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Phenotypic and genotypic screening of brown planthopper resistance in rice

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Abstract

Molecular characterization of selected 50 rice genotypes, including landraces and germplasm has been performed to identify the resistance sources against brown planthopper (BPH) using eight SSR markers linked to five BPH resistance genes. The results revealed that, the presence of all five BPH resistance genes *viz*., *Bph3, Bph32, Bph17, Bph6* and *Bph3* in the genotype, KMR-3. Likewise, the landraces *viz*., Kelivikua, Ram vaidya, Dubri budda, Dahi gurmatia, Chak hou poidon and Tilwo contained three BPH resistant genes (*Bph17, Bph3* and *Bph6*). Further, Mahasuri, Vargol, Pateni and IET-31591 also exhibited three BPH resistant genes (*Bph32, Bph17* and *Bph6*). Contrarily, Jeerige sanna, Navara, Ratnachoodi-1, Rajamudi, TN-1 (SC) and Jyothi exhibited only one gene (*Bph17*). A total of ten accessions registered four to five BPH resistant genes and such accessions could be efficiently utilized as source of planthopper tolerant genes in formulating upcoming rice breeding programmes.

Keywords: Brown planthopper, rice germplasm, phenotypic screening, SSR markers, molecular characterisation

INTRODUCTION

Rice (*Oryza sativa*) serves as the primary source of nourishment for over 68 per cent of the world population and about 85% of the Indian population. Asia produces and consumes more than 92 per cent of the world's rice (FAO, 2019). Due to its importance, the United Nations (UN) declared the year, 2004 as 'International year of rice'. Unintended rice farming in recent decades exposes rice yield potential to biotic and abiotic factors, which result in significant reductions in both quantity as well as quality of the produce. Among various biotic constraints, brown planthopper, *Nilaparvata lugens* (Stal.) (BPH) is a major threatening pest which belongs to the family Delphacidae, suborder Homoptera, series Auchenorrhyncha and superfamily Fulgoidea. It is a potential phloem sap feeder and most serious and destructive pest of rice by causing severe yield loss in Asia as well as in rice growing areas of other subcontinents (Heong and Hardy, 2009).

Both nymph and adult stages of the pest desap from the leaves and leaf sheaths. Under sever attack of the pest results in wilting and fatality of the plant characterized as circular, dried and burnt up concentric patches known as 'hopper burn'. The development of tolerant varieties against BPH relying on host plant resistance (HPR) gained the momentum globally to improve the potential yield of rice through integrated pest management (IPM) practices. Further, the propensity of BPH to quickly become virulent on novel plant genotypes and to generate new biotypes for the breakdown of resistant varieties significantly increased the difficulties of rice breeding for its resistance. This has provoked to seek additional sources of resistance in traditional rice landraces and germplasm which are superior over cultivated rice varieties. Hence, well precised phenotyping and genotyping is indispensable to generate information on BPH resistance traits in both rice germplasm and landraces.

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Unlike morphological traits, molecular markers can illustrate variations among accessions at DNA level by providing a more reliable and efficient method of characterization, conservation and management of germplasm. Since, these markers are unaffected by external variables, ensuring steady and accurate characterization, they can be been widely utilized for the identification and introgression of BPH resistance genes into susceptible cultivars of rice (Karkousis *et al*., 2003). In order to develop the tolerant rice cultivars against prevailing highly virulent and destructive BPH biotype 4 which is synonymously known as 'South Asian biotype', it is important to invigorate the resistance breeding through validating highly resistant donors (Heinrichs, 1986). In this context, the present study was executed to evaluate 260 traditional rice landraces along with 705 rice germplasm lines phenotypically and to subject the selected accessions for molecular screening to identify major QTLs (Quantitative trait loci) of BPH tolerance using SSR markers. This intern helpful in developing location specific management strategies and BPH resistant rice cultivars.

MATERIALS AND METHODS

Rearing of BPH colony and raising of test genotypes

The colony of brown planthoppers was maintained on jaya, variety of rice seedlings in the aluminium cages specifically meant for rearing under greenhouse conditions by following the protocol developed by Heinrichs *et al*. (1985). Such cages of planthopper cultures were maintained throughout the studies in order to obtain sufficient planthoppers for conducting screening experiment. The test genotypes used in the phenotypic screening consisted of 260 traditional rice landraces supplied through DBT (Department of Biotechnology, Ministry of Science and Technology, Government of India) sponsored project. Besides, it also consisted of 705 rice genotypes obtained from IIRR (Indian Institute of Rice Research), Hyderabad, India under multiple resistance screening trial (MRST-2023), Planthopper special screening (PHSS-2023) and national screening nursery-2 (NSN 2-2023) through All India Co-ordinated Rice Improvement Project (AICRP). The seeds were sown in the cement compartments specifically meant for screening containing well puddled soil in rows of 3.5 cm apart.

Phenotypic screening for BPH resistance

The phenotypic screening of rice landraces and germplasm accessions was performed during August-2023 by following Standard Seedbox Screening Test (SSST) developed by Heinrichs *et al*. (1985) by replicating the each accession thrice to know their reaction against a biotic stress, brown planthopper in rice entomology glasshouse with 27^{o}C±2^{o}C, 75%±5% relative humidity and 13 h light and 11 h dark photoperiod conditions positioned at ZARS, (Zonal Agricultural Research Station), VC-Farm, Mandya, Karnataka state of India which lies between a longitude 76^{0}82’ E and latitude 12^{0}56’ N and 690 m above mean sea level. The genotypes, PTB33 and TN1 were utilized as standard check varieties as resistance and susceptible sources, respectively in the screening experimentation. The release of BPH was synchronized in such a way that 2nd to 3rd instar nymphs were released artificially on 12 days old test seedlings approximately at a rate of 10-12 hoppers per plant and were observed daily for burning symptom caused by the brown planthoppers. At the same time, observation of the percentage dead seedlings was documented for each landrace / genotype when 90% of seedlings of susceptible check, TN1 died due to the de-sapping by the planthoppers. Further, the tested landraces / germplasm accessions were scored phenotypically on individual plant basis based on the burning symptoms to attain a ‘damage score’ by following the ‘Standard Evaluation System’ (SES) of rice (IRRI, 2014). Upon phenotypic screening under glasshouse, around 50 rice landraces and genotypes which exhibited resistant, moderately resistant and susceptible reaction were opted for molecular characterization studies.

Genotypic screening

The molecular characterization of the selected rice genotypes for major QTLs of BPH tolerance has been accomplished using genomic SSR markers linked to BPH resistance genes at Genetic Purity and Seed Health Laboratory, Zonal Agricultural Research Station, V.C. Farm, Mandya, Karnataka, India. A total of 50 rice genotypes including landraces that exhibited moderately resistant, resistant and susceptible reaction during phenotyping screening were used for molecular characterization studies. The genomic DNA was isolated from selected promising genotypes by following the protocol of Doyle and Doyle (1987). Further, the quantity of DNA and its purity was checked using Nanodrop. The accessions selected for molecular characterization were studied with the help of molecular makers, especially SSR markers. For these markers, the primer sequences were obtained from the Gramene database (www.gramene.org) and other published studies on BPH resistance genes with associated markers. The primer sequences were used to synthesize oligos through a commercial facility (Eurofins, Bengaluru, India). The process of PCR amplification was performed by using thermal cycler. Further, loading of PCR products, documentation and scoring of gels and analysis of the scored data were performed as per the standard set of protocols.

RESULTS AND DISCUSSION

Phenotypic screening

The screening results revealed that, out of 260 traditional rice landraces and 705 rice germplasm accessions subjected for phonotyping against BPH under greenhouse, only 50 accessions were selected for further molecular characterization (**Table 1**). Off which, the resistance pattern consisted of 15 resistant (DS 1), 26 moderately resistant (DS 3), 3 moderately susceptible (DS 5), 2 susceptible (DS 7) and 4 highly susceptible (DS 9) accessions. Among the selected 50 accessions, the landraces *viz*., Maharuri, Kalambudhiya, Kichadi, Local halwa, Ram vaidya and Deshibenika exhibited resistance reaction against planthoppers by registering the phonotypic score of ‘1’. Contrarily, the accessions Rajamudi, Jeerige sanna, Rathnachoodi-1 and Navara were found susceptible to the planthoppers which recorded the phenotypic score of either ‘7’ or ‘9’. The resistant check, PTB33 and the susceptible check, TN1 varieties utilized in the phenotypic screening exhibited the phenotypic score of ‘1’ and ‘9’, respectively. Some reports in the literature indicate that few traditional landraces have the important source of planthopper resistance genes (Jena, 2010).

Table 1. Phenotypic reaction of rice landraces / genotypes selected for molecular characterization

S. No.	Landraces / Germplasm accessions	Dead seedlings (per cent)	Damage Score (DS)	Resistance category[*]
1	Mahasuri	3.13	1	R
2	Kalambudhiya	4.55	1	R
3	Kichadi	9.68	1	R
4	Local halwa	5.00	1	R
5	Ram vaidya	9.09	1	R
6	Deshibenika	7.41	1	R
7	RP 2068-18-3-5	19.13	3	MR
8	RP Bio 4918-230	9.09	1	R
9	IET-31635	13.33	3	MR
10	IBT-BPHM 23	4.81	1	R
11	IET-31582	7.14	1	R
12	IET-31591	3.57	1	R
13	IET-31665	9.70	1	R
14	BPT 3194	3.85	1	R
15	IET-30233	0.00	1	R
16	IET-30602	3.45	1	R
17	Bheema sale-1	29.14	3	MR
18	Nellur sanna	16.89	3	MR
19	Sannakki	17.14	3	MR
20	Coimbatore sanna-1	16.00	3	MR
21	Budda	12.50	3	MR
22	Kaniarbaul	10.41	3	MR
23	Kelivikua	9.52	3	MR
24	Sathaka	12.00	3	MR
25	Kudesar	11.63	3	MR
26	Lalluchei	16.67	3	MR
27	Chak hou poidon	17.39	3	MR
28	Vargol	10.05	3	MR
29	Pateni	12.12	3	MR
30	Mansara	10.00	3	MR
31	Jaintoli	17.65	3	MR
32	Dahi gurmatia	14.29	3	MR
33	Dubri	21.74	3	MR
34	Tilwo	6.67	3	MR
35	Garfa	11.34	3	MR
36	Babawee	73.16	7	S
37	KMR 3	23.07	3	MR
38	CRCPT-7	17.39	3	MR
39	Suraksha	22.72	3	MR
40	IET-31658	12.12	3	MR
41	IET-32034	11.25	3	MR
42	IET-31778	29.41	5	MS
43	Rajamudi	76.19	7	S
44	Jeerigesanna	100.00	9	HS
45	Rathanachoodi-1	90.00	9	HS
46	Navara	86.67	9	HS
47	Jaya	50.00	5	MS
48	Jyothi	54.54	5	MS
49	PTB 33 (Resistant check)	7.83	1	R
50	TN 1 (Susceptible check)	89.28	9	HS

[*] R - Resistant, MR -Moderately Resistant, MS - Moderately Susceptible, S - Susceptible, HS - Highly Susceptible (As per SES, IRRI, 2014)

Genotypic screening

Based on the results of phenotypic screening under green house, over 50 rice genotypes and landraces that exhibited moderately resistant, resistant and susceptible reactions by Standard Seed box Screening Test (SSST) were subsequently used for molecular screening to identify major QTLs of BPH tolerance using SSR markers linked to BPH resistance genes. The molecular characterization study of the selected rice genotypes and landraces was performed using eight molecular markers associated with five BPH resistance genes / QTLs and having phenotypic effect value (PEV) ranging from 59.81 to 83.90 per cent were utilized to analyse the genetic basis of BPH resistance in the selected genotypes. The five resistant genes *viz*., *Bph3*, *Bph6*, *Bph17*, *Bph31* and *Bph32* selected for genotyping study were reported to be effective against biotype 4 of BPH, prevalent in India, and the SSR markers linked to above five genes are highly informative and have higher phenotypic variation. Further, these genes were frequently used in marker assisted breeding for introgression of BPH resistance genes. In cases where a gene / QTL associated with multiple markers, more than single SSR marker was also used to enhance the effectiveness of parental selection

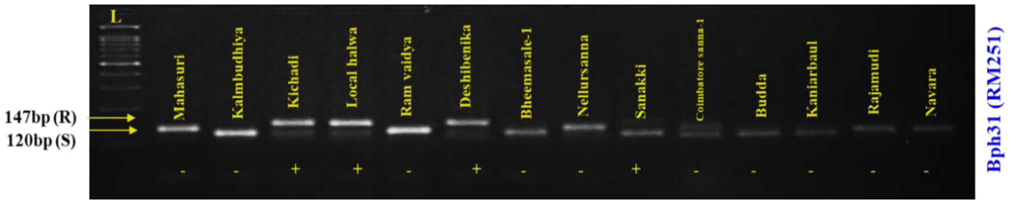

Fig. 1a: Primer RM 251(*Bph31*) showing expected amplicon size (146 bp)

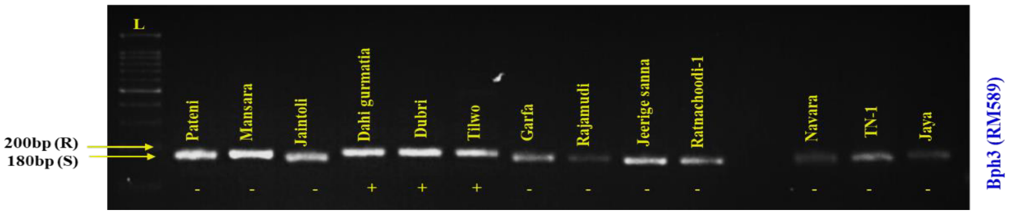

Fig. 1b: Primer RM589 (*Bph3*) showing expected amplicon size (200bp)

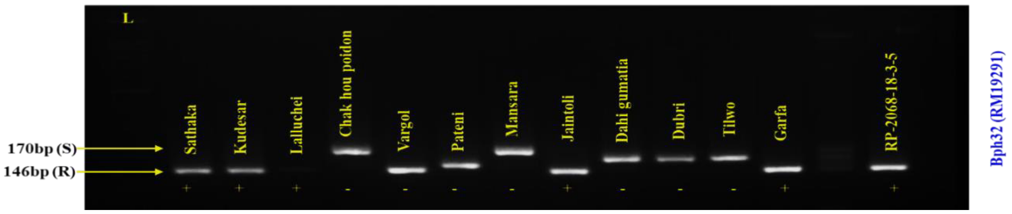

Fig. 1c: Primer RM19291 (*Bph32*) showing expected amplicon size (146 bp)

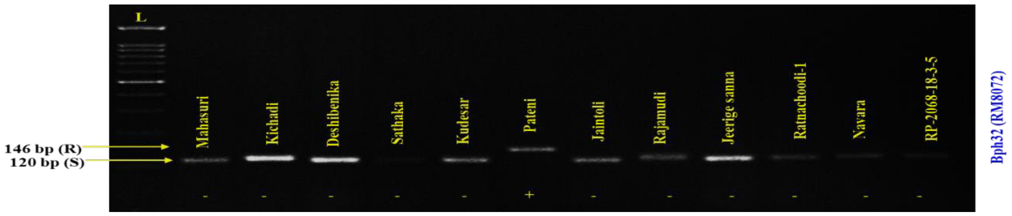

Fig. 1d: Primer RM 8072 (*Bph32*) showing expected amplicon size (146 bp)

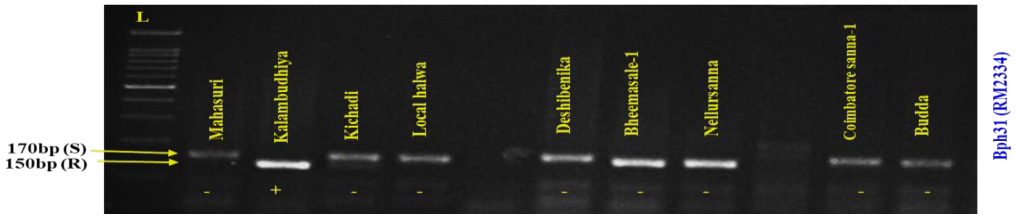

Fig. 1e: Primer RM 2334 (*Bph31*) showing expected amplicon size (150 bp)

Fig. 1. Amplification pattern of microsatellite primers RM 251, RM 589, RM 19291, RM 8072 and RM 2334
Note: + : Presence of resistant allele; - : Absence of susceptible allele

because of their close linkage specific gene / QTL. Meanwhile, the polymorphic pattern of RM 251, RM 589, RM 19291, RM 8072 and RM 2334 is also presented in **Fig. 1a - Fig. 1e**. Such type of work on diagnosing the genetic basis of BPH resistance and authentication of several quantitative trait loci (QTL) has been previously performed by Yang *et al*. (2004), Ishwarya Lakshmi *et al*. (2021) and Vignesh *et al*. (2023) in rice.

Molecular screening results indicated that among 50 genotypes studied, all the genotypes possessed at least one or more genes conferring resistance to brown planthopper. Specifically, *Bph3* was present in twenty one genotypes, while *Bph6* was present in thirty six genotypes*, Bph17* was present in fifty genotypes, *Bph31* was present in fifteen genotypes and *Bph32* was present in twenty five genotypes (**Table 2** and **Table 3**). Among 50 genotypes included in the study, KMR3 harboured all the five BPH resistant genes *viz*., *Bph3*, *Bph31, Bph32, Bph17* and *Bph6.* Overall, out of 50 genotypes, only one genotype contained five resistance genes, eighteen genotypes contained four genes, fourteen genotypes had three genes, eleven genotypes had two genes, and six genotypes had only one gene (**Fig. 2**).

Kalambudhiya, Kichadi, Local halwa, IBT-BPHM23, IET-31582, IET-31665, BPT-3194, IET-30233, IET-30602, and PTB-33 (Resistance check) showed a resistant reaction in phenotypic screening with a damage score of 1 and possessed four resistance genes in molecular profiling (**Table 1** and **Table 3**). Meanwhile, Mahasuri, Ram vaidya, Deshibenika, IET-31591, and RP-2068-18-3-5 showed a resistant reaction in phenotypic screening with a damage score of 1 and possessed three resistance genes. Similarly, KMR-3 displayed a moderately resistant reaction in phenotypic screening with a damage score of 3, despite having five resistance genes. Bheemasale-1, Nellursanna, Sannakki, Sathaka, Lalluchei, Garfa and IET-31778 displayed moderately resistant reaction with a damage score of 3 and had four resistance genes. In contrast, Rajamudi showed a susceptible reaction in phenotypic screening with a damage score of 7 and had only one resistance gene. Jaya showed a highly susceptible reaction with a damage score of 9, despite having two resistance genes. Jeerige Sanna, Ratnachoodi-1, Navara, TN-1 (Susceptible check), and Jyothi showed highly susceptible reactions with a damage score of 9 and possessed only one resistance gene. Mahantashivayogayya *et al*. (2024) reported similar findings, with the genotypes Kempu battha, Nari kela, Burma black selection 2 and Mukanna ratnachudi exhibiting all six resistance genes / QTLs—*Bph17*, *Bph3*, *QTL*, *Qbph3*, *Qbph6* and *Bph21* when amplified using gene-linked markers RM8213, RM589, RM410, RM154, RM314 and RM5479, respectively. These genotypes demonstrated resistance in both phenotypic and molecular assessments. Likewise, Jena *et al*. (2015) conducted genetic diversity analysis of 58 rice genotypes including 39 landraces through 22 gene-linked markers of BPH resistance categorized them into 4 major clusters with the 40% level of genetic similarity.

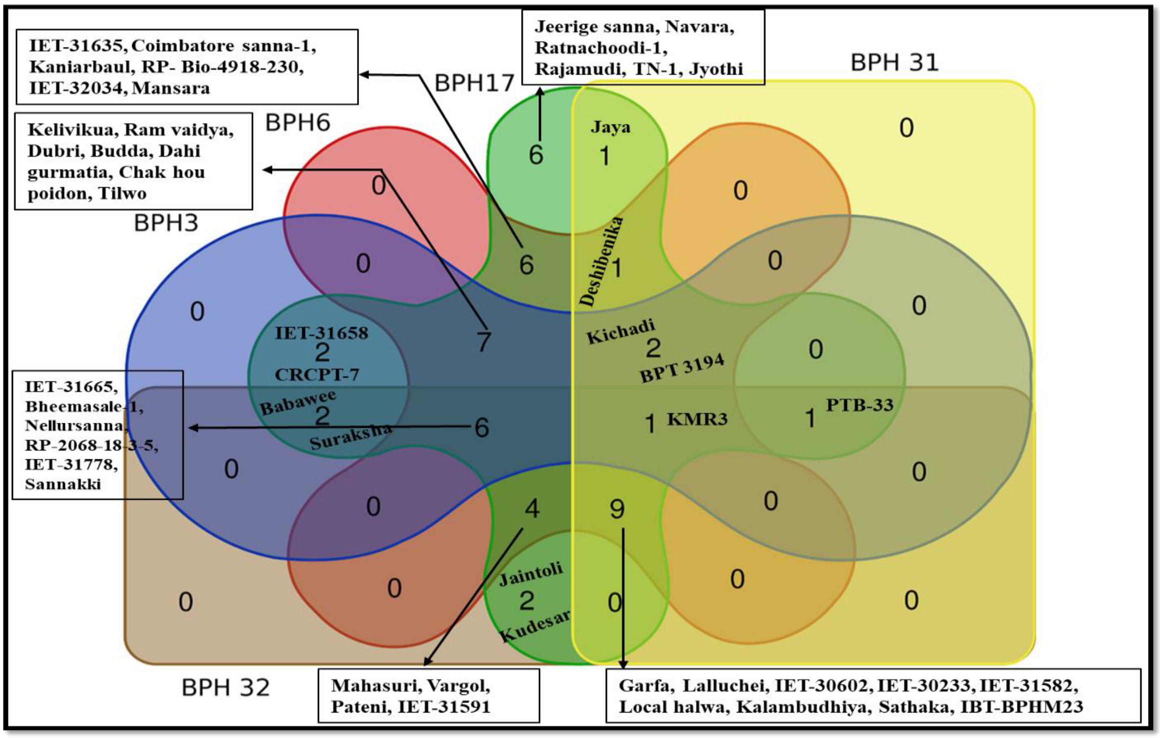

Fig. 2. Venn diagram representing combination of BPH resistant genes present in the selected 50 rice genotypes

Table 2. Scores of promising genotypes for the presence of brown planthopper resistance genes following genotypic evaluation with markers

S. No.	Designation	Phenotypic score	MARKERS								Total number of resistant genes
			RM589	RM119	RM8213	RM5953	RM251	RM2334	RM19291	RM8072	
			Bph3	*Bph6*	*Bph17*	*Bph17*	*Bph31*	*Bph31*	*Bph32*	*Bph32*	
1	Mahasuri	1	0	1	0	1	0	0	1	0	3
2	Kalambudhiya	1	0	1	1	1	0	1	1	0	4
3	Kichadi	1	1	1	1	1	1	0	0	0	4
4	Local halwa	1	0	1	0	1	1	0	1	0	4
5	Ram vaidya	1	1	1	1	1	0	0	0	0	3
6	Deshibenika	1	0	1	0	1	1	0	0	0	3
7	RP -2068-18-3-5	1	1	1	0	1	0	0	1	0	3
8	RP -Bio-4918-230	1	0	1	0	1	0	0	0	0	2
9	IET-31635	1	0	1	0	1	0	0	0	0	2
10	IBT-BPHM23	1	0	1	0	1	1	0	1	0	4
11	IET-31582	1	0	1	0	1	1	0	1	0	4
12	IET-31591	1	0	1	0	1	0	0	1	0	3
13	IET-31665	1	1	1	0	1	0	0	1	0	4
14	BPT 3194	1	1	1	0	1	1	0	0	0	4
15	IET-30233	1	0	1	1	1	1	0	1	0	4
16	IET-30602	1	0	1	1	1	1	0	1	0	4
17	Bheemasale-1	3	1	1	0	1	0	0	1	0	4
18	Nellursanna	3	1	1	0	1	0	0	1	0	4
19	Sannakki	3	1	1	0	1	0	0	1	1	4
20	Coimbatore sanna-1	3	0	1	0	1	0	0	0	0	2
21	Budda	3	1	1	0	1	0	0	0	0	3
22	Kaniarbaul	3	0	1	0	1	0	0	0	0	2
23	Kelivikua	3	1	1	1	1	0	0	0	0	3
24	Sathaka	3	0	1	1	1	1	1	1	0	4
25	Kudesar	3	0	0	1	1	0	0	1	0	2
26	Lalluchei	3	0	1	1	1	0	1	1	0	4
27	Chak hou poidon	3	1	1	1	1	0	0	0	0	3
28	Vargol	3	0	1	0	1	0	0	1	0	3
29	Pateni	3	0	1	0	1	0	0	0	1	3
30	Mansara	3	0	1	0	1	0	0	0	0	2
31	Jaintoli	3	0	0	1	1	0	0	1	0	2
32	Dahi gurmatia	3	1	1	1	1	0	0	0	0	3
33	Dubri	3	1	1	1	1	0	0	0	0	3
34	Tilwo	3	1	1	1	1	0	0	0	0	3
35	Garfa	3	0	1	0	1	1	1	1	0	4
36	Babawee	3	1	0	0	1	0	0	1	0	3
37	KMR3	3	1	1	0	1	1	0	1	0	5
38	CRCPT-7	3	1	0	1	1	0	0	0	0	2
39	Suraksha	3	1	0	0	1	0	0	1	0	3
40	IET-31658	3	1	0	0	1	0	0	0	0	2
41	IET-32034	3	0	1	0	1	0	0	0	0	2
42	IET-31778	3	1	1	0	1	0	0	1	0	4
43	Rajamudi	7	0	0	0	1	0	0	0	0	1
44	Jeerige sanna	9	0	0	0	1	0	0	0	0	1
45	Ratnachoodi-1	9	0	0	1	1	0	0	0	0	1
46	Navara	9	0	0	0	1	0	0	0	0	1
47	Jaya	9	0	0	0	1	1	0	0	0	2
48	Jyothi	9	0	0	0	1	0	0	0	0	1
49	PTB 33 (Resistant check)	1	1	0	1	1	0	1	1	0	4
50	TN 1 (Susceptible check)	9	0	0	1	1	0	0	0	0	1

Table 3. BPH resistant genes identified in rice genotypes during molecular characterization

Genes	Total number of genes	Total number of genotypes	Names of the genotypes
Bph31, Bph32, Bph17, Bph3, Bph6	5	1	KMR3
Bph31, Bph17, Bph3, Bph6	4	2	Kichadi, BPT 3194
Bph32, Bph17, Bph3, Bph6	4	6	IET-31665, Bheemasale-1, Nellursanna, RP -2068-18-3-5, IET-31778, Sannakki
Bph31, Bph32, Bph17, Bph3	4	1	PTB-33 (RC)*
Bph31, Bph32, Bph17, Bph6	4	9	Garfa, Lalluchei, IET-30602, IET-30233, IET-31582, Local halwa, Kalambudhiya, Sathaka, IBT-BPHM23
Bph17, Bph3, Bph6	3	7	Kelivikua, Ram vaidya, Dubri, Budda, Dahi gurmatia, Chak hou poidon, Tilwo
Bph32, Bph17, Bph3	3	2	Suraksha, Babawee
Bph31, Bph17, Bph6	3	1	Deshibenika
Bph32, Bph17, Bph6	3	4	Mahasuri, Vargol, Pateni, IET-31591
Bph17, Bph3	2	2	IET-31658, CRCPT-7
Bph17, Bph6	2	6	IET-31635, Coimbatore sanna-1, Kaniarbaul, RP -Bio-4918-230, IET-32034, Mansara
Bph31, Bph17	2	1	Jaya
Bph32, Bph17	2	2	Jaintoli, Kudesar
Bph17	1	6	Jeerige sanna, Navara, Ratnachoodi-1, Rajamudi, TN-1 (SC)**, Jyothi

*RC = Resistance Check **SC = Susceptible Check

The most promising BPH tolerant rice accessions identified in the study are presented in **Table 4**. The comparative analysis of phenotypic and molecular screening for BPH resistance underscores the complexity of this trait, which is influenced by both the number of resistance genes and their specific interactions. The high resistance observed in varieties like Kalambudhiya, Kichadi, Local halwa, IBT- BPHM23, IET-31582, IET-31665, BPT-3194, IET-30233 and IET-30602 with a damage score of 1 and four resistance genes implying that these genes may have a strong, synergistic effect that provides robust protection. Meanwhile, KMR-3, with five resistance genes, showed only moderate resistance (DS 3), which implies that gene number alone is not always predictive of phenotypic resistance. Specific combinations or environmental factors likely play a role in how these genes express resistance traits. Notably, the *Bph31* gene frequently appears in genotypes with a phenotypic score of 1 or 3.

The findings also witnessed that some varieties with fewer resistance genes (two or three) can still achieve strong resistance levels, as observed in Mahasuri and RP-Bio-4918-230, signifying that certain genes may be particularly potent. Conversely, genotypes like Jaya and Rajamudi, with one or two resistance genes, showed high susceptibility, emphasizing that some resistance genes may not be effective against the tested BPH biotypes or may require complementary genes to express a resistant phenotype. Thus, both the number and effectiveness of resistance genes are crucial, highlighting the importance of specific gene interactions over mere gene count in conferring BPH resistance. Additionally, gene interactions, gene expression levels and environmental factors could modulate resistance, leading to the observed variability. This suggests that resistance in these rice accessions may involve a wider spectrum of *bph* genes or gene networks beyond the five genes studied. The genotypes exhibiting resistant reaction could be utilised as donors in the crop improvement programme for evolving new cultivars with BPH resistance through Marker Assisted Selection (MAS). Plant breeders should use diverse cultivars to broader the genetic background of the improved cultivars, but most of the breeding programmes of rice have a narrow genetic variability of breeding resources. The present results revealed that ten accessions harboured four to five BPH resistant genes and these accessions could be used as source of resistant genes in designing future breeding programme. Additionally, pyramiding of resistance genes for BPH is a reliable approach for obtaining enhanced resistance.

Evaluation of germplasm for resistance genes contributes immensely in selection of parental lines, monitoring of germplasm and development of new breeding material. Information on target gene obtained from markers will facilitate the use of germplasm efficiently. The results further foreshow that since the SSR markers are neutral and codominant, they are powerful tools to access the genetic variation among the cultivars. Use of markers

Table 4. Promising BPH tolerant rice genotypes

Genotypes	Damage score	Total number of Resistant genes / QTLs	Gene combination
Kalambudhiya	1	4	*Bph31, Bph 32, Bph17, Bph6*
Kichadi	1	4	*Bph31, Bph17, Bph6, Bph3*
Local halwa	1	4	*Bph31, Bph 32, Bph17, Bph6*
IBT- BPHM23	1	4	*Bph31, Bph 32, Bph17, Bph6*
IET-31582	1	4	*Bph 32, Bph17, Bph6, Bph 3*
IET-31665	1	4	*Bph 32, Bph17, Bph6, Bph*3
BPT-3194	1	4	*Bph31, Bph17, Bph6, Bph3*
IET-30233	1	4	*Bph31, Bph 32, Bph17, Bph6*
IET-30602	1	4	*Bph31, Bph 32, Bph17, Bph6*
KMR-3	3	5	*Bph31, Bph 32, Bph17, Bph6, Bph3*

and phenotypic data together offers a productive tool to the breeders in selecting parents for future breeding programs *viz*., mapping of genes and in the application of marker assisted selection (MAS).

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