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Identification of BPH resistant donors from the Global Rice Array- "Antenna IV Panel" genotypes in a glass house environment and Validation using SSR markers

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ABSTRACT

The brown plant hopper (*Nilaparvata lugens*) is a major phloem-feeding pest in rice cultivation across Asia, responsible for "hopper burn" and substantial yield reductions. Sustainable management strategies emphasize host-plant resistance to mitigate infestations while maintaining ecological balance. This study assessed the resistance of rice genotypes from the Global Rice Array—specifically, the "Antenna IV's Panel"—under controlled glasshouse and laboratory conditions. Additionally, SSR marker analysis was conducted to validate resistance-associated loci and identify potential donor lines for breeding programs.The results indicated that genotypes IRRI 154, Sahel 108, IR 93354:34-B-5-1-23-1RGA-1-B-B, along with the susceptible check TN1, exhibited the highest susceptibility to BPH, as reflected in their damage scores. Conversely, genotypes such as IRRI 147, SANHUANGZHAN NO 2, IRRI 104, and NANHI demonstrated the lowest levels of BPH damage, suggesting a higher degree of resistance. Among the 58 evaluated genotypes, 54 exhibited primary resistance, with varying numbers of resistance genes: 21 genotypes carried three resistance genes, 12 possessed five, and nine contained four. However, none of the tested genotypes harbored all major resistance genes, emphasizing the necessity for further breeding efforts to develop more robust BPH-resistant rice varieties.

Figure : 00 References : 30 Tables : 03
KEY WORDS : Brown plant hopper, Genotype, Rice, SSR

Introduction

In terms of grain crops, rice is the most important one (*Oryza sativa*), provides calories to the half of the population they need each day. Rice provides about 20% of the world's (total) energy needs. In addition, an annual increase in rice output of 8–10 million tonnes is required to meet demand[5]. In India, rice nourishes 70% of the people and provides 43% of their daily caloric needs[21]. In the upcoming few decades, there will be an urgent need to scale up rice production by over 50%, and increasing productivity will be essential to achieving this goal.[22,30] In some way, rice provides main source of calories for more than 50% of Indians[20]. In terms of production, India ranks second in rice production and it losses ~52% of its production every year due to biotic factors. One of the most harmful sap-sucking pests in Asia is the brown plant hopper. It can be found in all of the rice-growing nations in the area, but the Philippines is where it is most common[18]. BPH significantly harms the crop by sucking to create "hopper-burn" like symptoms and resulting in the reduction of output. To lessen the damages, various pest control techniques can be used, including chemical treatment, improved field procedures, and develop insect-pest resistant cultivars. Moreover, using too many chemicals to treat BPH puts patients at risk for health problems, is expensive, ineffectual, and bad for the environment and

TABLE-1 : Lists of SSR markers closely associated to key BPH resistance gene (s)

Presence on chromosome No.	Marker Nomen-clature	Genes	Expected Amplicon size (bp)		Sequences of primer (5'-3')	
					F: Forward primer	F: Reverse primer
6	RM217	Bph 4	133/140	Kawagachi	ATCGCAGCAA TGCCTCGT	GGGTGTGAAC AAAGACAC
3	RM545	Bph 13	233	Chen *et al*., 2006	CAATGGCAGAG ACCCAAAAG	CTGGCATGTAA CGACAGTGG
3	RM222	Bph 21 (t)	266	Jena *et al*., 2009	GGCTTACTGG CTTCGATTTG	CGTCTCCTTTGG TTAGTGCC
11	RM120		175	Jairin *et al*., 2007	CACACAAGCCCT GTCTCACGACC	CGCTGCGTCAT GAGTATGTA
2	RM401	Qbph4 (Bph 17)	157	Liu 2009	TCGAAGCCATC CACCAACGAAG	TCCGTACGCCGA CGAGGTCGAG
8	RM25		147	Sun 2005	GGAAAGAATGA TCTTTTCATGG	CTACCATCAAAA CCAATGTTC

biodiversity. Growing BPH-resistant cultivars is most environmentally friendly and economical form of production. There are currently 37 BPH resistance genes found in various sources of resistance[14]. There are primarily six chromosomes on which the majority of BPH resistance genes are found rather than all twelve (2, 3, 4, 6, 11, and 12). Biotypes 1 and 2 include a large number of BPH resistance genes, however they are ineffective against biotype[4,6,26]. The majority of key genes mapped using molecular markers, and several genes are used in real molecular marker-based rice breeding efforts[24]. A powerful tool, molecular markers can provide precise identity of resistant cultivars through high-resolution genetic mapping of important characteristics. To select resistant cultivars, Molecular markers are utilized[17]. MAS has become important methods in breeding since its introduction, 1970s. To date, it has been used to identify favorable characteristics in individuals or groups, such as insect resistance[2,16]. The application of molecular markers with BPH resistance would therefore be beneficial in molecular a series of MAS procedures are being used to select and clone genes for progeny. These procedures include map-based selection, gene pyramiding, and other techniques to search for these genes in offspring[1.]

Using SSR markers distributed across rice chromosomes, we can identify quantitative trait loci (QTLs) and BPH-resistant genes. This provides a significant opportunity in order to make traditional plant breeding more effective[10]. Antenna IV's Panel genotypes for resistance to BPH were tested in a lab setting and in a glasshouse. The identification of donors for forthcoming hybridization programs also includes the validation for BPH resistance by SSR markers.

Material and Methods

screening for BPH resistance on the Global Rice Array's exotic rice germplasm "Antenna Panel" in a glass house environment

GBPUAT Pantnagar farm was used for the collection of initial BPH populations, followed by rearing the seed through a standard seed box screening procedure (SSST)[8]. The experiment for genotype screening of rice was undertaken in two crop seasons: one in kharif-2021 (the main growing season) and another in the off-season in a glass house during 2022. It included 58 genotypes of rice from IRRI including susceptible check, TN1. A fundamental screening

TABLE-2: Under glasshouse conditions, BPH Damage scores for "Antenna Panel" genotypes

Genotypes	Damage Scores			Concl-usion	Genotypes	Damage Scores			Concl-usion
	RI	RII	Mean			RI	RII	Mean	
"IRRI 154"	9	9	9	HS	"Manaw Thukha"	3.3	3.9	3.6	MR
"MINGHUI 63"	7.6	7.8	7.7	S	"BR28"	5.5	5.4	5.4	MS
"ZHENSHAN 97 B"	3.8	4.2	4	MR	"TN1"	9	9	9	HS
"IR 64"	4.3	4.6	4.4	MR	"IR6"	5	6	5.5	MS
"IRBB 66"	7.5	7.1	7.3	S	"GSR IR2-9-R1-SU3-Y2"	5.3	5.5	5.4	MS
"IR 78222-20-7-148 -2-B-B-B-B"	5.5	_	5.5	MS	"zanton::IRGC 31248-1"	0	0	0	HR
"IR 69726-116-1-1"	4	4.2	4.1	MR	"URAIBOOL::IRGC 52785-1"	1.9	2.4	2.1	R
"IRRI 147"	0	0	0	HR	"Hokkai 188"	3.7	4.4	4.0	MR
"SANHUANGZHAN NO 2"	0	0	0	HR	"IR 126182-1-1-1"	7.7	7.6	7.6	S
"IR77186-122-2-2-3"	3	_	3	R	"IR10F360"	4	4.3	4.1	MR
"IR77298-14-1-2-10"	4.5	4.7	4.6	MR	"Sahel 108"	9	9	9	HS
"SAMBHA MAHSURI + SUB 1"	4.2	3.7	3.9	MR	"Sahel 134"	0	0	0	HR
"SUPA"	2.7	2.9	2.8	R	"Sahel 177"	2	2	2	R
"IRRI 104"	0	0	0	HR	"Giza 178"	3.2	4.9	4.0	MR
"N 22::IRGC 19379-1"	4.1	4.3	4.2	MR	"Moroberekan"	2.7	2	2.3	R
"MTU1010"	3.5	4	3.7	MR	"DJ123"	5.1	5.8	5.4	MS
"SWARNA"	3.8	3.9	3.8	MR	"Oryzica 1"	4	4.4	4.2	MR
"NANHI"	0	0	0	HR	"FEDEARROZ 50"	5.1	5.6	5.3	MS

Genotypes	Damage Scores			Concl-usion	Genotypes	Damage Scores			Concl-usion
	RI	RII	Mean			RI	RII	Mean	
“JASMINE 85”	4.3	4.7	4.5	MR	“TEQING”	8	8.4	8.2	S
“KINANDANG PATONG”	2	2.5	2.2	R	“MG 2::IRGC 79837-1”	7.8	7.6	7.7	S
“SADRI”	5.5	6	5.7	MS	“UPL RI 7::IRTP 9897-C1”	3.8	4.6	4.2	MR
“OM4900”	4.8	3.8	4.3	MR	“CT11891-2-2-7-M”	1.3	1.8	1.5	R
“IR 95042:13-B-7-11 -15-3”	4.1	4.2	4.1	MR	“Oryzica sabana 10”	3.6	4.4	4	MR
“IR 93340:14-B-21-17-12-1RGA-1-B-B”	4	4.5	4.2	MR	“Oryzica sabana 6”	4.2	3.8	4	MR
“IR 93354:34-B-5-1-23 -1RGA-1-B-B”	9	9	9	HS	“Oryzica Llanos 5”	4.9	4.7	4.8	MR
“Khao Hlan On”	2.1	2.8	2.4	R	“Chhomrong Dhan”	0	0	0	HR
“IR13F167”	0	0	0	HR	“NSIC Rc240”	0	0	0	HR
“IR84984-83-15-481-B”	0	0	0	HR	“Jamir”	0	0	0	HR
“M202”	0	0	0	HR	“IR10M300”	2.8	2.9	2.8	R

***The grading standards (rating and grade of damage) that the brown plant hopper used to rate the damage to seedlings of the “Antenna Panel” genotype:** HR is highly resistant=0; R is resistant=1; MR is moderately resistant=3; MS is moderately susceptible=5; S is susceptible=7; HS is highly susceptible=9

method specified by IRRI was used in the current experiment with a little modification[23]. Each genotype’s seeds were pre-soaked in petri plates. In a plastic tray, the pre-germinated seeds were planted one by one with equal spacing between each row. Along the width of the plastic tray, each row was planted in a row that was 20 cm long. Randomly planted varieties included a vulnerable and resistant (TN-1) check. From the test plants, around 20 plants per row were removed seven days after planting. In a controlled glass house room, the planted trays were put. The tray was kept with about 5 cm of standing water to maintain a high humidity level ideal for survival and eliminate the need to water the plants. Seedlings were invaded about 18 days after sowing by an abundance of insects that were strewn across them. The mass-rearing cages were tapped from heavily infected plants. The test varieties could have the insects as evenly as feasible. Nymphs of the second and third instars were typically employed for infestation. An ideal population for identifying resistant and susceptible lines consisted of five insects on average per seedling. It was noted whether or not the insects preferred the test kinds. However, the degree of harm that the various test kinds experienced determined the ultimate classification for resistance. The IRRI’s conventional scoring system, which ranges from 0 to 9, was applied in the investigation. The ultimate damage rating was determined when the susceptible check variety’s plants were killed to a level of roughly 90%, typically 7 to 10 days following infestation[28].

Use of closely related SSR markers for BPH resistance to Molecularly Characterize exotic lines

The Experimental site was Plant Biotechnology Laboratory of the Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar was used for the molecular marker analysis. All 58 genotypes "Antenna Panel" were used as experimental material for characterization investigations during 2022. Leaves from the 58 genotypes of the Global Rice Array-IV of IRRI "Antenna Panel" were all collected from the field at the three-leaf stage and preserved at -80^{0}C deep freezer until use in the lab. Deoxyribonucleic acid (DNA) isolation, purification, and microsatellite analysis were subsequently performed on these leaves.

Results

A *screening for BPH resistance on the "Antenna Panel" of the Global Rice Array exotic rice germplasm in a glass house environment*

The rice plant, *Oryza sativa*, is susceptible to a number of major and minor illnesses and insect pests, the most destructive of which is the brown plant hopper, which is brought on by *Nilaparvata lugens* and found in most of the regions. One of the most reasonably priced and efficient methods for the control of BPH is the adoption of host plant resistance. By using more chemicals and fertilizers on semi-dwarf rice varieties and better farming techniques, the green revolution era increased output, but it also made rice cultivation more susceptible to abiotic and biotic challenges (insects, diseases and weeds). Workers[7] estimated that cumulative losses from insect-pest damage to rice were 25%[8]. A brown planthopper epidemic could reduce rice yield by up to 60%. Both nymphs and adults of the BPH insect are detrimental to rice crops by sucking the phloem to cause hopper burn. They could also spread various viral illnesses. There are currently four different BPH biotypes known, namely Biotypes 1, 2, 3, and 4[1]. Both 1 and 2 biotypes are extensively spread in Southeast Asia, whereas 3 and 4 biotypes are developed in laboratories, respectively, in the Philippines and the Indian subcontinent. Although there have been reports of four primary biotypes, the pathogenicity of BPH populations to resistant cultivars varies from country to country and within nations. Evaluation of the Global Rice Commission's "Antenna Panel". Under glass-house conditions, an array of exotic rice germplasm for BPH resistance was tested. The experiment was repeated twice using the standard Seed Box Screening methodology between 0 to 9.0 was the median BPH damage score (Table-2). The genotypes of the "Antenna Panel" ("IRRI 154", "Sahel 108", "IR 93354:34-B-5-1-23-1RGA-1-B-B", and the susceptible check TN1) showed the greatest BPH damage scoring (BPH Damage Score- 9.0). The samples with the lowest BPH damage scores were IRRI 147, SANHUANGZHAN NO 2, IRRI 104, NANHI, IR13F167, IR84984-83-15-481-B, M202, zanton::IRGC 31248-1, Sahel 134, Chhomrong Dhan, NSIC Rc240, and Jamir. These samples were followed by CT11891-2-2-7-M (BPH Damage Score- 1.5), Sa (BPH Damage Score- 3.0).

In terms of efficiency and cost-effective ways to reduce the damages they cause is to produce resistant cultivars. However, conventional plant protection chemicals have a number of drawbacks, including environmental pollution due to the buildup of pesticide residues, disruption of the ecological balance caused by the killing of natural enemies, which results in pest resurgence and secondary pest outbreaks, and additional financial inputs that raise the overall cost of production[3]. It was discovered that the loss of biodiversity and disruption of the ecological balance were to blame for BPH outbreaks that took place in IRRI between 1971 and 1977. The diversity of resistance genes must therefore be preserved and used effectively to exploit host plant resistance. The insect biotypes found in Pantnagar are among the most dangerous and well-known in Northern India.

Use of closely related SSR markers for BPH resistance to Molecularly Characterize exotic lines

Any systematic breeding programme aimed at creating cultivars with long-lasting resistance is mostly dependent on the availability of numerous efficient resistance genes that have been well studied. Five preliminary genes and thirty significant resistance genes (Bph1 through Bph32) have been found and described thus far[9]. The *indica* species and its wild relatives are the source of every BPH resistance gene discovered thus far. Most of the genes discovered, however, cannot be used to inhibit North Western Indian BPH biotypes. Therefore, it is crucial for breeders to continuously uncover and characterise resistance genes and introduce them into top breeding lines given that the BPH population has the potential to evolve into more virulent biotypes. Due to their many applications in agricultural research, including genetic diversity analysis, marker-assisted selection, and plant variety protection, molecular markers have emerged as a crucial breeding tool. They offer quick and accurate target gene selection, which is why they are regarded as extremely efficient. The rice genome has many simple sequence repeat (SSR) marker sites that are simple to investigate using the polymerase chain reaction. SSR markers were deemed

TABLE-3: Genotyping of 58 'Antenna Panel' genotypes of Global Array Rice (GRA-IV) with allele specific SSR markers associated with rice Brown Plant Hopper (BPH) resistance genes

S. No.	Genotypes	*Bph 1* RM25	*Bph 3* RM 120	*Bph 21(t)* RM 222	*Bph 17* RM 401	*Bph 13* RM 545	*Bph 4* RM 217	No. of genes
G1	"IRRI 154"	-	_	_	_	_	_	0
G2	"MINGHUI 63"	-	_	_	+	_	_	1
G3	"ZHENSHAN 97 B"	-	+	_	+	+	_	3
G4	"IR 64"	+	-	_	+	+	_	3
G5	"IRBB 66"	-	_	_	+	_	_	1
G6	"IR 78222-20-7-148-2-B-B-B-B"	-	_	_	+	+	_	2
G7	"IR 69726-116-1-1"	-	+	_	+	+	_	3
G8	"IRRI 147"	+	+	_	+	+	+	5
G9	"SANHUANGZHAN NO 2"	+	+	_	+	+	+	5
G10	"IR77186-122-2-2-3"	+	_	_	+	+	+	4
G11	"IR77298-14-1-2-10"	-	_	_	+	+	+	3
G12	"SAMBHA MAHSURI + SUB 1"	-	-	_	+	+	+	3
G13	"SUPA"	+	_	_	+	+	+	4
G14	"IRRI 104"	+	_	+	+	+	+	5
G15	"N 22::IRGC 19379-1"	+	_	_	_	+	+	3
G16	"MTU1010"	-	+	_	_	+	+	3
G17	"SWARNA"	+	_	_	+	+	_	3
G18	"NANHI"	+	+	_	+	+	+	5
G19	"JASMINE 85"	+	_	_	+	+	_	3
G20	"KINANDANG PATONG"	+	+	_	+	+	_	4

S. No.	Genotypes	*Bph 1* RM25	*Bph 3* RM 120	*Bph 21(t)* RM 222	*Bph 17* RM 401	*Bph 13* RM 545	*Bph 4* RM 217	No. of genes
G21	"SADRI"	+	_	_	_	+	_	2
G22	"OM4900"	+	_	_	+	+	_	3
G23	"IR 95042:13-B-7-11-15-3"	+	_	_	+	+	_	3
G24	IR 93340:14-B-21-17-12-1RGA-1-B-B	+	_	_	+	+	_	3
G25	"IR 93354:34-B-5-1-23-1RGA-1-B-B"	-	_	_	_	_	_	0
G26	"Khao Hlan On"	-	+	_	+	+	+	4
G27	"IR13F167"	+	+	_	+	+	+	5
G28	"IR84984-83-15-481-B"	+	+	_	+	+	+	5
G29	"M202"	+	+	_	+	+	+	5
G30	"Manaw Thukha"	+	_	+	_	+	_	3
G31	"BR28"	+	_	_	_	+	_	2
G32	"TN1"	-	_	_	_	_	_	0
G33	"IR6"	+	_	_	+	_	_	2
G34	"GSR IR2-9-R1-SU3-Y2"	-	_	_	+	+	_	2
G35	"zanton::IRGC 31248-1"	+	+	_	+	+	+	5
G36	"URAIBOOL::IRGC 52785-1"	+	_	_	+	+	+	4
G37	"Hokkai 188"	+	_	+	_	+	_	3
G38	"IR 126182-1-1-1"	-	+	_	_	_	_	1
G39	"IR10F360"	+	+	_	_	+	_	3
G40	"Sahel 108"	-	_	_	_	_	_	0
G41	"Sahel 134"	+	+	_	+	+	+	5

S. No.	Genotypes	*Bph 1* RM25	*Bph 3* RM 120	*Bph 21(t)* RM 222	*Bph 17* RM 401	*Bph 13* RM 545	*Bph 4* RM 217	No. of genes
G42	"Sahel 177"	+	+	_	+	+	_	4
G43	"Giza 178"	-	+	_	+	+	_	3
G44	"Moroberekan"	+	+	_	+	+	_	4
G45	"DJ123"	+	_	_	_	+	_	2
G46	"Oryzica 1"	+	_	_	+	+	_	3
G47	"FEDEARROZ 50"	-	_	_	+	+	_	2
G48	"TEQING"	-	_	_	+	_	_	1
G49	"MG 2::IRGC 79837-1"	-	+	_	_	_	_	1
G50	"UPL RI 7::IRTP 9897-C1"	+	+	_	_	+	_	3
G51	"CT11891-2-2-7-M"	+	+	_	+	+	_	4
G52	"Oryzica sabana 10"	+	_	_	+	+	_	3
G53	"Oryzica sabana 6"	+	_	_	+	+	_	3
G54	"Oryzica Llanos 5"	+	_	_	+	+	_	3
G55	"Chhomrong Dhan"	+	+	_	+	+	+	5
G56	"NSIC Rc240"	+	+	_	+	+	+	5
G57	"Jamir"	+	+	_	+	+	+	5
G58	"IR10M300"	+	+	_	+	+	_	4

to be the most common in rice, due to their abundance, information, and affordability[27]. In BPH, SSR markers are widely used to locate disease and resistance genes. High yielding varieties (HYVs) for BPH resistant might be tracked using molecular markers closely linked to BPH resistance genes. With this backdrop, 58 genotypes derived from the "Antenna Panel" were used for molecular validation for some of the key R- genes ("Bph 21(t)", "Bph 17", "Bph 13", "Bph 4" etc.). None of the genotypes could be seen to have "Bph 21(t)", "Bph 17", "Bph 13", "Bph 4", and other important R genes (Table 5). The diversities for the R-genes studied among the 'Antenna Panel' genotypes for Brown Plant Hopper (BPH) resistance gene(s) studied is as follows.

Genetic diversity for Bph 1 gene

In terms of resistance to BPH, the BPH 1 gene has a wide range of tolerances. The visualization of an amplicon of 147 bp of positive fragments using primer RM 25 allowed for an estimation of the PCR findings for the Bph 1 rice BPH resistance genes[29]. On chromosome 12, the Bph 1 gene was scored, yielding 39 genotypes.

The genotypes that tested positive for the gene-based marker RM 25 with an amplicon of 147 bp were "IR 64", "IRRI 147", "SANHUANGZHAN NO 2", "IR77186-122-2-2-3", "SUPA, IRRI 104", "N 22::IRGC 19379-1", "SWARNA", "NANHI", "JASMINE 85", "KINANDANG PATONG", "SADRI", "OM4900", "IR 95042:13- *Oryzica sabana* 10", *"Oryzica sabana* 6", *"Oryzica llanos* 5", "Chhomrong Dhan", and "NSIC Rc240" are among the species (Figure 2). Kim & Sohn and Park et al. also got comparable results[19].

Genetic diversity for Bph 3 gene

In studies, the Bph3 gene segregated independently from the Bph1 gene. The afore mentioned gene was discovered in Rathu Heenati. In 1982, prominent strains like IR 56 and IR 60 were developed in the Philippines using Rathu Heenati as donors for BPH resistance genes, specifically Bph3. More cultivars bearing the Bph 3 gene were made available in the years namely, IR 68, IR 70, IR 72, and IR 74 variants. It has been demonstrated that cultivars carrying the gene Bph 3 are resistant to BPH-biotype 3. In South-East Asia, resistance breeding projects have commonly used genes like BPH 1, BPH 2, BPH 3, and BPH 4[11]. The genotypes that tested positive in the current study for the gene-based marker RM 120 with an amplicon of 175 bp were "ZHENSHAN 97 B", "IR 69726-116-1-1", "IRRI 147", "SANHUANGZHAN NO 2", "MTU1010", "NANHI", "KINANDANG PATONG", "Khao Hlan On", "IR13F167", "IR84984-83-15-481-B", "M202", "zanton: Chhomrong Dhan", "NSIC Rc240", "Jamir", "2::IRGC 79837-1", "UPL RI 7", "IRTP 9897-C1", "CT11891-2-2-7-M", and "IR10M300" (Figure 3). Similar outcomes have been found in earlier investigations[11]. In this investigation, amplicon size specific for the Bph 3 gene with a 175 bp fragment was positive in 25 genotypes.

Genetic diversity for Bph 21(t) gene

Bph1, Bph2, Bph9, Bph10, Bph18, and Bph21 genes were discovered to be located on chromosome 12[11,29]. The Bph 21 gene found on the longer arm of the 12th chromosome. It was located in an area of 194.0 kb[11]. Using primer RM 222, the amplicon on 266 bp of positive fragments was seen to estimate the PCR findings for the Bph 21(t) rice BPH resistance genes. Three genotypes of the Bph 21(t) gene on chromosome 3 were identified: "IRRI 104", "Manaw Thukha", and "Hokkai 188". (Figure 4). Rest assured that no genotype displayed a positive amplicon. Earlier investigations reported similar findings[25].

Genetic diversity of Bph 17 gene

The Bph 12, Bph 15, Bph 17, and Bph 20 R-genes were found on chromosome 4. The BPH R-gene, or Bph 17, is more specifically found on short arm of chromosome number 4 and is bordered by the SSR markers "RM 401", "RM8213" and "RM5953", and "MS10" and "RM5953", respectively. In reality, "Bph 17" and "Bph 20" were found in "*Rathu Heenati*" and "*Oryza minuta*", the wild ancestor of cultivated rice, respectively. The two genes mentioned above have certain areas that overlap[25]. As RM 401 associates closely with rice resistance genes and can be used through amplification to select for BPH resistance, it is an ideal marker for marker assisted selection. In the current investigation, only fifteen genotypes ("MG 2::IRGC 79837-1", "UPL RI 7::IRTP 9897-C1", "N 22::IRGC 19379-1", "MTU 1010") demonstrated the absence of "Bph 17". These genotypes are: "Manaw Thukha", "BR28", "TN1", "Hokkai 188", "IR 126182-1-1-1", "IR10F360", "Sahel 108", "DJ123", and "IRRI 154". The genotypes showing positive amplicon of size of 157 bp –indicating for the presence of "Bph 17" are "MINGHUI 63", "ZHENSHAN 97 B", "IR 64", "IRBB 66", "IR 78222-20-7-148-2-B-B-B-B", "IR 69726-116-1-1", "IRRI 147", "SANHUANGZHAN NO 2", "IR77186-122-2-2-3", "IR77298-14-1-2-10", "SAMBHA MAHSURI + SUB 1", "SUPA", "IRRI 104", "SWARNA", "NANHI", "JASMINE 85", "KINANDANG PATONG", "OM4900", "IR 95042:13-B-7-11-15-3", "IR 93340:14-B-21-17-12-1RGA-1-B-B", "IR6", "GSR IR2-9-R1-SU3-Y2", "zanton::IRGC 31248-1", "URAIBOOL::IRGC 52785-1", "Khao Hlan On", "IR13F167", "IR84984-83-15-481-B", "M202", "Sahel 134", "Sahel 177", "Giza 178", "Moroberekan", "*Oryzica* 1", "FEDEARROZ 50", "TEQING", "CT11891-2-2-7-M", "*Oryzica sabana* 6", "*Oryzica sabana* 10", "*Oryzica llanos* 5", "Chhomrong Dhan", "NSIC Rc240", "Jamir and IR10M300". The results coincided with the previous study[15].

Genetic diversity of Bph 13 gene

Bph13(t), the BPH R-gene, was obtained from "IR54741-3-21-22" (an *O. officinalis* introgression line). Following the usage of a RAPD marker, the third chromosome was located. This gene was discovered to confer resistance in genotypes having them against the Indian biotype of BPH in numerous studies (biotype 4). Here, a single dominant gene regulates the resistance[15]. In the current investigation, visualisation of the amplicon on 233 bp of positive fragments using primer RM 545 was used to estimate the PCR findings for the Bph 13 rice BPH resistance genes. Bph 13 gene on chromosome 3 was scored in 48 genotypes viz., "ZHENSHAN 97 B", "IR 64", "IR 78222-20-7-148-2-B-B-B-B", "IR 69726-116-1-1", "IRRI 147", "SANHUANGZHAN NO 2", "IR77186-122-2-2-IR77298-14-1-2-10", "SAMBHA MAHSURI + SUB 1", "SUPA", "IRRI 104", "N 22::IRGC 19379-1", "MTU1010",

"SWARNA", "NANHI", "JASMINE 85", "KINANDANG PATONG", "SADRI", "OM4900", "IR 95042:13-B-7-11-15-3", "IR 93340:14-B-21-17-12-1RGA-1-B-B", "Khao Hlan On", "IR13F167, IR84984-83-15-481-B", "M202", "Manaw Thukha", "BR28", "GSR IR2-9-R1-SU3-Y2", "zanton::IRGC 31248-1", "URAIBOOL::IRGC 52785-1", "Hokkai 188", "IR10F360", "Sahel 134", "Sahel 177", "Giza 178", "Moroberekan", "DJ123", "Oryzica 1", "FEDEARROZ 50", "UPL RI 7::IRTP 9897-C1", "CT11891-2-2-7-M", *"Oryzica sabana* 10", "*Oryzica sabana* 6", "*Oryzica llanos* 5", "Chhomrong Dhan", "NSIC Rc240", "Jamir" and "IR10M300". Rest assured that no genotype displayed a positive amplicon. "TEQING", "MG 2::IRGC 79837-1", I"RBB 66", "IR 93354:34-B-5-1-23-1RGA-1-B-B", "TN1", "IR6", "IR 126182-1-1-1", "Sahel 108", "IRRI 154", and "MINGHUI 63" are the genotypes that lacked a positive amplicon. The findings were consistent with the earlier research 2[26].

Genetic diversity of Bph 4 gene

Bph 1 gene-carrying cultivars provide resistance to 1and 3 biotypes, but are vulnerable to biotype 2. All four biotypes of "BPH 3" are resistant to the gene, while the "BPH 2" gene confers resistance to only one and two biotypes, but not to biotype 3. Only biotype 4 is resistant to diseases caused by genes like "bph 5", "bph 6", and "bph 7"[13]. Using primer "RM 217", the amplicon on 140 bp of positive fragments was seen to estimate the PCR findings for the "Bph 4" rice BPH resistance genes. 20 genotypes of the Bph 4 gene were scored, including "IRRI 147", "SANHUANGZHAN NO 2", "IR77186-122-2-2-3", "IR77298-14-1-2-10", "SAMBHA MAHSURI + SUB 1", "SUPA", "IRRI 104", "N 22::IRGC 19379-1", "MTU1010", "NANHI", "Khao Hlan On", "IR13F167", "IR84984-83-15-481-B", "M202", "zanton::IRGC 31248-1", "URAIBOOL::IRGC 52785-1", "Sahel 134", "Chhomrong Dhan", "NSIC Rc240", and "Jamir". Rest assured that no genotype displayed a positive amplicon. The findings were consistent with the earlier research[12].

Conclusions

The 'Antenna Panel' genotypes - IRRI 154, Sahel 108, IR 93354:34-B-5-1-23-1RGA-1-B-B and the susceptible check TN1 exhibited the highest BPH damage score. The lowest BPH damage score was exhibited by – "IRRI 147", "SANHUANGZHAN NO 2", "IRRI 104", "NANHI", "IR13F167", "IR84984-83-15-481-B", "M202", "zanton::IRGC 31248-1", "Sahel 134", "Chhomrong Dhan", "NSIC Rc240", "Jamir".Out of 58 genotypes, more than 54 genotypes showed the primary BPH resistance to SSR-based characterization, with 9 genotypes having 4 genes, 12 having 5 genes, and 21 genotypes having 3 genes, respectively. The main R genes "Bph 1", "Bph 3", "Bph 21(t)", "Bph 17", "Bph 13", and "Bph 4" were not present in any of the genotypes. The following genotypes can be utilised as donors in BPH resistance breeding programmes: "IRRI 147", "SANHUANGZHAN NO 2", "IRRI 104", "NANHI", "IR13F167", "IR84984-83-15-481-B", "M202", "Zanton::IRGC 31248-1", "Sahel 134", "Chhomrong Dhan", "NSIC Rc240", and "Jamir".

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