PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–11
https://doi.org/10.14719/pst.5677

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Phenotypic and genotypic insights into rice germplasm resistance against a biotype of the brown planthopper *Nilaparvata lugens* (stal)

Bodireddy Thornavalli[1], Sheela Venugopal[1]*, RP Soundararajan[1], S Mohankumar[2], R Suresh[3], V Baskaran[1], M Alagar[1], B Geetha[4], S Vignesh[5] & S Manonmani[3]

[1]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[2]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[3]Department of Rice, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[4]Regional Research Station, Tamil Nadu Agricultural University, Vridhachalam 606 001, Tamil Nadu, India

[5]Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Email: sheelavenugopal@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY
Received: 09 October 2024
Accepted: 06 November 2024
Available online
Version 1.0 : 28 December 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Thornavalli B, Venugopal S, Soundararajan RP, Mohankumar S, Suresh R, Baskaran V, Alagar M, Geetha B, Vignesh S, Manonmani S. Phenotypic and genotypic insights into rice germplasm resistance against a biotype of the brown planthopper *Nilaparvata lugens* (stal). Plant Science Today.2024;11(sp4):01-11. https:/doi.org/10.14719/pst.5677

Abstract

This study explores the resistance of rice landraces, genotypes and wild rice to the South Asian brown planthopper (BPH) population, a significant pest affecting rice crops. Phenotypic screening identified a few landraces, genotypes and wild rice varieties with resistance to the South Asian biotype, including, PTB-33, *Oryza officinalis, O. nivara*, *Adukkan*, *Vellai Kombi Samba and Onamuttan* which exhibited damage scores ≤ 3 Moderate resistance was observed in IR 36, ARC 10550, T12, *Manvilayan,* Rathu Heenati, CR 2711, *Mysore Malli,* Swarnalata, Pokkali, Babawee and ASD 7, while other varieties demonstrated varying levels of susceptibility. Molecular analysis using SSR markers revealed the presence of BPH resistance genes across these genotypes. Notably, *Onamuttan* and *Vellai kombi samba* exhibited a broad resistance gene profile, while *Adukkan*, *Manvilayan* possess two BPH-resistant genes. Conversely, ASD 7 and Rathu Heenati, despite possessing several resistance gene markers, displayed moderate resistance. This study underscores the potential of integrating resistant genotypes into breeding programs, employing SSR markers for precise gene mapping and marker-assisted selection. The combined phenotypic and genotypic data provide valuable insights for advancing rice breeding strategies for sustainable BPH management and improved pest resistance.

Keywords

brown planthopper; cluster analysis; germplasm; molecular mechanism; resistant gene; rice

Introduction

Rice (*Oryza sativa L.*) is one of the most important staple foods for around four billion people worldwide. Globally, India is the second largest producer of rice after China (1). The BPH *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) is the most damaging biological barrier to rice production in most Asian countries. The BPH causes two main types of injury to rice: mechanical damage caused by sucking phloem from the plants, or transmission of viral disease, *viz*., ragged stunt phytoreovirus (RRSV), rice wilted stunt virus (RWSV), and rice grassy stunt virus (RGSV). In India, the first significant report of damage by the BPH came from Kerala in 1973, with subsequent reports from other states, including Andhra Pradesh, Bihar, Haryana, Orissa, Punjab, Tamil Nadu, and Uttar Pradesh (2, 3). In Andhra

Pradesh alone, the pest caused estimated losses of 1.33 to 2.11 lakh tonnes of rice, valued at Rs. 14.41 to 23.00 crores, during 1981 and 1982. Notable outbreaks were also recorded in 2007 in parts of the Cauvery command area in Karnataka and in 2008 in Haryana, Punjab, and Delhi (4). Plant resistance is the most practical and effective way of controlling pests in agricultural plants. Therefore, using a host-plant resistance approach to manage insects and increase yield is the most cost-effective, efficient, and ecologically safe approach. Host plant resistance is primarily caused by antixenosis, antibiosis and tolerance (5). Antixenosis avoids insect pest damage by repelling or disturbing the insects, thereby reducing pest colonization and oviposition. Antibiosis is a key mechanism that imparts resistance to BPH, primarily influencing insect behavior such as survival, feeding, and reproduction after infestation. The tolerance mechanism is peculiar, in which plants can produce good quality crops with little or no decrease in fitness despite being attacked (6). The genes provide their resistance by utilizing one or a combination of these three defense mechanisms.

Biotypes of BPH are defined as a population or an individual distinguished from others by non-morphological traits, such as adaptation and development to a particular host or host preference for feeding or oviposition, or both (7). The biotypes of BPH exhibit varying virulence patterns on different rice cultivars and landraces (8). Four distinct BPH biotypes are recognized: Biotypes 1 and 2 are commonly found in Southeast and East Asia, while Biotype 3 was developed in the laboratory by rearing the insects on the resistant variety ASD7, which carries the *bph2* resistance gene (9). The most destructive of these is Biotype 4, prevalent in the Indian subcontinent and often referred to as the South Asian biotype (10). Rice cultivars with the *Bph1* gene are resistant to Biotypes 1 and 3 but are susceptible to Biotype 2 (Table 1). Conversely, the *bph2/Bph26*gene provides resistance to Biotypes 1 and 2 but not to Biotype 3. *Bph3* gene, along with *bph4, bph8, bph9, Bph13(t),* and *BPH18(t),* confers resistance to all four biotypes. Additionally, the genes *Bph6, bph7*, *BPH31*, and *Bph34* specifically confer resistance to Biotype 4 (11). The variety PTB-33, which carries *Bph 3, 2, 17(t), 32* and some unknown genes, is resistant to nearly all known biotypes.

Plant genetic resistance is the most appropriate, eco-friendly, cost-effective method for managing *N. lugens*. A total of 70 BPH-resistant gene loci have been identified in rice;64 out of 70 genes/QTLs were mapped on chromosomes 1,2,3,4,6,8,10,11, and 12, respectively, with 17 of them successfully cloned and utilized in resistance breeding programs (12). The first dominant resistance gene, *Bph1*was discovered on the long arm of chromosome 12 in the indica rice cultivar Mudgo (12, 13). The first recessive gene, *bph2*, was also identified on the long arm of chromosome 12 in the *indica* cultivar ASD 7 (12, 14, 15). In the rice cultivars Rathu Heenati and Babawee, the resistance genes *Bph3* and *Bph4* were mapped to the short arm of chromosome 6 (16-18). Another resistance gene, *BPH6*, was localized on the long arm of chromosome 4 between markers RM 6997 and RM 5742. Furthermore, three resistance genes-*bph5*, *Bph6* and *bph7*were identified in the cultivars ARC 10550, Swarnalata and T12, respectively (19). According to a study, the *BPH6* gene is located on the long arm of chromosome 4, flanked by markers RM 6997 and RM 5742 (20). Another resistance gene, *Bph9*, was identified on the long arm of chromosome 12 in the cultivars Balamawee, Kaharamana and Pokkali (21, 22). Additionally, in the cultivar Rathu Heenati, the dominant gene *Bph17* was located on the short arm of chromosome 4. *Bph20(t)* was mapped to a 193.4-kb region on the short arm of chromosome 4, and another *Bph21 (t)* was mapped to a 194.0-kb region on the long arm of chromosome 12 in the IR-71033-121-15 (23).

Reports suggest that *N. lugens* biotype 4 has already overcome the resistance in well-known resistant genotypes like ASD 7 (*bph2*), IR 36 (*bph2*), Babawee (*bph4*), Chinsaba (*bph8*), Rathu Heenati (*Bph3*+ *Bph17*), Swarnalata (*Bph* 6) and T12 (*Bph7*) (7). IR71033-121-15, used in study also carries two new resistance genes, Bph20 and Bph21, making it a valuable source of BPH resistance (24). Moreover, being a monophagous pest of rice, *Nilaparvata lugens* can quickly overcome the resistant genes in its rice host through mutation, resulting in new virulence (25). Hence, it is necessary to find new and effective resistance sources from various resources, such as landraces and wild relatives, against this pest, as it continuously evolves with the host plants Table 1.

Landraces are frequently reported as treasures of valuable genes, which have become important sources of genetic variation that provide resistance and tolerance to biotic and abiotic stress (26). Understanding the genetics of rice resistance and BPH defense to their host plant is crucial for breeders in determining effective breeding strategies. Although numerous sources of resistance have been identified from various cultivars and wild species, the emergence of biotypes is compelling researchers to continue searching for new sources of resistance from germplasm to create tolerant and resistant varieties that possess desirable traits and resistance characteristics.

Table 1. Relationship between biotypes of brown planthopper and resistance genes

Germplasm	Gene	Reaction to Biotype			
		1	2	3	4
Mudgo	*Bph1*	R	S	R	S
ASD 7	*bph2*	R	R	S	S
Rathu Heenati	*Bph3*	R	R	R	R
Babawee	*Bph4*	R	R	R	R
ARC 10550	*bph5*	S	S	S	R
Swarnalata	*Bph6*	S	S	S	R
T12	*bph7*	S	S	S	R
Chin saba	*bph8*	R	R	R	-
Balamawee	*Bph9*	R	R	R	-
IR1154-243	*bph2/Bph26*	R	R	S	S
Kaharamana	*BPH9*	R	R	R	S
TN 1	*none*	S	S	S	S
IR65482-7-216 (O. australiensis)	*BPH18(t)*	R	R	R	R
IR54745-2-21 (*O. officinalis*)	*Bph13(t)*	R	R	R	R
O. officinalis (acc. 100896)	*Bph6,Bph13*	R	R	R	R
O. minuta (acc. 101141)	*Bph20, Bph21*	R	ND	ND	ND
O. latifolia (B14)	*Bph12*	ND	R	ND	ND
O. australiensis (acc. 100882)	*Bph18*	R	R	R	R

Materials and Methods

Phenotyping

Screening for BPH resistance: A total of 24 rice germplasm, including a susceptible check (TN1) and a resistant check PTB33 (Table 2), were screened against BPH using the Protray Screening. This method involved infestation of young seedlings (about 12 days old) of test entries grown in screening protrays (50 cm × 40 cm × 8 cm) filled with fertilizer-enriched puddled soil for uniform growth of seedlings and to avoid soil-related problems. Each screening protray includes the 22 test lines and susceptible TN-1 and resistant PTB-33 rice cultivars.

Field-collected BPH were inoculated into the TN-1 variety and kept in a cage to prevent escaping. They were also maintained at the paddy breeding station, TNAU, Coimbatore. From the culture, first and second-stage nymphs were released onto test entries 12-13 days after planting by shaking BPH-infested potted seedlings such that 4 - 5 nymphs were present per seedling. The screening protrays with BPH nymphs were kept inside the oviposition cages to prevent the nymphs from escaping. These infested protrays were regularly monitored for plant damage. After 7days of nymphs release, when TN-1 plants showed 90 percent of the wilting of leaves, the test entries were scored for damage reaction on a scale of 0-9 where 0 as Immune, 0-1 is classified as Highly resistant, 1-3 is classified as resistant, 3-5 as moderately resistant, 5-7 as moderately susceptible and 7-9 is classified as susceptible as per international standard evaluation system of IRRI as described in Table 3.

Honey dew excretion test

Honeydew excretion is a widely used method for assessing feeding activity and is a reliable indicator of a crop variety's resistance or susceptibility to homopteran pests. The honeydew excretion test was conducted using a feeding chamber to evaluate BPH feeding response on different rice landraces (27). This chamber consisted of an inverted, transparent plastic cup positioned over a filter paper placed on a plastic petri dish. Three 2- to 3-day-old gravid female adults, starved for 4 hours, were introduced into the chamber through a hole at the top of the cup to collect the honeydew. A cotton wad was then used to seal the hole, preventing the insects from escaping. The insects were permitted to stay for 24 hours. After 24 hours, the filter papers were collected and sprayed with a 0.1% ninhydrin solution in acetone. After being dried, the honeydew spots turned violet or purple due to the amino acids present. The area of the purple or violet, representing honeydew droplets, was measured in square millimeters (mm^2) using a graphical method (28). The feeding chambers were arranged in a randomized complete block design, with each cup serving as a replicate. The experiment included 25 treatments, each replicated and statistically compared data thrice.

Nymphal Preference

To study the settling response, pre-germinated seeds of the selected landraces were sown in protray of 45 cm × 30 cm with 50 wells of 4.5 cm diameter each, commonly used for raising vegetable seedlings. The wells of the protrays were filled with moist pulverized clay soil. The susceptible check (TN 1) was sown in two border rows, and the resistant check (PTB 33) was sown in the middle row. After 10 days of sowing, seedlings were thinned to 5 numbers/entry and the protray was covered with a cylindrical mylar film cage (20 cm diameter) with fine-mesh nylon. Sufficient second instar nymphs obtained from mass culture were released into protray (approximately at 10/seedling) and the number of nymphs settled on the base of the seedlings were manually recorded at 3 days on different rice germplasm without disturbances. To know the antixenosis basis of resistance mechanism where fewer nymphs were settled in resistant varieties compared to susceptible check. The experiment was set up in a completely randomized design with three replications.

Statistical analysis

The data obtained from various experiments related to Screening, Honeydew excretion and Nymphal preference were analyzed in a CRD design, subjected to necessary transformation (square root transformation), and statistically analyzed using one -way ANOVA (Analysis of Variance) and Dunncan's Multiple Range Test was performed for mean comparison by using STAR (Statistical Tool for Agricultural Research) developed by International Rice Research Institute (IRRI).

Table 2. Rice germplasm - varieties, landraces and wild genotypes used in the study

S. No.	Rice Germplasm	S. No.	Rice Germplasm	S. No.	Rice Germplasm
1.	ASD 7	9.	T12	17.	IR-71033-121-15
2.	IR 36	10.	Swarnalata	18.	*O. officinalis*
3.	PTB 33	11.	Rathu Heenati	19.	*O. nivara*
4.	*Adukkan*	12.	Babawee	20.	*Mapillai Samba*
5.	*Onamuttan*	13.	IR 64	21.	*Kavuni*
6.	*Manvilayan*	14.	CR 2711	22.	*Vellai kombi samba*
7.	*Panamara Samba*	15.	ARC 10550	23.	*Norungan*
8.	TN-1	16.	Pokkali	24.	*Mysore Malli*

Table 3. Damage score and category of resistance of rice to BPH

Score	Damage level	Resistance Category
0	No damage	Immune
0-1	Very slight damage	Highly resistant
1-3	Leaves partially yellow but with no hopper burn	Resistant
3-5	Pronounced yellowing and stunting or about 10 to 25% of the plants severely stunted or dying	Moderately resistant
5-7	More than half of the plants dead	Moderately susceptible
7-9	All plants are dead	Susceptible

(International Rice Research Institute-Standard Evaluation System, 2013)

Genotyping

Genomic DNA isolation: A laboratory experiment was carried out in the Molecular Ecology Laboratory, Department of Plant Biotechnology, Tamil Nadu Agricultural University to evaluate promising landraces for the presence of BPH-resistant genes. The selected germplasm was genotyped using selected gene-specific SSR markers for BPH-resistant genes.

The total genomic DNA was extracted from each germplasm's top three young leaves by the protocol using cetyltrimethylammonium bromide (CTAB) with minor modifications (29). The extracted DNA samples were then dissolved in buffer (10 mM Tris base,1 mM EDTA, pH: 8.00). The purity and concentration of DNA were determined spectrophotometrically at 260 and 280 nm using the NanoDrop ND 1000 Spectrophotometer. The DNA samples were diluted to a concentration of 20 ng/ml with TE (Tris-EDTA) for analysis.

Twenty-four SSR primer pairs linked to chromosomes 1,4,6,7 and 12 were obtained from a published rice microsatellite framework map (Gramene database) and other previously published research on BPH resistance with associated markers. The primer sequences were used and the oligos were synthesized from a commercial facility (Eurofins, Bengaluru, India). The markers used are listed in Table 4.

The PCR analysis and agarose gel electrophoresis: The polymerase chain reaction was conducted in an applied biosystems thermal cycler using 24 SSR primers with 10 µl reaction volume. This volume included template DNA of 2 µl, 0.5 µl each forward and reverse primer, PCR master mix of 3.0 µl and sterile distilled water of 4.0 µl. Prior to loading into a PCR thermal cycler with 96 wells, the PCR mix was centrifuged at 1000 rpm for 1 min. The PCR profile for amplification of genomic DNA begins with initial denaturation of 5 min at 94°C, followed by thirty-five cycles of denaturation for 30sec at 94°C, annealing for 10sec at 55°C, extension for 1 min at 72°C, and final extension for 10 min at 72°C. The amplified PCR products were resolved on a 3% agarose gel run for approximately 1 hour 30 min at a constant voltage of 70V. The DNA fragments were visualized under a UV-transilluminator and recorded using a BIORAD gel documentation system (30).

Results

Laboratory screening for BPH resistance was conducted on selected rice germplasm, including resistant varieties, landraces, and wild rice genotypes, to identify new resistant genotypes and assess the resistance status of known varieties against BPH. Evaluating these germplasm would help identify new resistant genotypes from landraces and determine the shift in resistance status of the already known resistant genotypes to this biotype of BPH, which would immensely help breeders develop improved rice varieties. These resistant varieties can then be integrated into pest management strategies that are both economically sound and environmentally safe, promoting more effective and sustainable pest management practices.

Protray screening

The results of the screening trials showed that none of the germplasm exhibited high resistance, evidenced by a damage score of 1. However, six genotypes - including one resistant variety, two wild rice and three landraces *viz*., PTB-33, *O. nivara, O. officinalis, Vellai Kombi Samba, Adukkan and Onamuttan* were classified as resistant, with a damage score of ≤3. Eleven genotypes, namely ARC 10550, T12, Rathu Heenati, CR 2711, Swarnalata, IR 36, *Mysore Malli*, *Manvilayan*, Babawee, Pokkali and ASD 7 showed a moderate level of resistance with an average damage score ranging from 3 to 5. Three genotypes, including IR-71033-121-15, IR 64 and *Panamara Samba* were moderately susceptible. The remaining four genotypes, *O. minuta*, *Norungan*, *Kavuni*, *Mapillai Samba* and TN-1, showed a susceptible reaction to BPH incidence (Table 5). PTB 33 recorded the lowest damage score, 1.5 and was on par with *O. officinalis*, and *O. nivara*. *Vellai Kombi Samba* and *Adukkan* were par on each other. The susceptible check, TN 1, showed the highest damage score of 9, similar to *Kavuni*.

Nymphal settlement – a measure of antixenosis

The settling behavior of nymphs on the third day after release differed significantly among the genotypes. Among different genotypes, the least number of nymphs settled on *O. officinalis*, *Onamuttan*, *Vellai Kombi Samba*, Rathu Heenati, followed by PTB 33 (1.8-4.33 nos./seedling) (Table 5). The moderately resistant

Table 4. Details of markers used for detection of R genes/QTLs for *Nilaparvata lugens* in PCR

Sl. No	Gene/QTL	Chromosome	Marker	Annealing temp (°C)	Forward (5' – 3')	Reverse (3'- 5')	Size (bp)	Reference
1	*BPH-2*	12	RM28493	55	ACCGTTAGATGACACAAGCAACG	GGTTAGCAAGACTGGAGGAGACG	259	(38, 49)
2	*bPH-2*	12	ID-161-2	55	ATCCTTTCGGACAGGGTGAT	GGACGGGATGATACCTCAGA	144	(38, 50)
3	*bPH-2*	12	RM28449	55	CACCCATTGATGTGAAACTCTGG	GGATTCATGATACAGTGTGCAACG	345	(38)
4	*BPH-3*	4	RM589	55	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	186	(51)
5	*bph-4*	6	RM190	55	TTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	124	(52)
6	*bPH-4*	6	RM225	55	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	140	(53)
7	*bph-4*	6	RM217	55	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC	133	(18, 52)
8	*BPH-6*	4	RM119	55	ATCCCCCTGCTGCTGCTGCTG	GCCGGATGTGTGGGACTAGCG	166	(20)
9	*Qbph-7*	7	RM500	55	GAGCTTGCCAGAGTGGAAAG	GTTACACCGAGAGCCAGCTC	259	(43)
10	*Bph -17*	4	RM6156	55	CGTCCGCACGCAAGAAGAAGG	CCGTACGTGTGGCTTCAGATTGG	280	(38)
11	*BPH17-ptb*	4	RM1305	55	GGTACTACAAAGAAACCTGCATCG	TCCTAGCTCAAATGTGCTATCTGG	117	(38, 49)
12	*BPH-20(t) Bph-17*	4	MS10	55	CAATACGAGAAGCCCCTCAC	CTGAAGGAACACGCGGTAGT	170	(39, 44)
13	*BPH-32*	6	RM19341	55	GCTACAAATAGCCACCCACACC	CAACACAAGCAGAGAAGTGAAGC	121	(38)
14	*BPH-32*	6	RM19291	55	CACTTGCACGTGTCCTCTGTACG	GTGTTTCAGTTCACCTTGCATCG	146	(38)
15	*Bph-33(t)*	1	RM488	55	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC	198	(48)
16	*Bph-33(t)*	1	RM11522	55	TAACTGCAGTGCTCAACAAAGG	CTAGGTACCGGATTAAGATTCACC	320	(48)
17	*Bph-33(t)*	1	RM212	55	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	140	(48)

varieties, *viz*., ARC 10550, Pokkali, ASD 7, had more nymphal settlements (13.33, 8.67, 7.67, nos./seedling, respectively). *Mapillai Samba* recorded the highest number of nymphs settled (13.67 nos./seedling) after 3 days after release. In the susceptible check, TN 1 recorded 7.67 nymphs/seedlings (Table 5).

Feeding rate of brown planthopper – a measure of antibiosis

The amount of phloem sap in the honeydew excreted by the insects in the selected germplasm was measured in mm^2. Among the twenty-four germplasms evaluated, *Adukkan, Manvilayan, Onamuttan*, *Vellai Kombi Samba*, showed a very low rate of honeydew excretion (5.3-16.0 mm^2), indicating the antibiosis effect of the germplasm against BPH, lower than the resistant check, PTB 33 which recorded 42.7mm^2. Moderately resistant entries *viz*., ASD 7, Swarnalata, Pokkali and *Mysore Malli* recorded honeydew areas greater than 200 mm^2. The highest honeydew excretion was recorded for TN 1 (600.3 mm^2) (Table 5).

Phenotypic cluster analysis

Agglomerative cluster analysis involved 23 germplasms alongside standard susceptible (TN 1) and resistant check (PTB 33). The study utilized phenotypic damage scores, nymphal settlement per seedling, and feeding rates as parameters. When the germplasm was subjected to agglomerative clustering using their BPH damage score, BPH nymphal settling and feeding rate, they were grouped into 4 clusters. The landrace *Kavuni* and susceptible check TN1, which exhibited high rates of damage score, nymphal settling, and feeding rate, were grouped in Cluster 1. Cluster 2 contained the highest number of germplasm (12 in total), including 6 resistant germplasm, 5 moderately resistant germplasms and 1 moderately susceptible. It was observed that the resistant germplasm viz., *Vellai Kombi Samba*, *Onamuttan*, *Adukkan*, *O. nivara, O. Officinalis,* and PTB 33 were grouped in this cluster. The moderately susceptible germplasm in Cluster 2 was *Panamara Samba,* which recorded lower honeydew (74.3 mm^2) despite higher damage score (7.0) and nymphal settlement (10 BPH/seedling). Cluster 3 consisted of 2 landraces *Mysore Malli*, Pokkali, with low damage score, high feeding rate, and a less number of nymphs settled, which would have made these germplasm to fall into this cluster. Cluster 4 comprised 8 genotypes, including 4 moderately resistant, 2 moderately susceptible and 2 susceptible genotypes. The germplasm having moderate resistance *viz*., ASD 7, Babawee, CR2711 and Swarnalata, fell into this category due to high honeydew deposition. All susceptible genotypes, such as *Kavuni*, *Mapillai Samba Norungan* and TN 1 fell into clusters 1 and 4 due to their high feeding rate, damage score and number of nymphal settlements (Fig. 1).

Detection of BPH-resistant genes using reported markers

The reported molecular markers associated with resistant genes were used in the current investigation to ascertain the presence of BPH resistance genes in the selected germplasm (Table 2). The set of markers used in this study was carefully chosen from the literature. Twenty-five markers linked to ten known BPH genes were used to screen the germplasm that demonstrated resistant and susceptible reactions in phenotyping screening. Gel band analysis was performed to understand the genetic background of the resistant and susceptible genotypic reactions. DNA fragments were amplified using PCR and analyzed via gel electrophoresis to identify the polymorphic reaction of the different genotypes to the selected markers. The gel banding pattern showed polymorphic banding in only 17 out of the 24 markers used, which were linked to 8 BPH genes viz., *Bph 2, 3, 4, 6, 7, 17, 32* and *33.* Hence, these were considered valid markers for comparison and subsequent analysis (Table 6).

Table 5. Response of selected rice germplasm to BPH feeding

Sl. No	Germplasm	Mean damage score*	Honeydew area (mm^2)*	Nymphal settlement*	Category
1	ASD 7	5.0n (2.34)^{cd}	232.0 (15.25)^{gh}	7.67 (2.82)^{bc}	MR
2	IR 36	3.5 (1.99)^{ef}	129.3 (11.39)^{i}	6.67 (2.67)^{cde}	MR
3	*Adukkan*	2.3 (1.65)^{gh}	5.3 (2.41)^{mn}	5.33 (2.41)^{hi}	R
4	*Onamuttan*	3.0 (1.87)^{fg}	14.3 (3.85)^{lmn}	3 (1.87)^{hi}	R
5	*Manvilayan*	4.0 (2.12)^{def}	13.7 (3.77)^{lm}	6 (2.55)^{ghi}	MR
6	*Panamara Samba*	7.0 (2.72)^{b}	74.3 (8.65)^{ijk}	10 (3.24)^{def}	MS
7	T12	3.7 (2.04)^{ef}	30.0 (5.52)^{jkl}	4.67 (2.27)^{gh}	MR
8	Swarnalata	4.2 (2.15)^{de}	272.5 (16.52)^{def}	5.67 (2.48)^{efg}	MR
9	Rathu Heenati	4.0 (2.12)^{def}	44.7 (6.72)^{ijk}	3.67 (2.04)^{ghi}	MR
10	Babawee	4.3 (2.17)^{de}	196.7 (14.04)^{fg}	5 (2.35)^{gh}	MR
11	IR 64	5.6 (2.46)^{bc}	248.7 (15.79)^{ef}	8 (2.92)^{bc}	MS
12	CR 2711	4.0 (2.12)^{def}	212.3 (14.59)^{fg}	5 (2.35)^{gh}	MR
13	ARC 10550	3.5 (1.99)^{ef}	63.3 (7.99)^{ij}	13.33 (3.72)^{a}	MR
14	Pokkali	4.3 (2.17)^{de}	358.0 (18.93)^{cd}	8.67 (3.03)^{b}	MR
15	IR-71033-121-15	6.0 (2.55)^{bc}	203.0 (14.27)^{fg}	8.67 (3.03)^{b}	MS
16	*O. officinalis*	2.0 (1.58)^{h}	91.3 (9.58)^{hi}	1.8 (1.52)^{j}	R
17	*O. nivara*	2.0 (1.57)^{h}	-	6.2 (2.58)^{j}	R
18	*Mapillai Samba*	9.0 (3.08)^{a}	222.0 (14.92)^{fg}	13.67 (3.76)^{a}	S
19	*Kavuni*	9.0 (3.08)^{a}	523.0 (22.88)^{ab}	9 (3.08)^{cd}	S
20	*Vellai Kombi Samba*	2.3 (1.65)^{gh}	16.0 (4.06)^{lm}	3.33 (1.96)^{i}	R
21	*Norungan*	9.0 (3.08)^{a}	219.3 (14.83)^{cde}	8.67 (3.03)^{cd}	S
22	*Mysore Malli*	4.0 (2.12)^{def}	365.7 (19.14)^{bc}	4.67 (2.27)^{ghi}	MR
23	TN-1	9.0 (3.08)^{a}	600.3 (24.51)^{a}	7.67 (2.86)^{bc}	S
24	PTB 33	1.5 (1.39)^{h}	42.7 (6.57)^{klm}	4.33 (2.20)^{ghi}	R
	SEd	0.142	1.423	0.248	
	CD (0.05)	0.286	2.862	0.50	

*Mean of three replication. Figures in the parentheses are square root transformed values. In a column, the mean followed by the same letter is not significantly different by DMRT at a 5 % level.

Genotypic variations in selected rice germplasm

Upon investigating the banding patterns of the selected germplasm (25 in total) using SSR markers (17 in total), it was observed that the bands in IR 36 are different from those in PTB-33 for the RM28493 (linked to *Bph 2* marker reported in IR 36). This marker also exhibited differential expressed in T12, Rathu Heenati, *O. officinalis*, and *Mysore Malli*, similar to PTB 33. The ID 161-2 marker for the *Bph2* gene showed differential banding, being absent in several genotypes, including ASD 7, PTB 33,

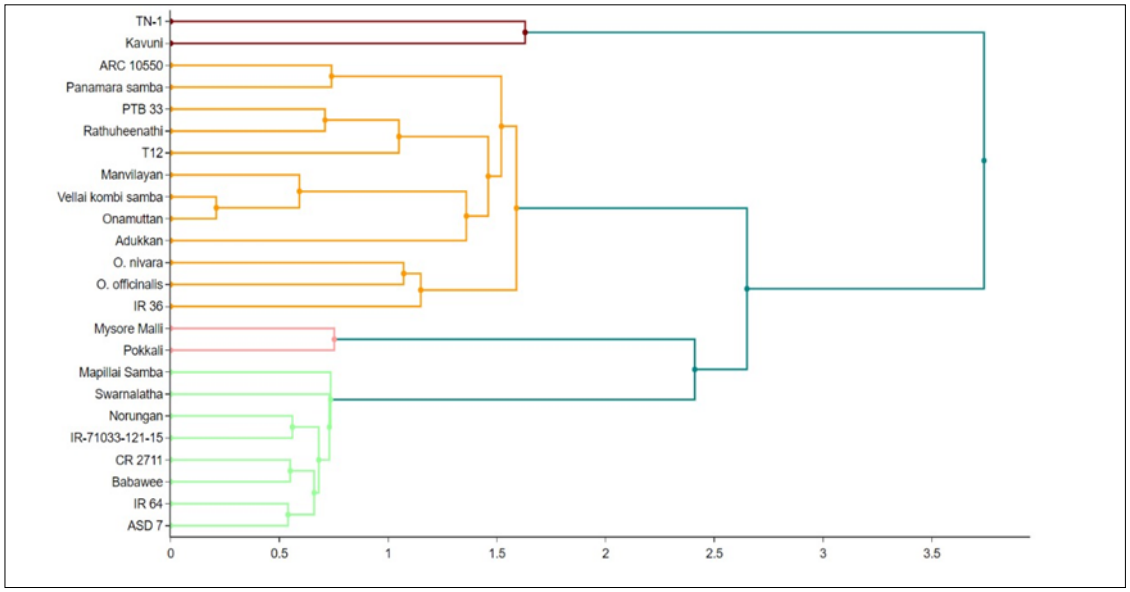

Fig. 1. Phenotypic clustering of rice germplasm.

Table 6. Genotypic data of rice germplasms using selective markers for specific BPH genes

Sl. no	Germplasm	RM 28493	ID 1612	RM 28449	RM589	RM 190	RM 225	RM 217	RM 119	RM 500	RM 6156	RM 1305	MS 10	RM 19341	RM 19291	RM488	RM 11522	RM212	Number of R genes/ QTLs present
		BPH2, BPH21	*Bph-2*	*bph2*	*Bph-3*	*Bph-4*	*Bph-4*	*Bph-4*	*BPH-6*	*Qbph-7*	*BPH17-ptb*	*BPH17-ptb*	*Bph20(t)*	*BPH-32*	*BPH-32*	*Bph33(t)*	*Bph33(t)*	*Bph33(t)*	
1.	ASD 7	+	+	+	+	+	+	+	-	+	+	^+	+	+	^^+	^+	~+	+	6
2.	IR 36	+	-	^+	~+	-	+	+	+	+	-	^+	+	-	+	^^+	~+	^+	7
3.	PTB 33	^^+	+	^+	^+	~+	+	+	+	+	-	+	^+	+	-	^^+	+	+	17
4.	*Adukkan*	^+	+	+	^+	~+	~+	+	+	+	-	^+	+	+	^+	+	+	^+	9
5.	*Onamuttan*	^+	-	^+	^+	~+	~+	+	+	+	-	+	+	+	^+	_	+	+	11
6.	*Manvilayan*	+	+	+	^+	~+	~+	+	-	+	-	^+	+	+	^+	+	+	^+	8
7.	*Panamara Samba*	+	+	^+	-	-	-	-	+	-	-	^+	+	-	^^+	-	-	-	4
8.	TN 1	-	+	+	-	-	+	-	+	-	-	^+	+	-	+	^+	+	+	6
9.	T12	^^+	+	+	+	~+	+	+	+	+	+	^+	+	+	+	^+	~+	+	9
10	Swarnalata	+	-	^+	~+	~+	+	+	+	+	-	^+	+	+	+	+	+	+	10
11.	Rathu Heenati	^^+	+	+	+	~+	+	+	+	+	+	^+	+	+	-	^+	+	+	11
12.	Babawee	^+	+	^+	+	+	+	+	+	+	-	^+	+	^+	+	+	+	+	9
13.	IR 64	+	-	^+	~+	~+	+	+	+	+	+	^+	+	+	+	^+	~+	+	8
14.	CR 2711	^+	+	+	+	~+	~+	+	+	+	+	^+	^+	+	^^+	~+	~+	+	8
15.	ARC 10550	+	-	^+	+	~+	+	+	+	+	-	^+	+	+	+	+	^+	+	9
16.	Pokkali	^+	-	+	^^++	+	~+	+	-	+	~+	^+	-	^+	-	+	~+	~+	3
17.	IR 71	+	-	^+	+	-	+	+	+	+	-	^+	+	+	+	+	~+	+	8
18.	*O. officinalis*	^^+	+	^+	^^+	~+	+	+	+	+	+	+	-	+	-	+	^+	~+	11
19.	*O. nivara*	+	~+	^+	+	+	+	^+	+	+	+	^+	+	^+	+	+	^+	~+	4
20.	*Mapillai Samba*	+	~+	^+	^+	~+	~+	+	+	-	+	^+	+	+	+	^+	+	+	8
21.	*Kavuni*	+	~+	^+	+	+	+	^+	+	+	-	^+	+	^+	+	^+	+	+	7
22.	*Vellai Kombi Samba*	+	+	-	^+	~+	+	+	-	+	-	+	^+	+	+	+	+	+	12
23.	*Norungan*	+	+	+	^+	~+	~+	+	+	+	+	^+	+	+	+	+	-	+	8
24.	*Mysore Malli*	^^+	+	+	^+	-	~+	+	+	+	-	^+	+	+	^+	^+	+	+	10

+ sign indicates the presence of a band, just as the product size of the primer

- sign indicates the absence of the band

~+ sign indicates the presence of a band slightly below the product size of the primer

^+ sign indicates a band a little more above the product size of the primer

^^+ sign indicates a band more above the product size of the primer

Adukkan, Manvilayan, Panamara Samba, TN 1, T12, Rathu Heenati, Babawee, CR 2711, *O. officinalis*, *Vellai Kombi Samba, Norungan* and *Mysore Malli.*

RM 28449 is another marker near RM 28493 for *Bph 2* gene present in ASD 7, showed differential banding in IR 36, *Onamuttan*, *Panamara Samba*, Swarnalata, Babawee, IR 64, ARC 13055, IR-71033-121-15, *O. officinalis*, *O. nivara, Mapillai Samba* and *Kavuni* together with PTB 33. Hence, for *Bph2* gene, out of the 3 markers, RM 28493 is considered a valid marker that differentiated the resistant check PTB 33 from other susceptible germplasms genotypically. This shows that the gene represented by RM 28449 would have contributed to the BPH resistance and other genetic regions responsible for resistance and could not be considered a valid marker for *Bph2* gene. This indicates that these genotypes possess different regions for the *Bph 2* gene marker ID 161-2, suggesting that ID 161-2 may not be

a valid marker for delineating the *Bph 2* gene responsible for resistance, as TN 1 could not be differentiated from PTB 33 using this marker.

MS 10, utilized as a marker for the *Bph17* gene in PTB33, exhibited differential expression in PTB33, CR 2711 and *Vellai Kombi Samba*, there by establishing its validity as a marker for *Bph17*. This also indicated that the rice germplasms CR 2711 and the landrace *Vellai Kombi Samba* have genes (*Bph17*) similar to PTB 33. RM 1305 marker for *BPH 17* gene, was differentially expressed in PTB 33, *Onamuttan, O. officinalis* and *Vellai Kombi Samba* further confirmed the presence of *Bph17* gene in the landrace *Vellai Kombi Samba* and considered to be valid marker for *Bph17* gene. The marker RM 6156, associated with the Bph17 gene, was present in moderately resistant germplasms such as ASD 7, T 12, Rathu Heenati, IR 64 and CR 2711 but was absent in others, including TN 1 and PTB 33. This suggests that the differential area of the *Bph17* gene may contribute to the induction of moderate resistance.

RM 119, a marker used to select *Bph 6* gene (Swarnalata), was expressed in all the tested germplasm, including the highly susceptible TN 1, *Kavuni*, *Mapillai Samba* and *Norungan* indicating the unsuitability of the marker in selecting *Bph 6* gene. Three markers Viz., RM 190, RM 225 and RM 217 were used to select *Bph4* gene present in Babawee. Among these, RM 190 exhibited differential expression in resistant and moderately resistant germplasms, but differential banding was also observed in the susceptible land races *Norungan* and *Mapillai Samba*. Thus, this region may complement the resistant reactions conferred by other major genes responsible for resistance. RM 225 showed banding even in the susceptible TN1 along with PTB 33. Still, it was differentially expressed in landraces *Adukkan, Onamuttan, Manvilayan, Mapillai Samba*, *Norungan*, *Mysore Malli*, CR 2711 and Pokkali. This indicates that this recessive resistant gene is present even in TN 1, which was otherwise considered not to possess any resistant genes. RM 217 selected the region invariably in all germplasm except in *Panamara Samba*, TN 1, *O. nivara*, *Kavuni* making it invalid.RM 500, a marker used for selecting *Bph 7* gene present in T12 was expressed in all the germplasms except TN 1, *Panamara Samba* and *Mapillai Samba*.

The presence of *Bph 3* gene in Rathu Heenati was better confirmed using the marker RM 589 than RM 508, the former of which got differentially expressed as 4 Polymorphic banding patterns in different germplasm. A similar banding pattern was observed in ASD 7, T 12, Rathu Heenati, Babawee, CR 2711, ARC10550, IR-71033-121-15, *O. nivara* and *Kavuni*. PTB 33, *Adukkan, Onamuttan*, *Manvilayan*, *Mapillai Samba*, *Vellai Kombi Samba*, *Norungan* and *Mysore Malli* showed a similar differential band. IR 36, Swarnalata, IR 64 formed another differential group. Pokkali and *o. officinalis* formed yet another group. The RM 589 marker for the *Bph 3* gene exhibited significant polymorphism and requires more investigation into its phenotypic responses in diverse genetic backgrounds.

Two markers RM19291 and RM19341 were used for selecting *Bph 32* gene present in PTB 33 of which RM 19291 showed 3 differential polymorphic bands among the germplasm along with the absence of band in PTB 33, Rathu Heenati, Pokkali and *O. officinalis*. IR 36, TN 1, T 12, Swarnalata, Babawee, IR 64, ARC 10550, IR-71033-121-15, *O. nivara*, *Mapillai Samba*, *Kavuni*, Velli Kombi Samba, and *Norungan* formed one group with similar banding. *Adukkan, Onamuttan, Manvilayan* and *Mysore Malli* formed another group. ASD 7, *Panamara Samba,* and CR 2711 formed yet another group. The second marker RM 19341 grouped ASD 7, PTB 33, *Adukkan, Onamuttan, Manvilayan*, T12, Swarnalata, Rathu Heenati, IR 64, CR 2711, ARC 10550, IR-71033-121-15, *O. officinalis*, *Mapillai Samba*, *Vellai Kombi Samba*, *Norungan* and *Mysore Malli* as single group and Babawee, Pokkali, *O. nivara* and *Kavuni* as another group. These two markers suggest that RM 19291 can be utilized to identify the *Bph 32* gene. Rathu Heenati, Pokkali and *O. officinalis* exhibit differential genetic make-up similar to PTB 33, while the landraces *Adukkan, Onamuttan, Manvilayan* and *Mysore Malli* demonstrate varied expression of the gene associated with their resistance to BPH (Table 6).

Three markers were used to select *Bph 33* gene present in RP2068 of which RM 11522 and RM 212 show banding even in the susceptible TN 1 as in the resistant RP 2068. However the marker RM 488 showed differential banding in resistant and susceptible germplasm. IR 36 and PTB 33 showed similar banding. ASD 7, TN 1, T 12, Rathu Heenati, IR 64, *Mapillai Samba*, *Kavuni,* and *Mysore Malli* showed similar banding. *Adukkan, Manvilayan,* Swarnalata, Babawee, ARC 10550, Pokkali, IR-71033-121-15, *O. officinalis, O. nivara*, *Vellai Kombi Samba*, *Norungan* and RP 2068 showed similar banding. No bands were present in *Onamuttan* and *Panamara Samba*. So it is observed that *Bph 33* gene is present in landraces *Adukkan, Manvilayan, Vellai Kombi Samba*, *Norungan* and germplasms like Swarnalata, Babawee, ARC 10550, Pokkali, IR-71033-121-15 and wild rice *O. officinalis* and *O. nivara* and hence contributes for this BPH resistance in combination with other resistant genes.

Genotypic cluster analysis

The Jaccard distance-based molecular cluster analysis was performed using this scoring while base pair scoring was applied for structure analysis (Table 6). The marker scorings were analyzed with the R-shiny-based package 'PBPERFECT' (Allan 2023) for the molecular cluster.

The germplasm was grouped into 4 clusters based on the Jaccard distance analysis. *Adukkan, Manvilayan, Onamuttan, Mysore Malli,* and *O. officinalis* were closely related to PTB 33. In contrast, Babawee TN 1, *Vellai Kombi Samba* and Pokkali were at a distance but grouped in the same cluster. *O. minuta* and *Panamara Samba* formed the second cluster.CR 2711, ASD 7, T 12 and Rathu Heenati were grouped in the third cluster and *O. nivara*, *Kavuni*, *Mapillai Samba* and *Norungan* were inclose proximity. IR 36, IR 64, Swarnalata, ARC 10550 and IR-71033-121-15 were at close distance but all of them remained in cluster four.

This cluster analysis indicates that the resistant landraces *Onamuttan*, *Vellai Kombi Samba*, *Adukkan,* and the moderately resistant landraces *Manvilayan* and *Mysore Malli* share a common genetic background with PTB 33. It was interesting to note that TN 1, a susceptible check, also showed some resistant genetic backgrounds and was grouped in the same cluster. The wildrace *O. officinalis* and the moderately resistant variety Pokkali were reported to possess the *bph9* gene and Babawee contained the *bph4* gene and was grouped with PTB 33.

O. minuta and *Panamara Samba* showed the least number of gene expressions while screening with these 24

markers and hence were grouped in a separate cluster CR 2711 (*BPH 31*), ASD 7 (*Bph 2*), T12 (*Bph 6*) and Rathu Heenati (*bph3*) were grouped in a separate cluster indicating their resistance mechanism is different from that of PTB 33. The susceptible landraces *Kavuni, Mapillai Samba* and *Norungan* were categorized alongside *O. nivara*, suggesting they may share some genetic traits with *O. nivara*; nonetheless, these traits are inadequate for complete resistance to BPH.

Lastly, moderately resistant varieties IR 36, IR 64, ARC 10550, Swarnalata and moderately susceptible IR-71033-121-15 were grouped with *O. nivara* and hence expressed to possess some of the major genetic background as *O. nivara* in resisting against BPH (Fig.2).

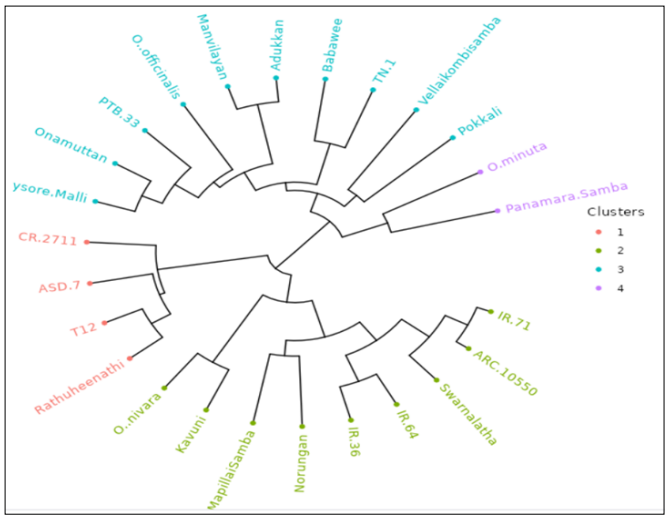

Fig.2. Genotypic clustering of germplasm based on Jaccard distance.

Discussion

The virulence of Biotype 4 in the South Asian population (Coimbatore, India) has been observed to be altered, as the resistance response, determined by the damage score, varies for the same germplasm over approximately 35 years. In previous research, Rathu Heenati, Babawee, ARC 10550 and Swarnalata were resistant to the same population in Coimbatore, India (31). However, the current study classifies all of these varieties as moderately resistant. A previous study indicated that IR-64 was resistant and IR-36 was moderately resistant to BPH. Still, in the current study, IR64 was found to be moderately sensitive, and IR36 was found to be moderately resistant (32). Furthermore, ASD 7 was previously reported as resistant, with T 12, Rathu Heenati, Swarnalata and Pokkali classified as moderately resistant (33). At the same time, in this study, ASD 7 was found to be moderately resistant, along with all the other mentioned varieties. Another study recorded the proportion of nymphs settled on RP2068-18-3-5 and Rathu Heenati are lower in relation to the susceptible control TN 1(34). At 72 hours after infestation (HAI), the lowest number of adults settled on PHSS 11 (0.23/ plant) and the highest on TN1 (6.15/plant) (35). In resistant genotypes, the insect population was reduced over time, whereas it increased in susceptible check TN1. The germplasm, which was found to be moderately resistant in the current study *viz*., Babawee, ARC 10550 and Pokkali was reported as susceptible to the Pantnagar, Punjab population, which is also considered as the South Asian Biotype (36).

A study indicated that the honeydew excreted by T12, PTB 33 and IR 36 was below 100 mm^2, although TN 1 exhibited a honeydew excretion area of 706.75 mm^2 (36). In the present investigation, analogous results were noted for T12 and PTB 33, while IR 36 demonstrated a moderate excretion area of 100 mm^2. ARC 10550 showed 286.5 mm^2 area (36), while in the current study, it was 63.33 mm^2. Another study also identified T12 as showing a high level of antibiosis, while Swarnalata, Babawee, Pokkali and IR 64 showed a moderate antibiosis level measured in terms of honeydew excreted (37).

T12, Rathu Heenati, *O. officinalis*, and *Mysore Malli* exhibited homozygous banding similar to PTB 33 at the RM 28493 marker. Among the three markers, ID-161-2, RM 28449, and RM 28493 were used for *Bph 2* gene and all these germplasm showed resistance or moderate resistance to the South Asian population of BPH at Coimbatore, India. The position of RM 28493 would be approximately 23.24 Mbp, as indicated in the substitution map of *Bph 2* on chromosome 12 (38). The same study reported that *bph2* was delimited in PTB 33 as approximately 247.5 kbp between RM 28449 and ID-161-2 on chromosome 12 at a physical location of roughly 22.69 to 22.94 Mbp. The location of *Bph2* partly overlaps that of *Bph1* (22.8-22.93 Mbp), *Bph9*(22.85-22.91 Mbp), *Bph10* (19.66-23.42 Mbp), and *Bph18* (22.87-22.90 Mbp). The same author reported earlier (39) that the location of *Bph21* was around 22.9 Mbp on chromosome 12 of IR71033-121-15 and was selected using RM1246 (19.2 Mbp) and RM28493 (23.3 Mbp). The delimited location of *Bph2* completely covers that of *BPH26* (22.77–22.91 Mbp) on chromosome 12, and RM 28493 was one of the markers used (40). Furthermore, it was reported that the amino acid sequences and resistance levels of *BPH2* from 'ASD 7' are identical to those of *Bph26* from 'ADR 52'.

Polymorphism for *Bph3* was observed as 4 bands at RM 589 and homozygous banding was found in ASD 7, T12, Babawee, CR 2711, ARC 10550, IR-71033-121-15, *O. nivara* and *Kavuni* as that observed in Rathu Heenati. *Bph 3* was initially documented in a Srilankan indica rice cultivar Rathu Heenati and a South Indian Indica cultivar PTB 33, which were resistant to four biotypes of BPH, including the South Asian biotype. Mapping studies placed the locus of *Bph3* between the two flanking SSR markers RM 589 and RM 588 on the short arm of chromosome 6, which was later remapped to chromosome 4S (41). The landraces *Adukkan, Onamuttan, Manvilayan*, *Mapillai Samba*, *Vellai Kombi Samba*, *Norungan* and *Mysore Malli* showed the second homozygous band similar to that seen in PTB 33. Still, none of the 4 polymorphic bands appeared in the susceptible TN1. Homozygosity of some of the susceptible germplasm viz., *Kavuni* with Rathu Heenati and *Mapillai Samba* and *Norungan* with PTB 33, for this *Bph 3* marker, indicates that even these susceptible landraces possess BPH resistant genes but which could not be fully expressed as BPH resistance is a polygenic trait. So, this polymorphic marker RM 589 for *Bph 3* gene could be explored further to understand the broad spectrum resistance offered by this gene.

Bph 6 is found in all germplasm, including susceptible landraces with marker RM 119. It was reported that the rice cultivar Swarnalata carried resistant alleles at RM 119 and RM 17004, designated as *Bph 6*. Later, a study used *Bph 6* linked SSR markers RM 16994 and RM 119 and found that these markers did not show polymorphism between their parents TN1 and Sinna Sivappu, which substantiates our results (42). RM 500 had caused

similar banding in all the tested germplasm except the susceptible TN 1, *Panamara Samba* and *Mapillai Samba* as a QTL flanked by RM 542 and RM 500 and located on chromosome 7 (43).

CR 2711 and *Vellai Kombi Samba* showed expressions similar to those in PTB 33when subjected to genotyping by marker MS10. (44-46) identified *BPH17(ptb)* position on chromosome 4 with markers, MS10-RM 5953(8.5-9.38 Mb). *Onamuttan, O. officinalis* and *Vellai Kombi Samba* showed a band similar to PTB 33 with an RM 1305 marker. RM 6156 also showed differential banding but selected susceptible entries like *Mapillai Samba* and *Norungan* with resistant PTB 33. Another study mapped *Bph 17 (ptb)*between two markers, RM 1305and RM 6156, at approximately 5.63 to 7.86 Mbp on chromosome 4S (38). The location of *Bph17-ptb* partially overlapped with those of *Bph12* (5.21-5.66 Mbp), *Bph15* (6.90-6.95 Mbp), *Bph17* (6.94-6.97 Mbp) and *Bph22*(t) (4.14-6.58 Mbp). The delimited region of *Bph17-ptb* is 2.23 Mbp and would, therefore, contain multiple BPH resistance genes and there is scope for further investigation in this region.

RM 19291, a marker used in genotyping *Bph32* gene, showed polymorphic banding that differentiated the resistant germplasm including PTB 33, Rathu Heenati, Pokkali and *O. officinalis*, with no banding. The other marker, RM 19341, also grouped germplasms differently. A study stated that *Bph32* gene for resistance to BPH was mapped in the interval between the markers RM 19291 and RM 8072 on the short arm of chromosome 6 (17, 47). The *Bph 32* gene has been located between RM 508 and RM 19341 on chromosome 6 (38). The *Bph 33(t)* gene(RP2068) is present in landraces *Adukkan, Manvilayan*, *Vellai Kombi Samba*, *Norungan* and germplasm like Swarnalata, Babawee, ARC 10550, Pokkali, IR-71033-121-15 and the wild rice *O. officinalis* and *O. nivara,* was identified when genotyped with the marker RM 488 but a differential banding was observed in others including PTB 33.Furthermore, the presence of *Bph 33(t)* in RP2068, conferring BPH resistance gene, was identified and delimited to chromosome 1 by markers RM 488 and RM 11522 (48).

The comparison of all the genotypic expression reveals that the resistant check PTB 33 has *Bph 2 gene* (RM 28493), *Bph 3 gene* (RM 589), *Bph 17* gene (MS 10 and RM 1305) and *Bph 32* (RM 19341) and presence of all these genes is responsible for the stable resistance exhibited by this variety against BPH over several decades. The landraces *Vellai Kombi Samba* and *Onamuttan* possess *Bph 3, 17* and 32 genes represented by RM 589, MS 10 RM 1305 and RM 19341, respectively, contributing to their resistance to BPH. *Adukkan* and *Manvilayan* were found to have *Bph 3* and *Bph 32* genes as their genetic basis of resistance upon investigation using the selective markers.

Conclusion

The study investigated the phenotypic and genotypic characterization of 24 rice germplasms to identify resistance against the South Asian population of BPH, a major rice pest. Through screening for BPH resistance using both field and molecular methods, it was found that some germplasms, such as PTB-33, *Adukkan*, *O. officinalis*, *Vellai Kombi Samba* and *Onamuttan* exhibit strong resistance to BPH. Low damage scores supported this resistance and reduced feeding rates. Additionally, it was found that such germplasm possesses resistant genes similar to PTB 33 when screened using BPH-specific resistant gene markers. Hence, these germplasm, especially the landraces, whose characteristics are more easily introgressed into popular varieties than wild rice germplasm, are suggested for breeding programs to develop more BPH-resistant rice strains. Future work could focus on fine-mapping resistance genes and using advanced techniques like CRISPR-Cas9 to accelerate breeding programs.

Additionally, exploring the molecular mechanisms of BPH resistance could reveal new defense genes or pathways. Monitoring the evolution of BPH biotypes and expanding research to include more diverse germplasms could aid in developing durable, widely applicable resistance strategies. Differential virulence of South Asian biotypes on resistant germplasm has also been documented, which helps select specific resistant genes for this biotype to breed for varietal evolution and gene pyramiding programmes. This research also highlights the importance of using phenotypic and molecular methods to discover new genetic sources for BPH resistance, ensuring effective pest management and sustainable rice production in the face of evolving pest biotypes.

Acknowledgements

The authors acknowledge the Department of Agricultural Entomology for providing fellowship for the conduct of research, the Department of Rice for providing rice germplasm and laboratory to conduct screening and phenotypic studies, the Department of Plant Biotechnology Tamil Nadu Agricultural University for facilitating genetic analysis of rice germplasm

Authors' contributions

SV conceptualized the theme, method of study, and interpreted the results.BT carried out experiments, analyzed the results and wrote the manuscript; RS, RPS and SM critically reviewed the manuscript and gave inputs for improvisation. SV helped in analyzing genotypic data using the software. The rest of the authors contributed by facilitating the conduct of the experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of Interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Yadav S, Sharma A. Shortcomings in rice production: with special emphasis on salinity stress. Acta Sci Microbiol. 2024;7(3):85-96. https://doi.org/10.31080/ASMI.2024.07.1361
2. Dyck VA, Thomas B. The brown planthopper problem. Brown Planthopper: Threat to Rice Production in Asia. 1979;3:3-17.
3. Raghavender B. Survey and mapping of brown planthopper, *Nilaparvata lugens* (Stål) incidence in Southern Telangana zone and phenotypic and genotypic evaluation of elite rice genotypes [Doctoral thesis]. Hyderabad: Department of Entomology, College

of Agriculture, Professor Jayashankar Telangana State Agricultural University; 2022.

4. Gajanan SM. Host plant resistance to brown plant hopper, *Nilaparvata lugens* (Stål), on selected rice genotypes with special reference to antibiosis traits [M.Sc. thesis]. Raipur (C.G.): Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya; 2010.
5. Alam S, Cohen MB. Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens,* in a doubled-haploid rice population. Theor Appl Genet. 1998;97:1370-79. https://doi.org/10.1007/s001220051031
6. Strauss SY, Agrawal AA. The ecology and evolution of plant tolerance to herbivory. Trends Ecol Evol. 1999;14(5):179-85. https://doi.org/10.1016/S0169-5347(98)01576-6
7. Anant AK, Guru-Pirasanna-Pandi G, Jena M, Chandrakar G, Chidambaranathan P, Raghu S, et al. Genetic dissection and identification of candidate genes for brown planthopper, *Nilaparvata lugens* (Delphacidae: Hemiptera) resistance in farmers' varieties of rice in Odisha. Crop Protection. 2021;144:105600. https://doi.org/10.1016/j.cropro.2021.105600
8. Sogawa K. Quantitative morphological variations among biotypes of the brown planthopper. International Rice Research New. 1978;3 (6):9-10.
9. Panda N, Heinrichs EA. Levels of tolerance and antibiosis in rice varieties having moderate resistance to the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). Environmental Entomology. 1983;12(4):1204-14. https://doi.org/10.1093/ee/12.4.1204
10. Jing S, Zhang L, Ma Y, Liu B, Zhao Y, Yu H, et al. Genome-wide mapping of virulence in brown planthopper identifies loci that break down host plant resistance. PLoS One. 2014;9(6):e98911. https://doi.org/10.1371/journal.pone.0098911
11. Muduli L, Pradhan SK, Mishra A, Bastia DN, Samal KC, Agrawal PK, et al. Understanding brown planthopper resistance in rice: Genetics, biochemical and molecular breeding approaches. Rice Sci. 2021;28 (6):532-46. https://doi.org/10.1016/j.rsci.2021.05.013
12. Athwal DS, Pathak MD, Bacalangco EH, Pura CD. Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L. Crop Science. 1971;11(5):747-50. https://doi.org/10.2135/cropsci1971.0011183X001100050043x
13. Hirabayashi H, Ogawa T. RFLP mapping of *Bph-1* (brown planthopper resistance gene) in rice. JpnJBreed. 1995;45(3):369-71. https://doi.org/10.1270/jsbbs1951.45.369
14. Murata K, Fujiwara M, Kaneda C, Takumi S, Mori N, Nakamura C. RFLP mapping of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph2* of indica rice introgressed into a japonica breeding lineNorin-PL4'. Genes Genet Syst. 1998;73(6):359-64. https://doi.org/10.1266/ggs.73.359
15. Murai H, Hashimoto Z, Sharma PN, Shimizu T, Murata K, Takumi S, et al. Construction of a high-resolution linkage map of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph2*. Theor Appl Genet. 2001;103:526-32. https://doi.org/10.1007/s001220100598
16. Laksminarayana A, Khush GS. New Genes for resistance to the brown planthopper in rice Crop Sci. 1977;17(1):96-100. https://doi.org/10.2135/cropsci1977.0011183X001700010028x
17. Jairin J, Phengrat K, Teangdeerith S, Vanavichit A, Toojinda T. Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3,* on rice chromosome 6. Mol Breeding. 2007;19:35-44. https://doi.org/10.1007/s11032-006-9040-3
18. Kawaguchi M, Murata K, Ishii T, Takumi S, Mori N, Nakamura C. Assignment of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph4* to the rice chromosome 6. Breeding Science. 2001;51(1):13-18. https://doi.org/10.1270/jsbbs.51.13
19. Kabis MA, Khush GS. Genetic analysis of resistance to brown planthopper in rice (*Oryza sativa* L.). Plant Breeding. 1988;100(1):54-58. https://doi.org/10.1111/j.1439-0523.1988.tb00216.x
20. Qiu Y, Guo J, Jing S, Zhu L, He G. High-resolution mapping of the brown planthopper resistance gene *Bph6* in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. Theor Appl Genet. 2010;121:1601-11. https://doi.org/10.1007/s00122-010-1413-7
21. Nemoto H, Ikeda R, Kaneda C. New genes for resistance to brown planthopper, *Nilaparvata lugens* Stal, in rice. Jpn J Beed. 1989;39 (1):23-28. https://doi.org/10.1270/jsbbs1951.39.23
22. Murata K, Fujiwara M, Murai H, Takumi S, Mori N, Nakamura C. Mapping of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene *Bph9* on the long arm of rice chromosome 12. Cereal Research Communication. 2001;29:245-50.https://doi.org/10.1007/BF03543667
23. Sun L, Su C, Wang C, Zhai H, Wan J. Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. Breeding Science. 2005;55(4):391-96. https://doi.org/10.1270/jsbbs.55.391
24. Rahman ML, Jiang W, Chu SH, Qiao Y, Ham T-H, Woo M-O, et al. High-resolution mapping of two rice brown planthopper resistance genes, *Bph20 (t)* and *Bph21* (*t),* originating from *Oryza minuta*. Theor Appl Genet. 2009;119:1237-46.https://doi.org/10.1007/s00122-009-1125-z
25. Horgan FG, Srinivasan TS, Naik BS, Ramal AF, Bernal CC, Almazan MLP. Effects of nitrogen on egg-laying inhibition and ovicidal response in planthopper-resistant rice varieties. Crop Protection. 2016;89:223-30. https://doi.org/10.1016/j.cropro.2016.07.033
26. Mahantashivayogayya K, Mahesh, Huruli S, Diwan JR, Lokesh GY, et al. Phenotyping and genotyping of rice genotypes for brown planthopper resistance. Int J Adv Biochem Res. 2024;8(3):132-41. https://doi.org/10.33545/26174693.2024.v8.i3b.689
27. SõGAWA K, Pathak M. Mechanisms of brown planthopper resistance in Mudgo variety of rice (Hemiptera: Delphacidae). Applied Entomology and Zoology. 1970;5(3):145-58. https://doi.org/10.1303/aez.5.145
28. Soundararajan RP, Gunathilagaraj K,Chitra N. Feeding rate of the brown planthopper, *Niiaparvata lugens* (Stal) (Hemiptera: Oelphacidae) on rice double haploid lines. Pest Management and Economic Zoology. 2001;9(2):165-70.
29. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980;8(19):4321-26. https://doi.org/10.1093/nar/8.19.4321
30. Sabina A, Shafiq WA, Ashraf BM, Shabir W, Nawsheeba W. Genetic diversity in cultivars and landraces of rice as revealed by SSR markers. Res J Biotech. 2015;10(1):17-25.
31. Velusamy R. Resistance of wild rices, *Oryza* spp., to the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). Crop Protection. 1988;7(6):403-08. https://doi.org/10.1016/0261-2194(88)90010-5
32. Pophaly DJ, Rana DK. Reaction of IR varieties to the brown planthopper (BPH) population in Raipur, Madhya Pradesh, India. Int Rice Research Notes. 1993;18(1):27-28.
33. Thamarai M, Soundararajan RP. Reaction of rice genotypes against specific population of brown planthopper, *Nilaparvata lugens* (stål). Ann Plant Prot Sci. 2017;25(1):74-77.
34. Sarao PS, Bentur JS. Antixenosis and tolerance of rice genotypes against brown planthopper. Rice Science. 2016;23(2):96-103. https://doi.org/10.1016/j.rsci.2016.02.004
35. Sable A, Suresh S, Mohan KS. Antixenosis mechanism of resistance to brown planthopper *Nilaparvata lugens* (Stal) in selected rice genotypes. Trends in Biosciences. 2014;7(13):1594-98.
36. Singh S, Tiwari S. Resistance in rice genotypes against brown planthopper, *Nilaparvata lugens*. Pantnagar J Res. 2021;19(1):46-49.
37. Bhanu KV, Reddy PS, Satyanarayana J. Studies on tolerance of rice cultures against brown planthopper, *Nilaparvata lugens*. Indian J Plant Prot. 2014;42(3):199-203.

38. .Nguyen CD, Zheng S-H, Sanada-Morimura S, Matsumura M, Yasui H, Fujita D. Substitution mapping and characterization of brown planthopper resistance genes from indica rice variety, 'PTB33' (*Oryza sativa* L.). Breeding Science. 2021;71(5):497-509. https://doi.org/10.1270/jsbbs.21034

39. Nguyen CD, Verdeprado H, Zita D, Sanada-Morimura S, Matsumura M, Virk PS, et al. The development and characterization of near-isogenic and pyramided lines carrying resistance genes to brown planthopper with the genetic background of japonica rice (*Oryza sativa* L.). Plants. 2019;8(11):498. https://doi.org/10.3390/plants8110498

40. Kamal MM, Nguyen CD, Sanada-Morimura S, Zheng S-H, Fujita D. Near-isogenic lines for resistance to brown planthopper with the genetic background of Indica Group elite rice (*Oryza sativa* L.) variety 'IR64'. Breeding Science. 2023;73(3):278-89. https://doi.org/10.1270/jsbbs.22093

41. Liu S, Liang QM, Zhou WW, Jiang YD, Zhu QZ, Yu H, et al. RNA interference of NADPH-cytochrome P450 reductase of the rice brown planthopper, *Nilaparvata lugens*, increases susceptibility to insecticides. Pest Manag Sci. 2015;71(1):32-39. https://doi.org/10.1002/ps.3760

42. Ramesh K, Padmavathi G, Deen R, Pandey MK, Lakshmi VJ, Bentur J. Whitebacked planthopper *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) resistance in rice variety Sinna Sivappu. Euphytica. 2014;200:139-48. https://doi.org/10.1007/s10681-014-1175-4

43. Liu Y, Su C, Jiang L, He J, Wu H, Peng C, et al. The distribution and identification of brown planthopper resistance genes in rice. Hereditas. 2009;146(2):67-73. https://doi.org/10.1111/j.1601-5223.2009.02088.x

44. Yang H, You A, Yang Z, Zhang F, He R, Zhu L, et al. High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). Theor Appl Genet. 2004;110:182-91. https://doi.org/10.1007/s00122-004-1844-0

45. Hirabayashi H, Kaji R, Okamoto M, Ogawa T, Brar DS, Angeles ER, et al. Mapping QTLs for brown planthopper (BPH) resistance introgressed from Oryza officinalis in rice. In: Khush GS, Brar DS, Hardy B, editors. Advances in Rice Genetics. Part 2. Singapore: World Scientific; 2003.p.268-70. https://doi.org/10.1142/9789812814319_0102

46. Huang D, Qiu Y, Zhang Y, Huang F, Meng J, Wei S, et al. Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). Theor Appl Genet . 2013;126:219-29. https://doi.org/10.1007/s00122-012-1975-7

47. Ren J, Gao F, Wu X, Lu X, Zeng L, Lv J, et al. *Bph32*, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. Sci Rep. 2016;6:37645. https://doi.org/10.1038/srep37645

48. Naik SB, Divya D, Sahu N, Sundaram RM, Sarao PS, Singh K, et al. A new gene *Bph33(t)* conferring resistance to brown planthopper (BPH), *Nilaparvata lugens* (Stål) in rice line RP2068-18-3-5. Euphytica. 2018;214:53. https://doi.org/10.1007/s10681-018-2131-5

49. McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, et al. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 2002;9(6):199-207. https://doi.org/10.1093/dnares/9.6.199

50. Tamura Y, Hattori M, Yoshioka H, Yoshioka M, Takahashi A, Wu J, et al. Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. indica cultivar ADR52. Sci Rep. 2014;4:5872. https://doi.org/10.1038/srep05872

51. Meshram P, Bhandarkar S, Banerjee S, Nair SK, Rana DK, Sarawgi AK. Screening and validation of known DNA markers for BPH resistance in germplasm of rice (*Oryza sativa* L.). Int J Chem Stud. 2018;6(6):1540-42.

52. Vang PTK, Lang NT, Chau LM, He TN. Determination of the presence of brown planthopper resistance genes (*Nilaparvata lugens* Stål.) in rice (*Oryza sativa* L.). Int J Environ Agric Biotechnol. 2020;5(3):796-804. https://doi.org/10.22161/ijeab.53.32

53. Melaku G, Zhang S, Haileselassie T. Comparative evaluation of rice SSR markers on different *Oryza* species. J Rice Res Dev. 2018;1(1):38-48. https://doi.org/10.36959/973/418