

Gene validation for Brown Plant Hopper resistance in rice varieties using SSR markers

ABSTRACT

The Brown Plant Hopper (BPH), *Nilaparvata lugens* (Homoptera: Delphacidae) is one of the most destructive insect pests causing significant yield loss in most of the rice cultivars of Asia. Marker-assisted backcrossing is used to introgress the genes for BPH resistance in which the selection of donor parents to confer durable resistance and validation of the presence of genes is a prerequisite. Jyothi the most popular, widely cultivated rice variety in Kerala is screened along with four parental lines viz., PTB33 (Variety released from RARS, Pattambi), IRR1 introgressed lines- IR65482-7-216-1-2, IR7103-121-15-B, and a breeding line RP2068-13-5-8 for validating the presence of genes *Bph3*, *BPH18*, *Bph20*, and *Bph21* using SSR markers. Parental polymorphism was shown for markers specific to *Bph3*, *BPH18*, and *Bph20*. Besides this, cluster analysis using the UPGMA method produced 4 clusters. The variety 'PTB33' was obtained in a separate cluster which can be attributed to its resistant character against BPH and can be considered as a potential donor. The IRR1 introgressed lines- IR65482-7-216-1-2, IR7103-121-15-B, and the breeding line RP2068-13-5-8 can also be well utilized as donors by proper standardized BPH screening tests for confirming the phenotypic expression of BPH resistance.

Keywords: *Brown Planthopper, Gene validation, Insect resistance, Polymorphism, Rice*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the primary source of food for more than half of the world's population and plays a significant role in the food security of Asia where more than 90 percent of the rice is produced [1]. Among the major cereal crops grown in India, rice alone contributes to 44.11 million hectares and produced 112.91 million tonnes during 2017-18 [2]. Rice encounters many biotic and abiotic constraints in production and productivity. Insects are the most important biotic stress causing significant yield loss. Among the insect pests, the brown plant hopper (*Nilaparvata lugens* Stal.) is a typical phloem sap-feeding pest of rice resulting in "hopper-burn" symptoms and its role as a vector of rice grassy stunt virus and ragged stunt virus have aggravated the posed by the insect. It accounts for a 20% to 80% yield loss in rice with an overall annual economic loss of around \$300 million in Asia [3].

Pest management strategies in managing BPH include chemical control, improving field practices, and developing resistant varieties. However excessive use of chemicals for BPH management is hazardous to human health, cost-ineffective, and unsafe for the environment and biodiversity. Alternatively, the most durable, precise, cost-effective, and environment-friendly strategy is to develop a sustainable host-plant resistance mechanism for BPH management. The quick evolution of BPH poses a primary concern. Hence, there is a need to attain durable and broad-spectrum resistance by the introgression of novel genes to build resistance against BPH [4]. Four biotypes of BPH have been recognized, in which biotypes 1 and 2 are the most predominant in East and Southeast Asia [5], biotype 3 originated in the laboratory from rearing the insect on a resistant variety [6], and biotype 4 in South Asia especially Indian sub-continent [7].

The first ever BPH-resistant gene, *bph1* was introgressed to the *indica* rice variety Mudgo, but the selection pressure on this gene leads to the involvement of new pathotypes in BPH which could tolerate the effect of *bph1* [8]. Accordingly, more than 38 major BPH resistance genes have been reported in several *Oryza sativa* sp. *indica* cultivars and wild relatives [9]. The genetics of BPH resistance revealed the presence of 32 major genes (*Bph1* to *Bph32*) in cultivated rice and seven wild relatives [10]. Whereas, cloning and characterization of nine potential genes (*Bph3/Bph17*, *Bph6*, *BPH9*, *Bph14*, *Bph15*, *BPH18*, *BPH26*, *BPH29*, and *Bph32*) were carried out to date [11,12,13].

Rice variety 'IR62' was observed with consistently high resistance to plant hoppers from screening in South and South East Asia [14] and was due to the genes for BPH resistance derived from PTB33, which is a popular source of resistance in modern rice breeding programs [15]. [16]

Introgressed BPH-resistant genes *Bph20* and *Bph21* into an elite variety CO43Sub1 using IR71033-121-15 as a donor and two introgressed lines 32-4-34 and 32-4-35 showed resistance to BPH population.

Identification of genes for BPH resistance from different rice cultivars, the study of resistant mechanisms, and the introgression of resistant genes to elite well-adapted but susceptible rice varieties become needful for sustainable economic yield under the BPH outbreak. Advancement in molecular genetics plays an important role in the search and confirmation of resistant genes from various genotypes and helps in efficient and fast insect-resistant breeding methods. Molecular markers are employed for the selection of plants with desired genomic regions that control the expression of the trait of interest. Marker-assisted selection in plant breeding enables the selection of individual plants based on their genotype. Selection of parents in backcross breeding programmes and other breeding techniques is more efficiently done with the aid of molecular markers reducing the cost, time, and resources. Screening of rice varieties and validating the presence or absence of genes of interest is essential for the selection of donor and recipient parents in breeding programmes for the development of resistant varieties.

So that the present study was undertaken to validate the presence or absence of four genes that impart BPH resistance (*Bph3*, *BPH18*, *Bph20*, and *Bph21*) in five genotypes of rice varieties to identify the best variety with multiple resistant genes, that could be used as a donor for marker-assisted gene pyramiding breeding programs and confirmation of the absence of the genes in Jyothi variety which is high yielding but susceptible to be used as recipient parent.

2. MATERIALS AND METHODS

2.1. Plant materials

The high-yielding rice variety in Kerala namely Jyothi, PTB33 (Variety released from RARS, Pattambi), IRRI introgressed lines- IR65482-7-216-1-2, IR7103-121-15-B, and a breeding line RP2068-13-5-8 were screened for validating the presence of genes *Bph3*, *BPH18*, *Bph20*, and *Bph21*. The details of the markers used in the study are listed in Table 1.

Table 1. List of SSR markers used for gene validation

2.2. Genomic DNA extraction and quantification

Genomic DNA was isolated using the CTAB method [17]. About 100 mg of each leaf sample was

Gene	Marker	Sequence (5'-3')	Annealing temp. (° c)	References
<i>Bph 3</i>	RM589	F-GAGGTTGTTTGGATGGATAGATGG R- AATCCCGTCCTAGAGTTCTTCTACC	55.3	[22]
<i>Bph 18</i>	RM3331	F-CCTCCTCCATGAGCTAATGC R-AGGAGGAGCGGATTTCTCTC	55.3	[26]
<i>Bph 20</i>	RM8213	F-TGTTGGGTGGGTAAAGTAGATGCTG R-CCCAGTGATACAAAGATGAGTTGG	55.3	[16]
<i>Bph 21</i>	RM28561	F-CTTCAAGACTGGCCCAATATTACTGC R-TGACTGAAGCCTTCACTTGC	55.3	[26]

ground using a pestle and mortar with liquid nitrogen (-196°C), and 400 µL CTAB extraction buffer (2% CTAB, 100 mM Tris-HCL, 20 mM EDTA, 1.4 M NaCl, 1% PVP) was added, and the solution was transferred into a 2 ml microcentrifuge tube. The tubes were kept in the water bath at 60°C for 1 hr with manual shaking every 10 minutes. After that, the plant debris was removed by centrifuge (14,000 rpm, 10 minutes), and the supernatant was collected in a fresh centrifuge tube and 400 µl mixture, the ratio of phenol: chloroform: isoamyl alcohol (25:24:1%), was added. The mixtures were then well mixed by inverting and centrifuge (14,000 rpm, 5 minutes) at 4°C. The supernatant was then transferred into a fresh microcentrifuge tube and an equal volume of isopropanol was added. The mixtures were gently mixed by inverting the microcentrifuge tube and then incubated at -20°C overnight. Following, the next day the mixture was centrifuged again (14,000 rpm, 5 minutes) at 4°C.

The supernatant was then discarded and the pellets were washed with 70% alcohol two times. Subsequently, pellets were air-dried and re-suspended in 80 µl of TE buffer, and stored at -20°C. Agarose gel electrophoresis was carried out to resolve the genomic DNA in a horizontal gel electrophoresis Unit of BIO-SYS. An amount of 0.8% agarose was used for quantifying the genomic DNA in 0.5X TBE buffer. The gel was visualized under UV light using the SYNGENE gel documentation system in order to check the intactness and shearing of DNA and RNA contamination.

2.3. Gene validation using SSR markers

The genomic DNA of five rice varieties was amplified using four SSR markers. The reaction was executed in a 25 µl reaction mixture consisting of 20ng template DNA, 2 µl of dNTP mix, 2.5 µl of PCR buffer, 2.5 µl of MgCl₂ and 0.3 µl Taq DNA polymerase, and 1 µl of both forward and reverse primers. Amplification compassed in an Eppendorf master cycler nexus PCR. Following PCR conditions were used: An initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94 ° C for 30 seconds, the annealing temperature of 55.3 ° C for 30 seconds, primer extension at 72 ° for 1 minute, and final extension at 72°C for 8 minutes. The amplified DNA was separated on 4% metaphor-agarose gels, visualized by staining with ethidium bromide, and viewed under UV light using the SYNGENE gel documentation system and photographed.

2.4. Data analysis

The presence and absence of alleles were scored using the binary system '1' and '0' respectively. A genetic similarity matrix was constructed using Jaccard's coefficient method [18]. The distance matrix was subjected to cluster analysis employing UPGMA (unweighted paired group method with arithmetic averages) using the SAHN (Sequential Agglomerative Hierarchical and nested Cluster) module of the software NTSYS PC [19].

3. RESULTS AND DISCUSSION

The rice varieties Jyothi, PTB33, IRRI introgression lines IR65482-7-216-1-2, IR7103-121-15-B, and a breeding line RP2068-13-5-8 were validated for the presence or absence of BPH-resistant genes *Bph3*, *BPH18*, *Bph20*, and *Bph21* using SSR markers RM589, RM3331, RM8213, and RM28561. All the markers were amplified at an annealing temperature of 55.3°C (Fig. 1).

Jyothi (PTB 39) is the most widely accepted and popular high-yielding rice variety of Kerala released from RARS, Pattambi. At present, the variety is susceptible to brown plant hopper and was considered as a susceptible parent in the study. Parental polymorphic studies using the SSR marker 'RM589' which is linked with the *Bph3* gene, produced five alleles. The obtained band size varied from 180bp in Jyothi (susceptible parent) to 220bp in the breeding line 'RP2068-13-5-8'. The resistant donor variety PTB33 produced a band at 200bp (Table 2). Being resistant to all known biotypes of BPH, PTB33 can be used as a donor parent for BPH resistance [20] [21]. [22] Mapped *Bph3* using markers RM589 and RM588 on the short arm of chromosome 6. A three-gene encoded plasma membrane-localized lectin receptor kinases were identified as a cluster in the *Bph3* gene conferring durable, broad-spectrum resistance against BPH. Also, enhanced resistance to BPH was obtained by the introgression of *Bph3* into susceptible varieties [23].

The SSR marker 'RM3331' was used to detect the polymorphism between parental lines for the presence of the *Bph18* gene and produced five alleles with the band size of the screened varieties ranging from 110bp to 150bp (Table 2). The IRRI introgression line 'IR65482-7-216-1-2' derived from wild species *Oryza australiensis* showed a band size of 110bp and the susceptible parent 'Jyothi' produced a band of 130bp. The *Bph18* was mapped [4] on the sub-terminal region of the long arm of chromosome 12. A novel type of coiled-coil nucleotide-binding site and leucine-rich repeat motif (CC-NBS-NBS-LRR) in the introgression line 'IR65482-7-216-1-2' confers the resistance against BPH [24]. Being an indica donor line, it can be efficiently used to transfer the *Bph18* to other indica cultivars with reduced linkage drag.

The IRRI introgression line 'IR7103-121-15-B' derived from an interspecific cross between IR31917-45-3-2 and a wild species *Oryza minuta*, mapped with two genes *Bph20* and *Bph21* imparts resistance to BPH biotypes [25]. The 'RM8213' marker was employed in screening the rice varieties for the gene *Bph20* producing four alleles and the band size ranged from 110-140bp (Table 2). A band size of 130bp and 140bp was produced by the susceptible parent 'Jyothi' produced and the introgression line 'IR7103-121-15-B' respectively. The *Bph21* gene was screened using the marker 'RM28561' which produced only two alleles among the varieties screened. The band size obtained

was 290bp in PTB33 and 280bp in Jyothi, PTB33, IRRI introgression lines IR65482-7-216-1-2, IR7103-121-15-B, and a breeding line RP2068-13-5-8 (Table 2). Monomorphic bands were obtained in the susceptible variety Jyothi and the resistant variety IR7103-121-15-B using the marker 'RM28561'.

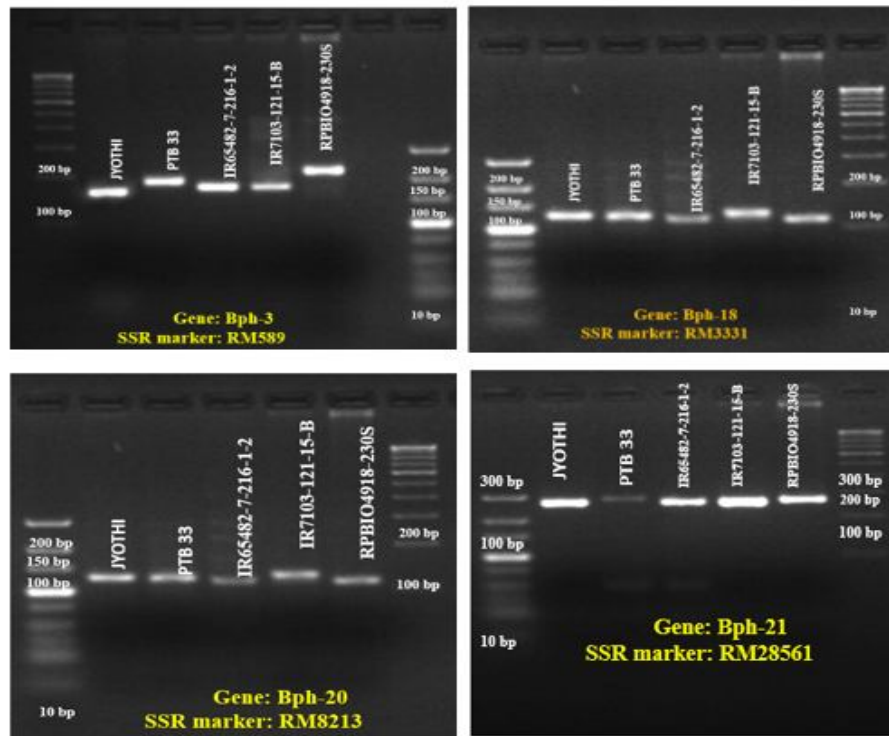


Fig 1. The banding pattern of SSR makers in rice varieties

Table 2. Details of marker amplification in rice varieties

Marker	Genotypes	bp	Number of alleles
RM589	Jyothi	180	5
	PTB33	200	
	IR65482-7-216-1-2	195	
	IR7103-121-15-B	190	
	RP2068-13-5-8	220	
RM3331	Jyothi	130	5
	PTB33	125	
	IR65482-7-216-1-2	110	
	IR7103-121-15-B	150	
	RP2068-13-5-8	120	
RM8213	Jyothi	130	4
	PTB33	120	
	IR65482-7-216-1-2	110	
	IR7103-121-15-B	140	
	RP2068-13-5-8	110	
RM28561	Jyothi	280	2
	PTB33	290	
	IR65482-7-216-1-2	280	
	IR7103-121-15-B	280	
	RP2068-13-5-8	280	

The similarity coefficient as shown in the dendrogram varied from 0.53 to 0.68 (Fig. 2). Dendrogram reflects the similarity among the genotypes using seriation methods with most similar

objects placed together [27]. All the genotypes used for the study were grouped into 4 clusters at 0.64 similarity coefficient. Group 1 consists of the genotypes Jyothi and RP 2068-13-5-8 and groups 2, 3, and 4 consist of single genotypes namely IR 65482-7-216-1, IR 7103-121-15-B, and PTB33 respectively. Clustering analysis reveals the similarity between the genotypes evaluated for a number of traits [28]. Each genotype showed dissimilarity with the variety Jyothi in terms of BPH resistance and carrying the resistant gene. The genotypes, IR 7103-121-15-B and IR 65482-7-216-1 were grouped into separate clusters at 0.56 and 0.63 similarity coefficients respectively. The rice variety 'PTB33' which is resistant to almost all biotypes of BPH was placed in a separate cluster from 0.53 similarity coefficient onwards. This revealed the distinct nature of the variety 'PTB33' in imparting resistance to BPH which can be screened using gene-specific markers making it suitable to be used as a potential donor. Cluster 1 consists of two genotypes, Jyothi (susceptible variety) and RP2068-13-5-8 (breeding line with BPH resistance) may be inferred as there exists some extent of genetic similarity between the two genotypes but the latter exhibited significant resistance in BPH screening tests [29] suggesting that it is phenotypically different. Varieties that show genotypic similarity may differ phenotypically depending on environmental and geographical conditions as reported [30] [31].

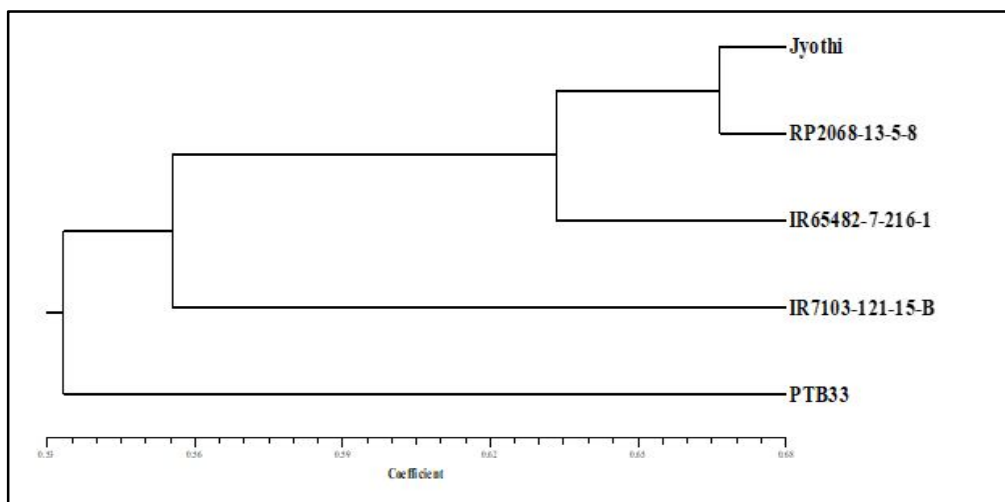


Fig.2 Cluster diagram using the UPGMA method showing the relationship among the rice varieties

4. CONCLUSION

India is home to the majority of BPH-resistant sources, especially landraces. Genetic studies in rice varieties had revealed valuable sources of BPH resistance. With the current improvements in plant molecular biology, it is now easier to identify the individuals with the gene of interest using molecular markers and incorporate it into the susceptible cultivars through marker-assisted backcrossing. It is important to detect parental polymorphism prior to the initiation of any breeding programme. Among the molecular markers, simple sequence repeats (SSRs) are widely employed in detecting polymorphism among parental lines for genes specific to BPH resistance. In this study, SSR markers were used for screening rice parental genotypes for validating the presence of genes in genotypes considered donor parents and the absence of genes in the susceptible parent 'Jyothi'. The varieties PTB33, IRRI introgression lines IR65482-7-216-1-2, IR7103-121-15-B showed polymorphism for the markers specific to the genes *Bph3*, *BPH18*, and *Bph20*. These genotypes may be effectively used in marker-assisted backcrossing as donor parents to transfer the genes for resistance to the high-yielding but susceptible variety 'Jyothi'.

ACKNOWLEDGEMENTS

We are thankful to Kerala Agricultural University, Thrissur for providing research funding and the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, for providing laboratory facilities for the research work.

COMPETING INTERESTS

The authors have declared that no competing interests exist

AUTHORS' CONTRIBUTIONS

Arun Chacko conducted the experiment, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Jayalekshmy, V.G. designed the study and provided scientific advice, Karthikeyan K. provided genetic material for the study, Beena Thomas and Shanas, S. provided scientific advice, and Shahiba, A.M. managed literature searches and manuscript editing. All authors read and approved the final manuscript.

REFERENCES

1. Khush, GS. What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, 2005; 59:1-6.
2. GOI [Government of India]. 2018. Year End Review [on-line]. 2018; Accessed 26 December 2018. Available: <https://pib.gov.in/newsite/PrintRelease.aspx?relid=186796>.
3. Min S, Lee SW, Choi BR, Lee SH, Kwon DH. Insecticide resistance monitoring and correlation analysis to select appropriate insecticides against *Nilaparvata lugens* (Stål), a migratory pest in Korea. *Journal of Asia-Pacific Entomology*, 2014;17(4): 711-716.
4. Jena KK, Jeung JU, Lee JH, Choi HC, Brar DS. High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18(t)*, and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 2006;112:288-297.
5. Brar DS, Virk PS, Jena KK, Khush GS. Breeding for resistance to planthoppers in rice. In: *Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia*. Los Banos, the Philippines: International Rice Research Institute: 2009;401-427.
6. 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):1204-1214.
7. Jing, S L, Zhang L, Ma YH, Liu BF, Zhao Y, Yu HJ, Zhou X, Qin R, Zhu LL, He GC. Genome-wide mapping of virulence in brown planthopper identifies loci that break down host plant resistance. *PLoS One*, 2014; 9(6): e98911.
8. Myint KKM, Yasui H, Takagi M, Matsumura M. Virulence of long-term laboratory populations of the brown planthopper, *Nilaparvata lugens* (Stål), and whitebacked planthopper, *Sogatella furcifera* (Horváth)(Homoptera: Delphacidae), on rice differential varieties. *Applied Entomology and Zoology*, 2009; 44(1): 149-153.
9. Badhini P, Ulaganathan K. Identification and introgression of *bph3* resistance gene into rice elite lines. *Plant Cell Biotechnology and Molecular Biology*. 2019; 20(21-22):1009-1019.
10. Sundaram RM, Madhav MS, Neeraja CN, Balachandran SM, Mangrauthia SK, Barbadikar KB, Divya PS, Rao LV, Hariprasad AS. Multi-trait improvement in rice through marker-assisted breeding. *ORYZA-An International Journal on Rice*, 2018; 55:24-37.
11. Muduli L, Pradhan SK, Mishra A, Bastia DN, Samal KC, Agrawal PK, Dash M. Understanding brown planthopper resistance in Rice: Genetics, biochemical and molecular breeding approaches. *Rice Science*, 2021; 28(6):532-546.

12. Alam SN, Cohen MB. Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. *Theoretical and Applied Genetics*, 1998; 97(8), p.1370.
13. Strauss SY, Agrawal AA. The ecology and evolution of plant tolerance to herbivory. *Trends in ecology & evolution*, 1999;14(5): 179-185.
14. Horgan FG, Ramal AF, Bentur JS, Kumar R, Bhanu KV, Sarao PS, Iswanto EH, Van Chien H, Phyu MH, Bernal CC, Almazan MLP. Virulence of brown planthopper (*Nilaparvata lugens*) populations from South and South East Asia against resistant rice varieties. *Crop Protection* 2015;78:222-231.
15. Ren J, Gao F, Wu X, Lu X, Zeng L, Lv J, Su X, Luo H, Ren G. Bph32, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. *Scientific Reports* 2016;6:37645.
16. Mohanapriya B, Jeyaprakash P, Raveendran M, Soundararajan RP, Ramchander S, Subashini G, Prabhakaran P, Manonmani K, Robin S. Marker assisted introgression for brown planthopper resistance genes Bph20 and Bph21 in CO43Sub1 variety of rice. *Electronic Journal of Plant Breeding* 2019;10(2):645-652.
17. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. *Focus*, 1990; 12,13-15.
18. Jaccard P. Nouvelles recherches sur la la distribution florale. *Bull. Soc. Vandoise des Sci. Naturelles* 1908;44: 223-270
19. Rohlf RJ. NTSYS-PC Version 2.0, Exeter Software, Setauket, New York. 1998.
20. Akanksha S, Lakshmi VJ, Singh AK, Deepthi Y, Chirutkar PM, Ramdeen, Ram T. Genetics of novel brown planthopper *Nilaparvata lugens* (Stål) resistance genes in derived introgression lines from the interspecific cross *O. sativa* var. *Swarna* × *O. nivara*. *Journal of Genetics* 2019; 98:1-10.
21. Nguyen CD, Zheng SH, 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.
22. Jairin J, Phengrat K, Teangdeerith S, Vanavichit A, Toojinda T. Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. *Molecular Breeding*, 2007; 19:35–44.
23. Liu Y, Wu H, Chen H, Liu Y, He J, Kang H, Sun Z, Pan G, Wang Q, Hu J, Zhou F. A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nature biotechnology*, 2015;33(3): 301-305.
24. Ji H, Kim SR, Kim YH, Suh JP, Park HM, Sreenivasulu N, Misra G, Kim SM, Hechanova SL, Kim H, Lee GS. Map-based cloning and characterization of the BPH18 gene from wild rice conferring resistance to brown planthopper (BPH) insect pest. *Scientific Reports*, 2016;6(1):1-14.
25. Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam MS, Chin JH, Jeung JU, Brar, DS. High-resolution mapping of two rice brown planthopper resistance genes, Bph20 (t) and Bph21 (t), originating from *Oryza minuta*. *Theoretical and Applied Genetics*, 2009;119: 1237-1246.
26. Jiang H, Hu J, Li Z, Liu J, Gao G, Zhang Q, Xiao J, He Y. Evaluation and breeding application of six brown planthopper resistance genes in rice maintainer line Jin 23B. *Rice*, 2018;11(1):1-11.

27. Arief VN, DeLacy IH, Basford KE, Dieters MJ. Application of a dendrogram seriation algorithm to extract pattern from plant breeding data. *Euphytica*, 2017;213:1-11.
28. Shahiba AM, Jayalekshmy VG, Sajeena A, Thomas B, Chacko A. Molecular Characterization of Fusarium and Screening of Cowpea [*Vigna unguiculata* (L.) Walp.] Germplasms against Fusarium Wilt (*Fusarium oxysporum* f. sp. *Tracheiphilum*). *Legume Research*. 2022; DOI: 10.18805/LR-5015.
29. Naik SB, Divya D, Sahu N, Sundaram RM, Sarao PS, Singh K, Lakshmi VJ, Bentur JS. A new gene Bph33 (t) conferring resistance to brown planthopper (BPH), *Nilaparvata lugens* (Stål) in rice line RP2068-18-3-5. *Euphytica*, 2018; 214;1-12.
30. Muduli L, Dash M, Das Mohapatra S, Mohapatra KK, Nayak HS, Bastia DN, Pradhan B, Tripathy SK, Jena RC, Pradhan SK. Phenotypic and genotypic assessment of elite rice varieties for brown plant hopper (*Nilaparvata lugens* Stål.) resistance. *Cereal Research Communications*, 2023; 1-13.
31. George J, Jayalekshmy VG, Shahiba AM, Chacko A, Lachyan TS. Physical and nutrient characterization of the grains from traditional rice varieties of Kerala and validation of the trait-linked molecular markers. *ORYZA-An International Journal on Rice*. 2021; 58(3):394-408.