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RESEARCH

Mapping of a novel recessive brown planthopper resistance gene *bph46* from wild rice (*Oryza nivara*)

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Abstract Rice production is severely threatened by frequent outbreaks of Brown planthopper (BPH), Nilaparvata lugens (Stål.) biotypes globally. On this account, host-plant resistance serves as an important strategy to reduce the damage caused by BPH. The wild species of rice *Oryza nivara* accession IRGC 93198 showed consistent resistance reaction against BPH biotype 4 for 5 consecutive years of screening under the greenhouse conditions. The mapping of the BPH resistance gene from *Oryza nivara* accession IRGC 93198 was conducted using BC2F2 and BC2F3 progenies. Out of 239 BC2F2 plants, 65 plants were resistant (1-3 score), and 174 plants (5, 7, and 9 score) were susceptible, thus fitting the segregation ratio of 3:1 (Susceptible: Resistant). The BC2F3 progenies segregated in 1:2:1 confirming that the resistance from *O. nivara* is governed by a single recessive gene. Bulked segregant analysis (BSA) identified genomic region on the short arm of chromosome 4 to be associated with BPH resistance. Molecular mapping performed on BC2F2 population identified a QTL on chromosome 4 within the marker interval RM16285 and RM6314 explaining phenotypic variance of 27% at LOD 22.34. The linked marker RM6659 was found efficient in demarcating the susceptible from resistant lines when applied on the panel of rice cultivars, hence can be used for marker assisted selection in crop breeding. The previously identified BPH-resistant genes located on chromosome 4 were found susceptible to the BPH biotype 4 screening test. This specifies bph46 to be a novel gene that can be deployed as a valuable donor in BPH resistance breeding programs.

Keywords Brown plant-hopper · *Oryza nivara* · Bulked segregant analysis · QTL · Marker-assisted selection

Introduction

Rice is a staple food for more than half of the world's population (Sen et al. 2020). Rice yield is compromised by various biotic and abiotic stresses. Of these, Brown planthopper (BPH) has turned into the most devastating insect/pest of rice, damaging the rice plants by feeding on the phloem sap and transmiting rice grassy stunt virus (RGSV) and rice ragged stunt (RRSV) virus (Bao and Zhang 2019; Cabauatan et al. 2009; Wang et al. 2021). The typical symptoms of BPH infestation include "hopper burn" which is characterized by circular patches of drying and lodging of mature rice plants (Nguyen et al. 2021). In recent years heavy yield loss due to the BPH outbreak is

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reported in India, Indonesia, China, Japan, Vietnam, Korea, Bangladesh and other South East Asian rice-growing countries (Sogawa 2015; Mishra et al. 2022). Presently, chemical control measures are advocated to diminish this insect, but they owe to the higher cost and adverse effects on the environment (Hu et al. 2018; He et al. 2013). In addition, the continuous application of pesticides often leads to pest reappearance. Identification of resistant genotypes and implementation of new genes or a combination of genes for providing durable BPH resistance in high-yielding genotypes is a vital and preferred approach to manage this insect.

There are four different biotypes in which BPH populations have been characterized (Khush et al. 1985) i.e., Biotype 1 and Biotype 2 (East and Southeast Asia), Biotype 3 (IRRI) and Biotype 4 (South Asia). Nevertheless, only a few BPH resistance genes showed broad-spectrum resistance in monogenic rice lines (Horgan et al. 2015) and the majority of the identified genes are ineffective against the evolving BPH biotype(s) prevalent in North-Western India (Sarao et al. 2016). For instance, IR26, the first BPH-resistant variety with a single resistant gene, *Bph1*, was ineffective against new biotype 2 (Khush and Coffman 1977). In 1976, the BPH resistant varieties like IR36 harboring *bph2* were also found to be susceptible to the new BPH population (Alam and Cohen 1998; Ketipearachchi et al. 1998). Currently, many of the deployed genes against BPH biotypes 1 and 2 are not effective for BPH biotype 4 (Deen et al. 2017). The continuously evolving nature of the BPH biotypes necessitates the exploration of new broad-spectrum BPH resistant sources and furthers their deployment in the elite cultivars either singly or in combination for the creation of durable resistance.

Till date, more than 44 major resistance genes and 22 quantitative trait loci (QTLs) are identified from wild rice and cultivated varieties, most of which are usually present in clusters on chromosomes 3, 4, 6, and 12 (Du et al. 2020). Only a few BPH-resistant genes have been utilized from the wild species of rice i.e. *O. australiensis*, *O. officinalis*, *O. latifolia, O. minuta* and *O. rufipogon.* Among them, *Bph14, Bph3/Bph15, Bph26/bph2, bph29, bph7/Bph9/Bph10/Bph21, Bph18/Bph1, Bph32, Bph6, Bph30/Bph40,* and *Bph37* have been isolated and characterized via map-based cloning approach (Cheng et al. 2013; Ji et al. 2016; Ren et al. 2016; Du et al. 2009; Tamura et al. 2014; Wang et al. 2015; Zhao et al. 2016; Liu et al. 2015; Jing et al. 2017; Guo et al. 2018; Shi et al. 2021; Zhou et al. 2021). Despite the identification and deployment of these gene(s) to the elite cultivars, frequent outbreaks of BPH have been observed in recent years (Bottrell and Schoenly 2012; Sani et al. 2020). Hence there is an imperative need to explore diverse genetic sources conferring resistance against the new virulent Biotype 4.

The wild species of rice *O. nivara* IRGC 93198 (2n = 2X = 24) was identified as a promising BPH resistance source against Biotype 4 over the past 4 years of screening at Punjab Agricultural University, Ludhiana, India. A mapping population was developed by hybridizing *O. nivara* accession IR93198 (BPH resistant) and Punjab Rice 122 (PR122) (BPH susceptible) to identify genetic nature, chromosomal location and markers linked to the resistance genes in *O. nivara*.

Materials and methods

Plant materials and development of mapping population

The *O. nivara* accession (IR93198) (Score 1) was selected from a pool of 1003 wild accessions of rice screened against BPH biotype 4 at PAU. The *O. nivara* accession IR93198 was crossed with a BPH-susceptible rice cultivar PR122. The resulting F_1 plants were backcrossed with PR122 to generate the BC_1F_1 and BC_2F_2 populations. The BC_2F_2 population and BC_2F_3 progenies were used for the genetic analysis and mapping of the BPH-resistance locus (Fig. 1). Further generation was advanced to BC_2F_8 to select agronomically superior introgression lines with BPH resistance.

BPH insect raising

Seeds of the susceptible rice cultivar Taichung Native 1 (TN1) were sown in the earthen pots filled with puddled clay soil to maintain BPH biotype 4 insect culture. For insect rearing, 30 days old TN1 plants were placed in the rectangular cages of steel (0.68 × 0.50 × 0.50 m), covered with nylon net stitched along all sides except the top. The 2nd and 3rd instar

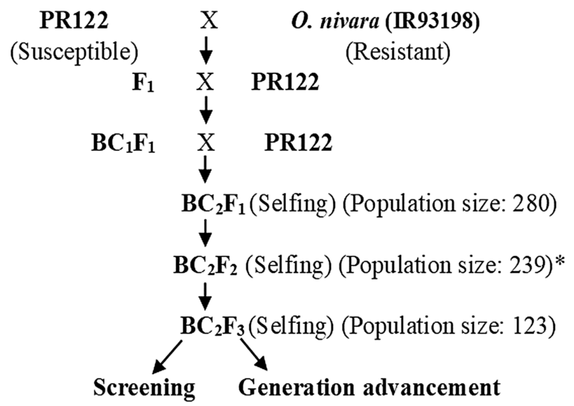

Fig. 1 Crossing scheme for generation of mapping population (*Due to sterility, 116 plants didn't have the seed set, therefore 123 BC_2F_3 progenies were used to confirm the inheritance of BPH resistance)

nymphs obtained thereafter were then released on the screening tray (Sarao et al. 2016).

Phenotypic evaluation of BPH resistance

For screening of the BC_2F_2 population, 239 seeds of each line along with parents were sown in well-puddled soil containing plastic trays (45 cm × 35 cm × 10 cm) using the standard seed box screening technique (SSST) proposed by Heinrichs et al. (1985). An insect-proof greenhouse maintained at 30 ± 2 °C, 80 ± 5% relative humidity, was used to raise the progenies. After 14 days, the 2nd to 3rd instar nymphs of hopper @ 10–12 per seedling were released on the plastic trays before enclosing it with a fine-gauge nylon net on the iron cage. When all of the seedlings of TN1 died, each BC_2F_2 individual was scored on a 0–9 scale using the Standard Evaluation System (SES) for rice by IRRI, (1996). For the evaluation of the BC_2F_2 population, the plants with 1–3 damage scores were scored as resistant and 7–9 scores as susceptible. Further, BC_2F_3 phenotypically superior lines with desirable characteristics were screened by sowing 30 seeds derived from single BC_2F_2 plants in each row. The evaluation of the BC_2F_3 family along with the donor and recurrent parent was done by averaging the damage score of 30 seedlings planted in each row. Based on SES, the mean score of each family with 0–4, 4.1–7, and 7.1–9.0 were regarded as resistant, segregating, and susceptible, respectively (Heinrichs et al. 1985). The experiments were conducted with at least three biologically independent replicates. The chi-square test for goodness of fit was used to study the inheritance pattern of BPH resistance.

DNA extraction, marker analysis

Total genomic DNA was extracted from fresh leaves of the BC_2F_2 population using the standard CTAB protocol (Saghai-Maroof et al. 1984). The extracted DNA was dissolved in 1X Tris EDTA buffer. Each DNA sample was diluted to 50 ng/µl for PCR analysis. The parental polymorphism survey between PR122 and *O. nivara* was conducted using 558 Simple sequence repeat (SSR) markers reported by Orjuela et al. (2010) and Temnykh et al. (2000) distributed over the 12 chromosomes of rice. Bulked segregant analysis (BSA) was performed to identify markers linked to BPH resistance (Michelmore and Paran 1991). According to the phenotypic screening of the BC_2F_2 population, resistant bulks (RB) and susceptible bulks (SB) were made consisting of 10 extremely resistant and 10 extremely susceptible individuals respectively. The polymorphic markers were used for BSA to identify the linked markers for genotyping the BC_2F_2 population. The Polymerase chain reaction (PCR) was applied on two bulked genotypes i.e., RS and SB along with PR122 and *O. nivara*. The SSR markers were amplified in a total of 10 µl PCR reaction as previously described (Kaur et al. 2022). PCR amplified products were separated on 3% agarose gel and individual alleles were scored in correspondence to parental alleles. Further, the identified linked markers were applied to the mapping population for QTL analysis.

Linkage map construction and QTL mapping

The linkage map was constructed using ICI Mapping software ver. 4.0 (Meng et al. 2015). The Kosambi function was used to estimate map distance and default parameters were used to construct the genetic linkage map. A LOD score of 3.0 was used as the threshold for detecting QTL location. Inclusive Composite Interval Mapping (ICIM) function was used to identify the position of QTL in correspondence to the phenotypic and genotypic data of 239 BC_2F_2 individuals.

Agronomic trait measurement

The agronomic traits of the developed BC_2F_3 progenies were measured by planting each line composing five rows and each row included ten plants (20 cm spacing between plants and 30 cm spacing between rows distance). Observations on agronomic traits, (i.e., 1000-grain weight, plant height, the number of grains per panicle, tiller number and effective panicle size) were recorded. Field management followed normal agronomic practices. Data recorded for the above traits were subjected to statistical analysis as per the analysis of variance for RBD using SAS software ver. 9.2 (SAS Institute Inc., 2003) and interpretations were made accordingly.

Results

Bioassay and genetic analysis for BPH resistance

The mode of segregation of the BPH resistance derived from *O. nivara* was carried out using the BC_2F_2 population and BC_2F_3 progenies (Supplementary Table 1). The inheritance pattern of genetic loci conferring BPH resistance was confirmed using screening of 239 individual BC_2F_2 plants, out of which 63 plants were found resistant and 176 plants were susceptible fitting to 3 (Susceptible): 1 (Resistant) segregation ratio ($\chi^2_c = 0.613 \le 3.8$, $\chi^2_{0.05,1}$) (Fig. 2). The segregation ratio states the recessive nature of the BPH resistance gene from *O. nivara*. Due to linkage drag, 116 plants didn't have the seed set, therefore 123 BC_2F_3 progenies were used to confirm the inheritance of BPH resistance where 25 plants were resistant, 58 were segregating and 40 were susceptible. The segregation ratio was in accordance with 1:2:1 ratio ($\chi^2_c = 4.04 \le \chi^2_{0.05,2} = 5.99$), thus confirming a single major QTL controlling the BPH resistance in the mapping population.

Molecular mapping of the BPH resistance gene

Out of the 475 SSR markers applied, 164 SSR (34.52%) distributed on 12 chromosomes of rice were found polymorphic between PR122 and the *O. nivara* accession IRGC 93198. The polymorphic SSR markers spanning all the chromosomes were applied to the BC_2F_2 population for the saturation of the genetic map. Three markers (RM16285, RM6314 and RM6659) of chromosome 4 were found linked/ associated with BPH resistance through BSA. The identified BPH-resistant locus was mapped using 239 BC_2F_2 individuals on the short of chromosome 4 flanked by SSR markers RM16285 and RM6314 at 22.34 LOD score exhibiting 27% phenotypic variance having RM6659 as peak marker (Table 1). The SSR marker RM6659 was co-segregating with the trait (Fig. 3). In this region 50 more markers were applied and three of them were found polymorphic between the parents. Additional markers were applied to

Fig. 2 Evaluation of BC_2F_3 progenies against BPH biotype 4

the population to further narrow down the region. Out of 239 BC_2F_2 plants, 22 recombinants within the RM16282-RM16643 interval were selected having breakpoints as RM16282 (4), RM16285 (5), RM6659 (1), RM6314 (1), RM17487 (5) and RM16643 (10) respectively (Table 2). This showed that the gene of interest lies within RM6659 and RM 6314 marker interval.

Table 1 Statistics of QTL mapping for *bph46*

Trait Name	Chromosome	Position	Left marker	Right marker	LOD	PVE (%)	Add	Dom
BPH	4	12.00	RM16285	RM6659	22.34	27.49	−2.21	1.57
BPH	4	19.00	RM6659	RM6314	21.27	27.45	−2.22	1.62

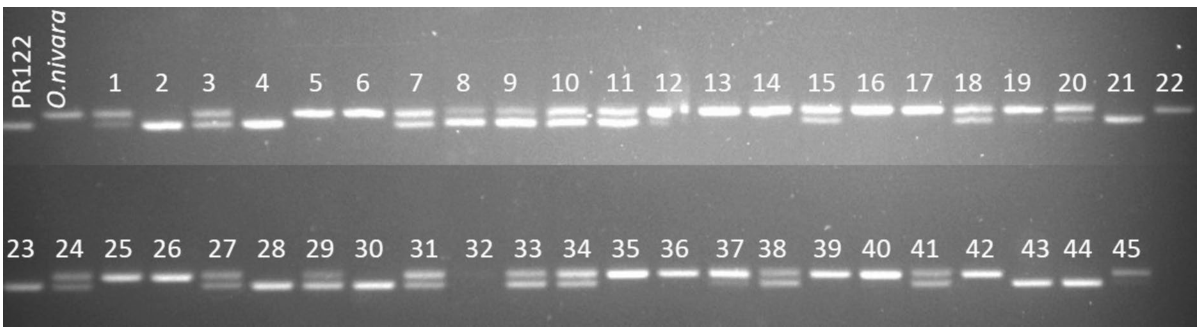

Fig. 3 Genotyping of BC_2F_2 population with peak SSR marker RM6659 (PR 122: susceptible parent, *O. nivara*: resistant parent, BC_2F_2 individulas from 1-45: The screening against BPH biotype 4 of the corresponding BC_2F_3 progenies indicated that the individuals with *O. nivara* alleles had the damage score between 1 to 3, with H alleles had damage score between 3.1 to 6.9 whereas the with PR122 alleles had damage score between 7 to 9)

Table 2 Selected recombinants indicating recombination events in the *bph46* region along with their mean BPH resistance scores, where A-allele is homozygous for PR122; B-allele is homozygous for *O. nivara* acc. IRGC 93198 and H- heterozygotes

Parents & Recombinants	BPH	RM16282	RM16285	RM6659	RM6314	RM17487	RM16643
PR122	9	A	A	A	A	A	A
1	9	B	A	A	A	A	A
2	7.9	B	H	H	A	A	A
3	8.14	B	B	H	A	A	A
4	1	B	B	B	H	A	A
5	3	B	B	B	B	A	A
6	4.9	B	B	H	A	A	A
7	5.5	B	B	H	A	A	A
8	6.9	B	B	H	A	A	A
9	3.08	H	H	B	B	H	A
10	6.7	A	A	A	A	B	B
11	7.2	B	B	A	A	A	A
12	9	A	A	A	A	A	B
13	9	A	A	A	A	A	B
14	9	A	A	A	A	A	H
O. nivara IRGC93198	1	B	B	B	B	B	B

Validation of linked marker RM 6659 in a panel of rice cultivars

A selected panel of elite cultivars: Non-Basmati (PR122, Pusa44, PR106, PR114, PR115, PR118, PR120, PR121, PR123, PR124, PR126, PR127, PR128, PR129, PR130, PR131, IR24, IR64, IRBB8, IRBB13, Kitaake) and Basmati (Vasumati, Tarori Basmati, Basmati 370, Basmati 386, Basmati 6004, Ranbir Basmati, Basmati Type 3, Pusa Basmati 1, Champaran Basmati, Tapovan Basmati, PB 2, PB 3, PB 5, PB 1509, Pusa 1121, Pusa 1718, Ryt 3315, Ryt 3388, Ryt 3432, Ryt 3677) showed susceptible reaction against BPH biotype 4. To validate the results, the linked marker was applied to the above described panel (Fig. 4). All of the cultivars showed an amplicon size of 250 bp as that of PR122 (susceptible parent) whereas *O. nivara* had the amplicon size of 270 bp with the linked marker RM6659 indicating the absence of the novel gene *bph46* in the panel of cultivars. Hence, this marker can be used for market-assisted transfer of the BPH resistance to the elite rice cultivars.

Identification of superior introgression lines

The BC_2F_3 progenies derived from PR122/*O.nivara* accession IRGC 93198 showed significant improvement for different desirable agronomic traits such as high tiller number and thousand-grain weight compared to PR122 (Supplementary Fig. 1). Considerable variation was observed for plant height (65.33–101.50 cm), tiller number (33.50–41.0), grains/panicle (148.50–167.0), and thousand-grain weight. (23.60gm-27.23gm). Line-1988 and Line-1989 were the best lines with the highest tiller number (28.13% increment), grains/panicle (22.79% increment) and thousand-grain weight (26.92% increment) (Table 3). The outperforming lines can be used in crop improvement programs as pre-breeding resources. Transgressive segregation reported for plant height, tiller number, grains/panicle, and thousand-grain weight indicates the contribution of beneficial alleles from the wild parent. These results indicate that the developed breeding lines acquired BPH resistance values and exhibited similar agronomic traits as the recurrent parent.

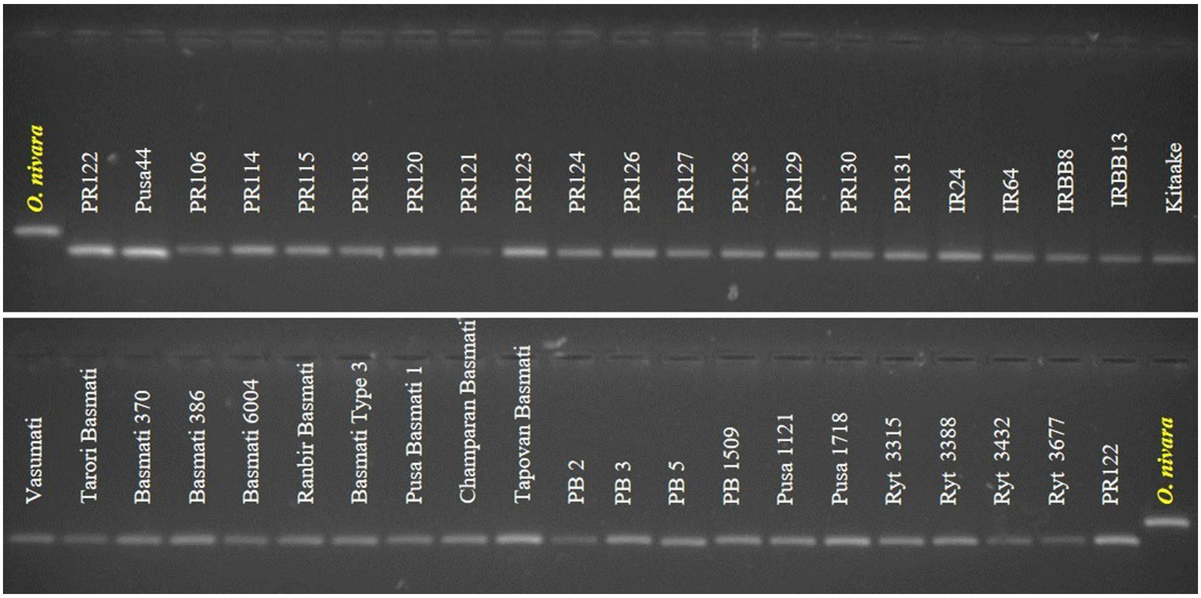

Fig. 4 Validation of the linked marker RM6659 on a panel of cultivars. This marker efficiently demarcates the susceptible and resistant lines and hence can be used for MAS

Table 3 Identification of agronomically superior BPH-resistant introgression lines

Line-ID	Traits (mean values are given for each trait)									
	Thousand grain weight(gm)	% Increment	Plant height (cm)	% Increment	Grains per panicle	% Increment	Tiller number	% Increment	Panicle size (cm)	% Increment
1898	26.55^{f}	24.60	86.50^{de}	– 16.0	157.55^{bc}	15.80	33.50^{ab}	0.04	24.25^{bc}	13.30
1900	26.13^{ef}	22.69	93.67^{f}	–9.06	151.50^{b}	11.40	34.50^{b}	7.81	23.00^{b}	9.39
1906	26.67^{f}	25.20	87.17^{de}	– 15.37	154.40^{bc}	13.53	35.00^{b}	9.38	25.00^{c}	18.78
1915	26.50^{f}	24.41	88.17^{e}	14.30	158.50^{bc}	16.50	34.33^{b}	7.28	24.50^{c}	14.40
1943	25.17^{de}	18.15	90.33^{e}	– 12.30	152.50^{b}	12.13	39.83^{d}	24.48	24.00^{bc}	14.08
1945	24.25^{d}	13.80	88.75^{e}	– 13.89	148.50^{b}	8.82	38.50^{cd}	20.31	23.75^{bc}	10.98
1962	27.23^{fg}	27.85	65.33^{a}	–36.57	162.00^{c}	19.12	34.33^{b}	7.29	26.00^{cd}	23.47
1970	25.33^{de}	18.93	90.50^{e}	– 12.14	158.00^{bc}	16.18	35.50^{bc}	10.94	26.00^{cd}	23.47
1988	25.70^{e}	20.65	88.75^{e}	– 13.83	157.75^{bc}	15.99	33.50^{ab}	4.68	27.20^{de}	27.10
1989	27.03^{f}	26.92	98.50^{fg}	–4.37	167.00^{c}	22.79	41.00^{e}	28.13	24.00^{bc}	14.08
1990	23.60^{c}	10.80	92.33^{ef}	– 10.36	159.00^{bc}	16.91	36.50^{c}	14.06	25.00^{c}	18.78
2005	25.63^{e}	18.90	101.50^{g}	– 1.48	152.50^{b}	12.13	35.16^{bc}	9.87	25.66^{cd}	20.46
2007	24.75^{d}	15.00	89.75^{e}	– 12.80	154.70^{bc}	13.78	34.50^{b}	7.81	24.75^{c}	15.65
2010	25.70^{e}	20.66	88.67^{e}	– 13.92	167.00^{c}	22.79	37.00^{c}	15.63	22.33^{ab}	6.26
PR-122	21.3^{a}		103^{gh}		136^{a}		32^{a}		21.40^{a}	
C.D	1.09		5.93		13.61		2.43		1.76	
SE(m)	0.38		2.10		8.36		1.57		0.78	
SE(d)	0.54		2.97		11.82		2.22		1.11	
C.V	2.78		3.95		15.18		8.34		7.20	

Means within a column followed by the same lowercase letters are not significantly different at $P \leq 0.05$ according to the Duncan's multiple range test

Discussion

O. nivara as a source of resistance to biotic and abiotic stresses

In our study, *O. nivara* was identified as a source of BPH resistance for the enhancement of the elite cultivars. Wild species are an important reservoir of valuable genes to widen the genetic diversity of modern cultivated rice. The crossing ability of *O. nivara* with cultivated rice has made it a good resource of favorable alleles to enrich the diversity in the existing gene pool for rice improvement. The accessions of *O. nivara* are identified as an ideal source of resistance to biotic stresses, Grassy stunt virus (GSV), Bacterial leaf blight (BLB), Sheath blight (ShB), neck blast, BPH and tolerance to many abiotic stresses. Several genes and major effect QTLs have been identified for pest and disease resistance, yield and yield component traits, grain quality, and nutritional traits from different accessions of *O. nivara*: *Xa*38 (Bhasin et al. 2012), *Xa*33 (Kumar et al. 2012), *Grh6* (Fujita et al. 2004), *qShb1, qShb3, qShb6, qShb7, qBlast8, qBlast12* (Eizenga et al. 2016).

Novelty of *bph46*

To date, a total of 13 BPH resistance QTLs have been mapped into two clusters on chromosome 4. Nine of these genes namely *Bph3/Bph17*, *Bph12(t)*, *Bph15*, *Bph20(t)*, *Bph30*, *Bph36*, *Bph41*, *bph42* and *QBph4.3* are clustered on the short arm of chromosome 4 (Sun et al. 2005; Yang et al. 2002, 2004; Rahman et al. 2009; Wang et al. 2018, 2021; Li et al. 2019; Kaur et al. 2022; Mohanty et al. 2017) whereas, 5 genes (*Bph6*, *Bph27*, *bph18(t)*, *Bph27(t)*, and *Bph34)* are grouped on the long arm of chromosome 4 (Qiu et al. 2010; Huang et al. 2013; Li et al. 2006; He et al. 2013; Kumar et al. 2018). To prove the novelty of *bph46*, the available sources of BPH-resistant genes located on chromosome 4 were screened against prevalent BPH biotype 4. Along with PR122 and *O. nivara* accession IRGC 93198, Babawee (*bph4*) (Jairin et al. 2010), Swarnalata (*Bph6*), IR71033-121-15 (*Bph20[t]*), Balamwee (*Bph27*) and TN1 (Susceptible check) were sown in plastic trays and scored based on the SES. The *O. nivara* accession IRGC 93198 showed a resistant reaction (score 1), whereas other sources showed a susceptible reaction (score 9) against BPH biotype 4 demonstrating the novelty of *bph46* against BPH biotype 4 (Fig. 5). Further, based on the marker information, *Bph3* [4:3995250–3997677]/*Bph17* [4:6942716–6939046], *bph4* [4:14481667], *Bph6* [4:19322356–19324388], *Bph20* [4:5019886–7179115] and *Bph27* [4:19121439] location doesn't coincide with the position of newly identified *bph46* [4:6541821]. To date only seven (*bph2*, *bph4, bph5, bph7, bph8, bph25* and *bph29)* recessive BPH resistance genes are available in the breeding program and none of them lies on chromosome 4. Therefore, the newly identified recessive *bph46* gene would be useful in broadening the genetic pool for BPH resistance.

Generation of pre-breeding lines as a valuable resource

Introgression lines (ILs) derived from crop wild relatives are powerful pre-breeding resources for identification, dissection and validation of QTLs underlying complex traits, thus playing a significant role in broadening the genetic pool of cultivars. ILs carrying desirable traits from *O. rufipogon*, *O. glaberrima*, *O. nivara*, *O. minuta*, *O. longistaminata* and other wild species are being utilized for raising rice yields and stress resistance (Singh et al. 2016). Reciprocal ILs were used to identify background-independent QTLs (BI-QTLs) and epistatic QTLs (E-QTLs) for quality traits (Takai et al. 2014; Qiu et al. 2017; Hori et al. 2021), salt tolerance (Cheng et al. 2012), lodging resistance (Ookawa et al. 2016). In the present study, a set of BPH-resistant ILs with superior agronomic traits were developed from wild species of rice *O. nivara*. Being a close wild relative of rice, the annual diploid species, *O. nivara* holds great potential to introgress disease resistance and yield associated QTL(s) into elite backgrounds of cultivated rice varieties (Khush 1977; Brar and Singh 2011; Swamy et al. 2014). 131 ILs were developed by introducing *O. nivara* segments into the 93–11 background, and 65 QTLs for 13 yield-related traits were detected to improve yield-associated traits (Ma et al. 2016). In addition, backcross introgression lines (BILs) of 'Swarna' containing wild introgressions from *O. nivara* were used to map QTLs for BB resistance (Balakrishnan et al. 2022). All these facts provide strong support to the fact that wild species of rice

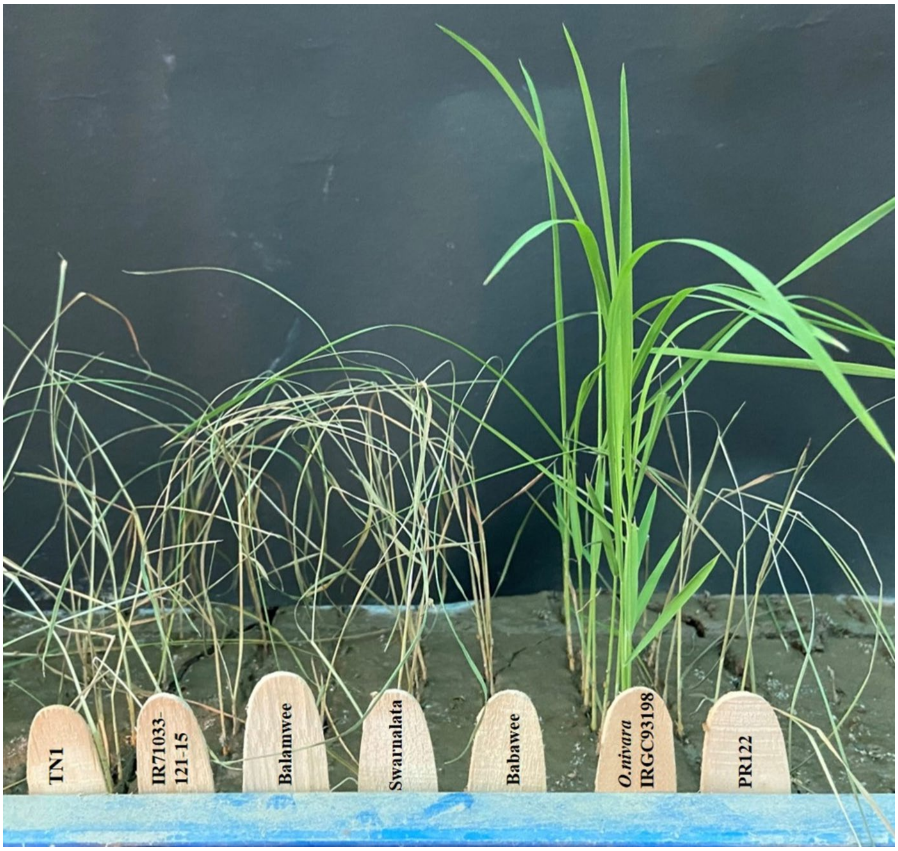

Fig. 5 BPH screening of Babawee (*bph4*), Swarnalata (*Bph6*), IR71033-121-15 (*Bph20[t]*), Balamwee (*Bph27*) and TN1 (Susceptible check), along with parental lines: PR122 and *O. nivara* accession IRGC 931983 demonstrating the novelty of *bph46* against BPH biotype 4

harbor favorable alleles for rice improvement that can be utilized for generating pre-breeding lines.

Conclusion

The research reveals a new recessive locus for BPH resistance originating from wild rice, *O. nivara*, located within the BPH resistance gene cluster on chromosome 4's short arm. With recessive genes against BPH biotype 4 being scarce, the identification of *bph46* expands the repertoire of recessive resistance genes available for breeding initiatives.

Author contributions Authors' contribution All authors contributed to the study. Kumari Neelam, Kuldeep Singh, Yogesh Vikal conceptualized the research, proofreading of the manuscript, Preetinder Singh Sarao maintained the BPH biotype 4 and helped in the screening of mapping population, Pavneet Kaur, Kumari Neelam, renu Khanna: Genotyping, data analysis, writing of the original draft, Pavneet Kaur, Yashika Walia Dhir, Navneet Singh Saini performed phenotyping of mapping population against BPH biotype 4. All co-authors approved this manuscript before submission.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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