*International Journal of Plant & Soil Science*

***Volume 35, Issue 18, Page 1491-1496, 2023; Article no.IJPSS.104349***
***ISSN: 2320-7035***

# Brown Planthopper, *Nilaparvata lugens* Stål Resistance in Backcross Derived Rice Lines

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/IJPSS/2023/v35i183449

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/104349

***Original Research Article***

***Received: 29/05/2023***
***Accepted: 02/08/2023***
***Published: 03/08/2023***

## ABSTRACT

A prominent rice pest, brown planthopper (BPH) significantly reduces the grain yield in rice across the globe and employing chemical pesticides leads to unwarranted environmental issues. Breeding for BPH resistance is an essential strategy to mitigate the losses caused by them. Host plant resistance through marker assisted selection is a chief strategy to lessen harms caused by BPH and boost rice production. In this study, we have analyzed BPH resistance in the $BC_1F_5$ population, which is a backcross derivative of improved CO51 and Ptb33. Improved CO51 has already been introgressed with bacterial blight resistant genes *xa5, xa13 and Xa21* and blast resistant gene *Pi54* via marker assisted selection (MAS). Ptb33 was used as the donor parent to incorporate BPH resistant genes *bph2* and *Bph32* to this CO51 background. The genotypically and phenotypically

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selected 26 lines of $BC_1F_5$ generation were screened against BPH along with parents and checks. The bioassay of the population exhibited a range of variation for BPH resistance. Among the 26 near isogenic lines, 18 (2 resistant and 16 are moderately resistant) and eight showed susceptible to moderate susceptible reaction. The 18 resistant lines were further multiplied and are now in hot spot screening.

*Keywords: Brown planthopper resistance; phenotypic screening; protray screening test; MAS; rice.*

## 1. INTRODUCTION

Rice is one of the important cereal crops and a vital source of energy for the growing population but its production is constrained by a range of factors, including pests and diseases. Over a hundred varieties of insects are known to infect rice, with around twenty of them posing significant threat to rice crops due to the extent of damage they can inflict [1]. One of the most devastating pests of rice is the brown planthopper (BPH), *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is highly prevalent in tropical Asia where rice crops are continuously cultivated**.** It is a monophagous pest that causes damage via phloem sap-feeding behaviour by BPH nymphs and adults from the lower part of the plant, further causing yellowing of the leaves , reduced plant height and more unfilled grains. The severe infestation leads to 'hopperburn' and ultimately leads to death of the plant [2-4]. BPH also acts as a vector by transmitting viruses like rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV), which result in significant losses. BPH infestation has increased across Asia in recent years [5,6].

To mitigate the incidence of pest infestation, host-plant resistance mechanism can be exploited via marker assisted selection(MAS) and resistant rice varieties can be developed. It is cost-effective, eco-friendly way to control BPH population below economic injury thus identification of BPH resistance genes are crucial [7,6]. More than 40 BPH resistance genes have been identified against 4 virulent biotypes in India [8]. Among them nine genes, *Bph3/Bph17, Bph14, Bph9, Bph15, Bph18, Bph26, Bph29, Bph32* have been cloned successfully and characterised for BPH resistance [9-16]. Reports suggested that incorporating multiple resistance genes into rice varieties results in stronger and more sustainable resistance [17]. A detailed review on BPH management is available [18]. Successful introgression of multiple resistance gene has been reported in several crops [19]. Thus improved CO51 was crossed with Ptb33 to introgress BPH resistance genes, *bph2* and *Bph32* [20,8,15,21]. *bph2* is located in the long arm of chromosome12 [22]. *Bph32* is located in the chromosome 6 [23]. The backcross derived lines were screened for both phenotype and genotype.

## 2. MATERIALS AND METHODS

CO51 is a high yielding, semi dwarf variety with short duration. Improved CO51 was developed by introgressing bacterial blight (*xa5, xa13* and *Xa21)* and blast (*Pi54)* resistance gene [24] and it was crossed with Ptb33 to incur BPH resistance genes *bph2* and *Bph32* [15]. The $F_1$, $BC_1F_1$, $BC_1F_2$ and $BC_1F_3$ were developed by marker assisted backcross breeding and forwarded [25]. A total of 585 plants were raised in $BC_1F_4$. Based on genotype and phenotype, 26 superior lines were identified and forwarded to $BC_1F_5$. These lines were screened against BPH to confirm their resistance.

For foreground selection, the genomic DNA was isolated from leaves of young, disease and pest free plants. The DNA was isolated from three week old plants. Modified CTAB method was used for DNA isolation [26]. The isolated DNA quality was determined in nanodrop. The isolated crude DNA was diluted to 100ng/µl with respect to their concentration for further usage in PCR. The PCR reaction mixture was prepared using 1µl of template DNA, 0.5µl each of forward and reverse primers, 4µl of Emerald Takara master mix, and 4µl of nuclease free water, with a total reaction volume of 10µl. The PCR protocol involved 35 cycles with an initial denaturation step at 94°C for 5minutes, followed by denaturation at 94°C for 1 minute and primer annealing at 56°C for BPH18-ind2; 57°C for PASH6 and extension at 72°C at 1minute. A final extension step was performed at 72°C for 7 minutes, followed by an infinite hold at 4°C. The PCR products were analysed using gel electrophoresis with ethidium bromide for band visualization in a BIO- Rad Doc EZ Imager under UV light. The gel was loaded into an agarose gel electrophoresis unit with 1X TBE buffer. The foreground selection was done with the help of SSR markers PASH6 and BPH18-ind2 as mentioned in Table 1.

**Table 1. List of linked/ functional markers used for foreground selection**

| Gene | Chromosome | Marker | Primer sequence | AT ($^{o}$C) | Size (bp) | Reference |
|---|---|---|---|---|---|---|
| bph2 | 12 | BPH18-ind2 | F TGGGCTGACAAATGGGTCC<br>R CCTTGTCGGGTGTAGCCAA | 56$^{o}$C | 257 | Ji et al. [16] |
| Bph32 | 6 | PASH 6 | F CCGACAACAAGACCTCCAAT<br>R CTGAACTGCACCTGGGTTTT | 57$^{o}$C | 193 | Jena et al. [23] |

Protray screening method (PST) was followed to screen the lines against BPH resistance at the seedling stage, in greenhouse. The protrays were kept on a galvanized iron tray, inside the closed mesh cage. Roughly about 5cm standing water was sustained in the tray to maintain necessary humidity for insect survival and to prevent disturbing of insects by watering it. 15 seeds of each entry were sown in individual cells within the protray. The selected plants along with the parent lines CO51and Ptb33, as well as negative check varieties TN1 were sown. Negative checks were sown in either corner of the protray. Every genotype was sown in two replications in separate closed mesh cage. The seven days old seedlings (one to two leaf stage) were infested with 2$^{nd}$ and 3$^{rd}$ instar nymphs by uniformly scattering inside cage, with an average of 7-8 nymphs per plant. The damage rating for each entry was recorded when approximately 90% of the susceptible check had been dried, usually occurring 6-7 days after infestation. Seedlings were then scored based on the observed damage symptoms, with the average score of two replications of each line. The standard evaluation system (SES) for rice, developed by International Rice Research Institute (IRRI, 2004) was followed for screening.

## 3. RESULTS AND DISCUSSION

Insects pose a serious threat to cereal crops and significantly reduce crop productivity [27]. One of the most dangerous pests that impact rice is the brown planthopper(BPH). It is a monophagous pest and has a specialized feeding behaviour. As a vascular feeder, uses its stylet to extract sap from rice phloem. This can cause direct harm to rice plants and lead to 'hopper-burn' condition in the field. It also acts as vectors and cause viral diseases. Modern technological advancements have produced a number of control strategies to reduce crop output losses and host plant resistance is the most efficient and environmentally safe method to reduce pest damage and boost crop output potential [28,29]. One of the chief technique is marker assisted selection (MAS) paves way to develop durable resistance to biotic and abiotic stress. It is highly useful in gene pyramiding from multiple parents helps to develop combination of resistance [30].

The improved CO51 has already been introgressed with bacterial blight (*xa5, xa13* and *Xa21)*, blast resistance gene (*Pi54*) . The improved CO51 was now stacked with BPH resistance genes, *bph2* and *Bph32*. The $F_1$, $BC_1F_1$, $BC_1F_2$, $BC_1F_3$, $BC_1F_4$, $BC_1F_5$ were raised. Out of 585 plants in $BC_1F_4$ plants with similar agronomically traits to CO51 were identified and forwarded to next generation. Among the 585 plants, 26 individual plants with different combinations of introgressed homozygous resistance genes were selected. They were screened for BPH resistance with an objective to select the lines that confer resistance against the Brown Planthopper (BPH). The molecular markers BPH18-ind2 and PASH6 were used for foreground selection of the BPH resistance genes *bph2* and *Bph32*, respectively (Fig. 1). The use of these markers allowed for efficient identification of the resistance genes within the selected lines. Notably, Ptb33, which has been reported to carry both *bph2* and *Bph32* genes, was used as donor parent material and CO51 was used as the recurrent parent in the developed backcross population.

The Protray screening test of 26 lines of $BC_1F_5$ showed that two were resistant, 16 were moderately resistant, seven were moderately susceptible and one was susceptible to bph infestation, These results imply that BPH resistance in population varies widely with some individuals displaying significant levels of resistance to BPH infestation (Table 2).

The results of this study suggested that incorporating the *bph2* and *Bph32* genes into rice varieties through marker-assisted selection has enhanced resistance against BPH, which is a major constraint on rice production. Overall, these findings have implications for the development of improved rice varieties with enhanced resistance to BPH, an important step towards ensuring global food security. The identification of resistant and moderately resistant plants is promising for further research and development of BPH resistant genotypes.

**Table 2. Phenotypic screening and genotypic analysis of $BC_1F_5$ population**

| Plant Number | Damage Scoring | Rating | *bph2* | *Bph32* |
|---|---|---|---|---|
| 1. | 6.84 | MS | R | R |
| 2. | 7.25 | MS | R | R |
| 3. | 2.91 | R | R | R |
| 4. | 5.03 | MR | R | R |
| 5. | 5.30 | MR | R | R |
| 6. | 6.12 | MR | R | R |
| 7. | 6.25 | MR | R | R |
| 8. | 5.74 | MR | R | R |
| 9. | 4.85 | MR | R | R |
| 10. | 5.45 | MR | R | R |
| 11. | 4.98 | MR | R | R |
| 12. | 7.39 | MS | R | R |
| 13. | 5.27 | MR | R | R |
| 14. | 6.16 | MR | R | R |
| 15. | 3.7 | R | R | R |
| 16. | 5.83 | MR | R | R |
| 17. | 6.33 | MR | R | R |
| 18. | 5.63 | MS | R | R |
| 19. | 4.36 | MR | R | R |
| 20. | 5.01 | MR | R | R |
| 21. | 5.04 | MR | R | R |
| 22. | 5 | MR | R | R |
| 23. | 6.71 | MS | R | R |
| 24. | 6.3 | MS | R | R |
| 25. | 7.89 | S | S | S |
| 26. | 6.09 | MS | R | R |
| CO51 | 9 | S | S | S |
| Ptb33 | 3 | R | R | R |

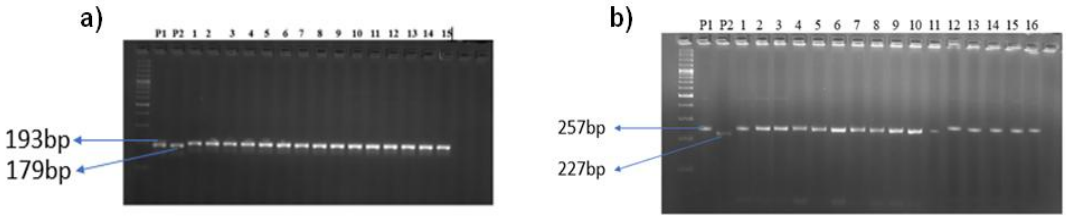


**Fig. 1. PCR amplification of (a) PASH6 (b) BPH18-ind2 in selected genotypes**

## 4. CONCLUSION

Two lines (3 and 15) showed good resistance to BPH and agronomic superiority over the parents. These lines can be grown in BPH endemic areas. The other promising lines 4 , 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21 and 22 showed moderate resistance to BPH which can be further utilized in breeding programmes. This study helped in identification of promising lines to be released as new variety and base material for host-pest interaction.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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