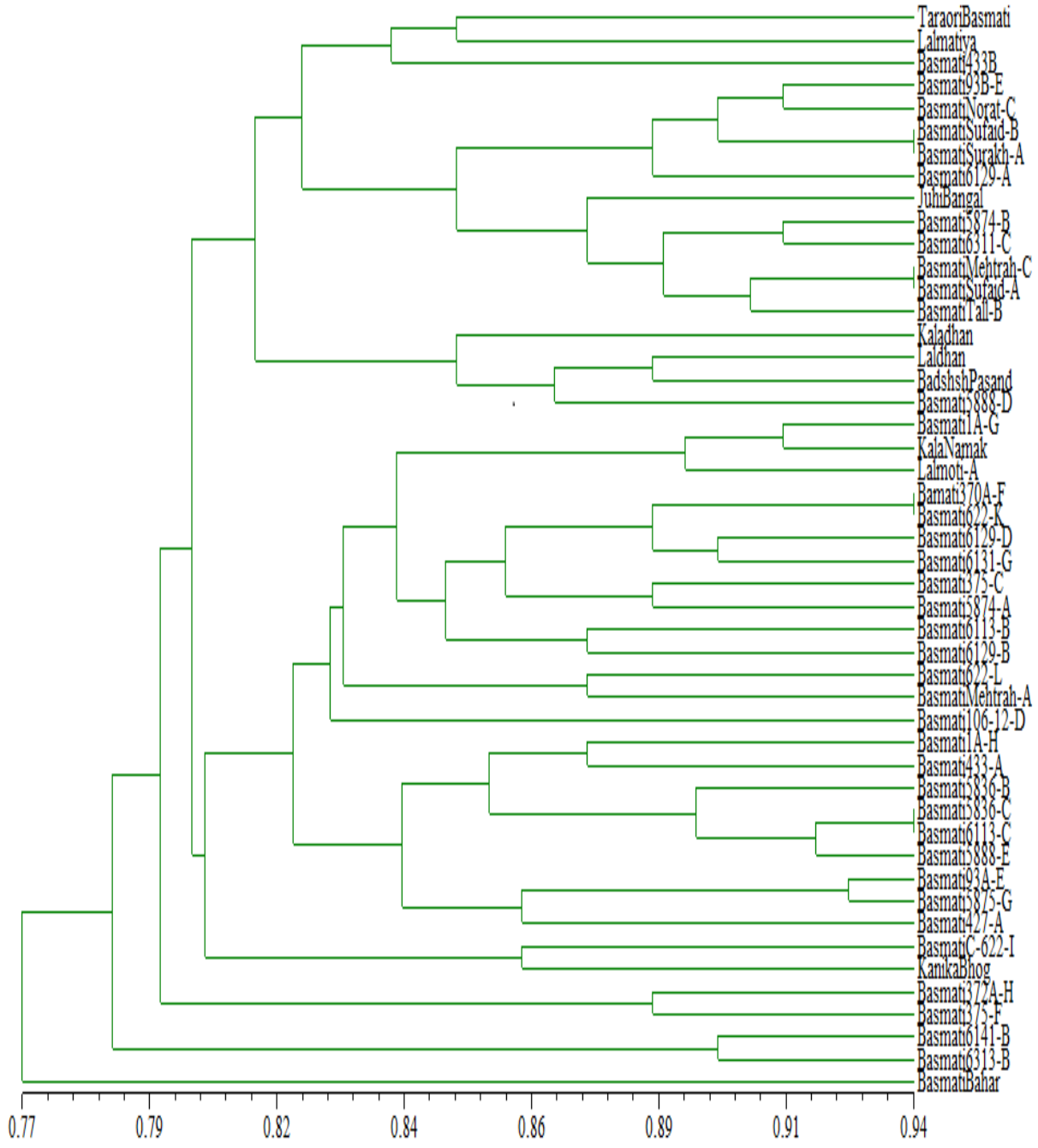
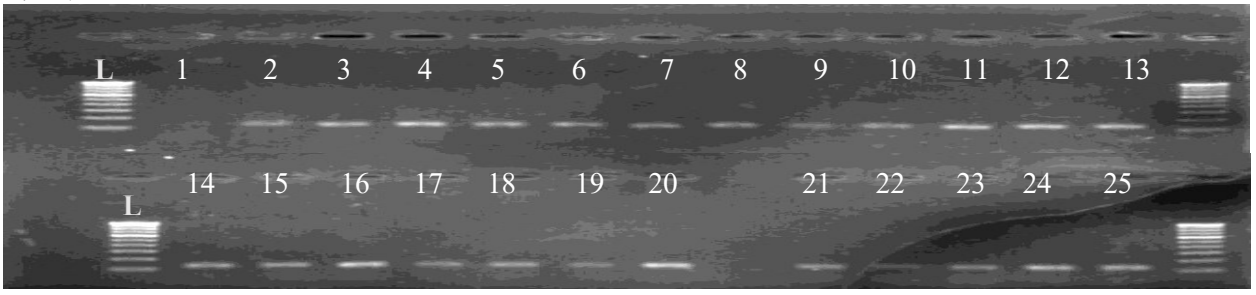


Fig- Dendrogram constructed using Jaccard's Similarity Coefficient

RM-519



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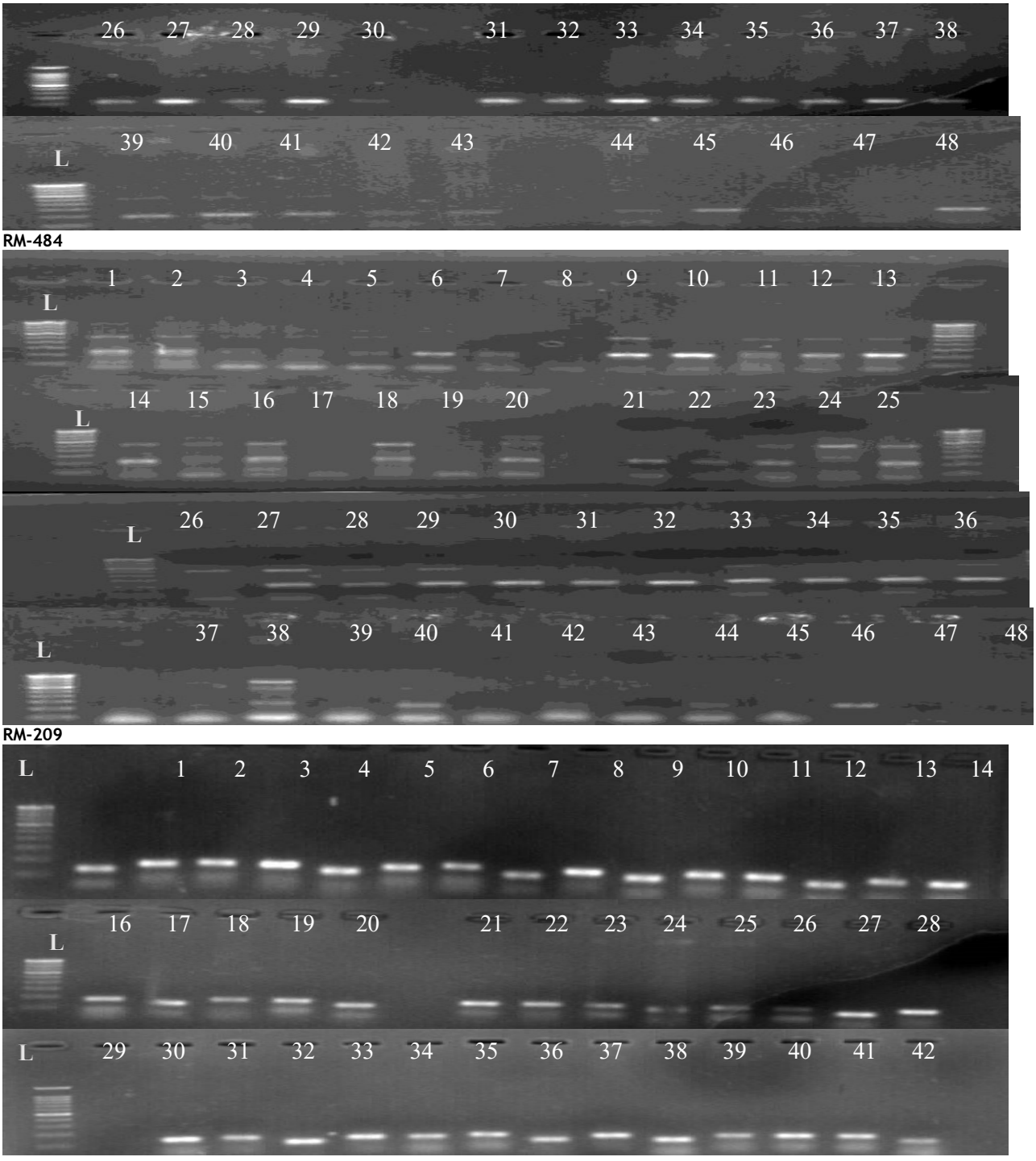

Amplified DNA fragments of forty-eight genotypes using SSR primer RM-519, RM-484, RM-209 linked to BPH resistance. L= 1000 bp ladder.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1	1.00																																																
2	0.83	1.00																																														
3	0.84	0.84	1.00																																													
4	0.81	0.79	0.83	1.00																																												
5	0.86	0.81	0.83	0.83	1.00																																										
6	0.85	0.90	0.84	0.81	0.81	1.00																																									
7	0.75	0.85	0.86	0.81	0.86	0.83	1.00																																								
8	0.76	0.74	0.85	0.83	0.83	0.79	0.76	1.00																																							
9	0.80	0.88	0.86	0.76	0.79	0.85	0.80	0.76	1.00																																						
10	0.85	0.70	0.86	0.84	0.81	0.80	0.78	0.84	0.78	1.00																																					
11	0.80	0.83	0.91	0.81	0.84	0.83	0.85	0.84	0.88	0.83	1.00																																				
12	0.83	0.78	0.89	0.84	0.84	0.78	0.78	0.86	0.85	0.85	0.90	1.00																																			
13	0.79	0.84	0.83	0.75	0.75	0.84	0.76	0.75	0.89	0.79	0.81	0.79	1.00																																		
14	0.86	0.84	0.83	0.80	0.88	0.86	0.81	0.73	0.84	0.81	0.84	0.84	0.75	1.00																																	
15	0.76	0.81	0.90	0.83	0.85	0.84	0.86	0.83	0.81	0.81	0.89	0.84	0.83	0.83	1.00																																
16	0.84	0.81	0.78	0.85	0.83	0.84	0.79	0.70	0.74	0.76	0.79	0.79	0.73	0.83	0.80	1.00																															
17	0.75	0.75	0.79	0.84	0.76	0.83	0.75	0.86	0.78	0.83	0.80	0.80	0.81	0.76	0.81	0.74	1.00																														
18	0.76	0.79	0.85	0.75	0.75	0.81	0.81	0.80	0.81	0.81	0.84	0.79	0.80	0.78	0.88	0.80	0.79	1.00																													
19	0.83	0.78	0.79	0.86	0.79	0.83	0.78	0.79	0.75	0.85	0.80	0.83	0.71	0.81	0.76	0.89	0.80	0.79	1.00																												
20	0.81	0.81	0.80	0.83	0.85	0.81	0.76	0.83	0.81	0.76	0.81	0.81	0.85	0.80	0.85	0.78	0.84	0.80	0.76	1.00																											
21	0.79	0.71	0.80	0.88	0.80	0.79	0.79	0.83	0.71	0.86	0.76	0.76	0.78	0.75	0.85	0.75	0.84	0.78	0.81	0.88	1.00																										
22	0.83	0.78	0.81	0.76	0.79	0.83	0.75	0.76	0.83	0.85	0.78	0.83	0.86	0.79	0.81	0.76	0.83	0.86	0.78	0.84	0.81	1.00																									
23	0.76	0.79	0.83	0.83	0.83	0.81	0.84	0.85	0.79	0.81	0.86	0.81	0.80	0.78	0.88	0.78	0.86	0.80	0.79	0.85	0.85	0.81	1.00																								
24	0.78	0.80	0.84	0.86	0.81	0.78	0.83	0.79	0.78	0.83	0.83	0.85	0.74	0.84	0.86	0.81	0.83	0.81	0.85	0.81	0.84	0.83	0.91	1.00																							
25	0.75	0.78	0.84	0.79	0.81	0.78	0.80	0.81	0.80	0.75	0.90	0.83	0.76	0.79	0.89	0.84	0.80	0.89	0.78	0.84	0.76	0.78	0.86	0.83	1.00																						
26	0.83	0.83	0.76	0.86	0.81	0.88	0.73	0.76	0.83	0.80	0.80	0.83	0.79	0.89	0.84	0.84	0.83	0.79	0.85	0.84	0.81	0.85	0.81	0.85	0.80	1.00																					
27	0.79	0.79	0.80	0.80	0.93	0.79	0.84	0.80	0.81	0.79	0.84	0.81	0.80	0.85	0.88	0.80	0.81	0.78	0.76	0.88	0.83	0.79	0.90	0.86	0.86	0.81	1.00																			
28	0.79	0.79	0.85	0.85	0.83	0.76	0.81	0.78	0.79	0.81	0.81	0.86	0.80	0.80	0.88	0.83	0.76	0.78	0.84	0.85	0.85	0.81	0.88	0.91	0.81	0.84	0.85	1.00																	
29	0.81	0.84	0.78	0.78	0.75	0.89	0.74	0.73	0.86	0.79	0.79	0.76	0.88	0.83	0.83	0.80	0.81	0.78	0.79	0.83	0.80	0.81	0.85	0.81	0.79	0.89	0.83	0.85	1.00																
30	0.79	0.79	0.88	0.78	0.83	0.81	0.81	0.83	0.79	0.76	0.84	0.84	0.75	0.78	0.90	0.80	0.76	0.83	0.74	0.83	0.78	0.76	0.80	0.79	0.86	0.76	0.80	0.83	0.78	1.00															
31	0.76	0.81	0.83	0.93	0.85	0.81	0.84	0.83	0.76	0.79	0.81	0.84	0.75	0.80	0.88	0.85	0.84	0.78	0.86	0.85	0.85	0.79	0.90	0.94	0.84	0.86	0.88	0.93	0.80	0.83	1.00														
32	0.83	0.80	0.79	0.81	0.79	0.90	0.73	0.86	0.80	0.80	0.78	0.78	0.81	0.79	0.81	0.74	0.88	0.76	0.80	0.86	0.86	0.83	0.81	0.75	0.75	0.88	0.76	0.76	0.86	0.81	0.79	1.00													
33	0.79	0.79	0.85	0.80	0.85	0.81	0.81	0.78	0.81	0.79	0.81	0.81	0.78	0.80	0.88	0.83	0.71	0.85	0.79	0.83	0.80	0.84	0.75	0.79	0.84	0.81	0.80	0.83	0.75	0.88	0.83	0.81	1.00											
34	0.76	0.79	0.83	0.78	0.80	0.79	0.81	0.75	0.76	0.74	0.86	0.79	0.75	0.78	0.90	0.83	0.76	0.83	0.74	0.80	0.75	0.74	0.85	0.84	0.86	0.76	0.83	0.83	0.80	0.88	0.83	0.74	0.80	1.00										
35	0.76	0.84	0.85	0.78	0.80	0.84	0.84	0.75	0.81	0.76	0.84	0.79	0.80	0.78	0.90	0.85	0.76	0.88	0.76	0.78	0.75	0.81	0.85	0.84	0.86	0.81	0.83	0.83	0.83	0.83	0.83	0.76	0.85	0.90	1.00									

36	0.80	0.80	0.84	0.71	0.84	0.83	0.83	0.76	0.83	0.80	0.80	0.78	0.84	0.76	0.86	0.74	0.73	0.79	0.70	0.76	0.76	0.83	0.81	0.75	0.75	0.75	0.81	0.79	0.81	0.81	0.74	0.80	0.84	0.81	0.89	1.00												
37	0.79	0.76	0.75	0.85	0.80	0.84	0.74	0.78	0.76	0.76	0.74	0.79	0.70	0.85	0.80	0.83	0.84	0.75	0.84	0.80	0.80	0.79	0.80	0.84	0.76	0.91	0.80	0.83	0.83	0.78	0.88	0.86	0.80	0.75	0.80	0.74	1.00											
38	0.78	0.75	0.81	0.74	0.81	0.75	0.78	0.81	0.75	0.80	0.83	0.83	0.79	0.74	0.84	0.71	0.78	0.74	0.73	0.79	0.79	0.78	0.84	0.78	0.75	0.75	0.79	0.84	0.79	0.81	0.76	0.80	0.74	0.81	0.81	0.90	0.74	1.00										
39	0.79	0.76	0.73	0.75	0.75	0.81	0.69	0.68	0.76	0.74	0.74	0.76	0.73	0.80	0.78	0.80	0.71	0.75	0.71	0.75	0.70	0.79	0.68	0.71	0.74	0.84	0.70	0.73	0.80	0.83	0.73	0.81	0.83	0.78	0.78	0.79	0.80	0.76	1.00									
40	0.73	0.83	0.84	0.84	0.81	0.83	0.85	0.79	0.80	0.75	0.85	0.80	0.81	0.79	0.94	0.81	0.83	0.81	0.78	0.84	0.81	0.75	0.89	0.88	0.88	0.80	0.89	0.86	0.84	0.86	0.91	0.78	0.81	0.89	0.86	0.80	0.79	0.78	0.76	1.00								
41	0.78	0.83	0.81	0.84	0.81	0.83	0.80	0.79	0.83	0.78	0.83	0.85	0.79	0.79	0.84	0.79	0.78	0.79	0.80	0.79	0.76	0.80	0.79	0.83	0.80	0.83	0.81	0.79	0.76	0.81	0.86	0.80	0.86	0.76	0.81	0.80	0.79	0.75	0.84	0.88	1.00							
42	0.88	0.83	0.84	0.79	0.81	0.93	0.78	0.79	0.85	0.83	0.83	0.80	0.81	0.86	0.86	0.79	0.83	0.81	0.78	0.81	0.79	0.83	0.81	0.78	0.80	0.88	0.81	0.76	0.89	0.86	0.79	0.88	0.79	0.81	0.81	0.83	0.84	0.78	0.84	0.83	0.80	1.00						
43	0.79	0.79	0.78	0.90	0.78	0.84	0.79	0.75	0.79	0.81	0.81	0.79	0.78	0.85	0.83	0.83	0.86	0.78	0.84	0.83	0.83	0.79	0.85	0.86	0.84	0.89	0.83	0.83	0.85	0.75	0.90	0.81	0.78	0.78	0.78	0.69	0.88	0.69	0.78	0.86	0.84	0.81	1.00					
44	0.81	0.84	0.75	0.78	0.78	0.91	0.79	0.78	0.81	0.76	0.79	0.74	0.83	0.80	0.80	0.75	0.84	0.75	0.74	0.80	0.78	0.79	0.83	0.74	0.76	0.84	0.80	0.70	0.85	0.80	0.78	0.89	0.75	0.75	0.78	0.79	0.80	0.74	0.80	0.81	0.84	0.91	0.88	1.00				
45	0.85	0.78	0.84	0.86	0.81	0.85	0.75	0.81	0.83	0.88	0.83	0.88	0.76	0.89	0.84	0.79	0.85	0.79	0.83	0.81	0.81	0.83	0.79	0.83	0.80	0.90	0.79	0.81	0.84	0.84	0.84	0.88	0.81	0.76	0.76	0.75	0.89	0.78	0.86	0.80	0.85	0.78	0.94	0.84	1.00			
46	0.88	0.83	0.84	0.79	0.81	0.93	0.78	0.79	0.85	0.83	0.83	0.80	0.81	0.86	0.86	0.79	0.83	0.81	0.78	0.81	0.79	0.83	0.81	0.78	0.80	0.88	0.81	0.76	0.89	0.86	0.79	0.88	0.79	0.81	0.81	0.83	0.84	0.78	0.84	0.83	0.80	0.85	0.91	0.91	0.90	1.00		
47	0.86	0.79	0.80	0.78	0.83	0.86	0.76	0.80	0.79	0.81	0.79	0.79	0.80	0.83	0.85	0.75	0.84	0.75	0.71	0.83	0.80	0.79	0.80	0.74	0.76	0.81	0.80	0.75	0.85	0.88	0.75	0.89	0.75	0.83	0.78	0.84	0.80	0.84	0.85	0.81	0.76	0.81	0.90	0.90	0.89	0.94	1.00	
48	0.79	0.79	0.78	0.90	0.78	0.84	0.79	0.75	0.79	0.81	0.81	0.79	0.78	0.85	0.83	0.83	0.86	0.78	0.84	0.83	0.83	0.79	0.85	0.86	0.84	0.89	0.83	0.83	0.85	0.75	0.90	0.81	0.78	0.78	0.78	0.69	0.88	0.69	0.78	0.86	0.84	0.81	0.90	0.85	0.91	0.84	0.80	1.00

CONCLUSION

In order to achieve food security, the green revolution has been instrumental in creating high-yielding and hybrid types. Even at the expense of eliminating natural predators, lowering plant intrinsic defense, and deteriorating the environment, BPH a threat to rice cultivation—is constantly controlled by the extensive use of synthetic pesticides. This study on the **genetic diversity of aromatic rice genotypes for Brown Planthopper (BPH) resistance** using **Simple Sequence Repeat (SSR) markers** has provided significant insights into the genetic structure and variability of the genotypes. The analysis revealed a high level of genetic diversity among the aromatic rice varieties, with distinct genotypic differences related to their resistance to BPH. The SSR markers used in this study proved to be effective tools for identifying molecular variations associated with BPH resistance, allowing the differentiation of genotypes based on their genetic makeup.

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