Uttar Pradesh Journal of Zoology

Volume 45, Issue 16, Page 1-7, 2024; Article no.UPJOZ.3803
ISSN: 0256-971X (P)

Genetic Validation of Advanced Backcross-derived Rice Lines for *Nilaparvata lugens* (Stal) Resistance via SSR Markers

R. Prathima Kumari [a*], Ch. Anusha [b++], M. A. Prajwal Gowda [a], D. Akhilandeshwari [a], R. Shravan Kumar [c#], K. Rajendra Prasad [d++] and Y. Hari [e†]

[a] *Department of Entomology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, India.*
[b] *Department of Entomology, Agricultural College, Warangal, PJTSAU, India.*
[c] *Regional Agricultural Research Station, Warangal, PJTSAU, India.*
[d] *Department of Genetics and Plant Breeding, Agricultural College, Warangal, PJTSAU, India.*
[e] *Department of Biotechnology, Regional Agricultural Research Station, Warangal, PJTSAU, India.*

Authors' contributions

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

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i164281

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://prh.mbimph.com/review-history/3803

Original Research Article

Received: 17/05/2024
Accepted: 20/07/2024
Published: 23/07/2024

[++] *Assistant Professor;*
[#] *Scientist, Rice Section;*
[†] *Scientist;*
**Corresponding author: Email: prazzzugowdru@gmail.com;*

Cite as: *Kumari, R. Prathima, Ch. Anusha, M. A. Prajwal Gowda, D. Akhilandeshwari, R. Shravan Kumar, K. Rajendra Prasad, and Y. Hari. 2024. "Genetic Validation of Advanced Backcross-Derived Rice Lines for Nilaparvata Lugens (Stal) Resistance via SSR Markers". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (16):1-7. https://doi.org/10.56557/upjoz/2024/v45i164281.*

ABSTRACT

Nilaparvata lugens (Stål), referred to as the brown planthopper (BPH), is a serious threat to paddy cultivation. It inflicts damage by feeding on rice plants and serves as a vector for the rice ragged stunt virus and the rice grassy stunt virus, both of which contribute to significant yield losses. The research was conducted during *kharif*-2021 at Regional Agricultural Research Station (RARS), Warangal. SSR markers RM 3766 (for the Bph3 gene) and RM 586 (for the Bph4 gene) were used for molecular confirmation of resistance in BPH. The study revealed the presence of resistance genes in all resistant and moderately resistant lines, except Siddhi-BC2F6 BPH-BL-24, which was heterozygous (partial resistance) for Bph3, and Siddhi-BC2F6 BPH-BL-11 and Siddhi-BC2F6 BPH-BL-52, which were heterozygous (partial resistance) for Bph4. This genetic validation using SSR markers not only confirms the successful introgression of resistance traits but also enhances the efficiency of breeding programs aimed at developing durable BPH-resistant rice cultivars.

Keywords: BPH; resistance; SSR markers.

1. INTRODUCTION

Oryza sativa, commonly known as rice, plays a key role in global food security by serving as a major staple crop [1]. With cultivation spanning 114 countries, it represents the principal economic support for over 100 million households in Asia and Africa. Rice, part of the Gramineae family, has a genome size of 430 MB. It is grown worldwide on 162.06 million hectares, producing 500 million metric tons with an average yield of 5.0 tons per hectare [2]. After China, India is the largest producer of rice. According to USDA [3], in India, rice is grown over an area of 45,500 thousand hectares, yielding an annual production of 125,000 thousand metric tons with an average productivity of 4.1 metric tons per hectare.

Biotic stresses lead to a loss of around 52% of the world's rice production, and insect pest attacks are responsible for 25% of these losses [4]. Atwal and Dhaliwal [5] stated that out of more than 100 insect species that target rice, 20 are identified as major pests. Out of those BPH, *Nilaparvata lugens* (Homoptera: Delphacidae), is one of the devastating pest affecting rice in Asia which is known for feeding on phloem sap [6]. Nymphs and adults suck sap from leaves and leaf sheaths causing leaves to turn yellow, less tillering, shorter plant stature, and more of chaffy grains. BPH infestation decreases chlorophyll and protein levels in leaves. Severe infestation of BPH can result in symptoms known as 'hopper burn' [7]. As of now, 38 resistance loci against BPH have been mapped from both cultivated and wild rice species. The Bph3 encodes a cluster of three Lectin Receptor Kinase genes (OsLecRK1-3) that confer broad-spectrum resistance [8]. SSR markers help identify and select resistant genes efficiently, accelerating breeding efforts towards developing pest-resistant rice varieties. Farmers commonly resort to chemical pesticides to manage insect attacks, despite their high cost and detrimental impact on the ecosystem. Indiscriminate usage of pesticides poses resistance and resurgence problems in BPH populations [9]. Hence, the present work has been aimed at exploring the genotypic resistance of various backcross-derived rice lines against *N. lugens.*

2. MATERIALS AND METHODS

2.1 Experimental Site & Setup

In the *kharif* season of 2021, this research was carried out at the RARS in Warangal. TN1 and BM71 were used as the susceptible and resistant checks, respectively. The promising lines found in phenotypic screening were selected for molecular studies to know the presence or absence of Bph3 and bph4 genes [10]. Two SSR markers RM 3766 (Bph3 gene) and RM 586 (bph4 gene) were used for molecular confirmation of test lines.

DNA isolation: Leaf samples were collected from 10-15 days-old test seedlings during the morning hours and freezed in liquid nitrogen, at -80^{0} C for further use. Plant genomic DNA of promising lines were extracted from leaf tissue along with checks by CTAB (Cetyl-Tetra Methyl Ammonium Bromide) method [11].

2.2 Tools & Components used for Isolation of DNA

1. Micro pestle 2. Micro centrifuge tubes 3. Holding stands 4. Water bath 5. 1000 µl, 200 µl and 20 µl micropipettes 6. 1000 µl, 200 µl and 20 µl tips 7. Micro centrifuge 8. Freezer (-80°C).

2.3 Preparation of Chemicals- stock Solutions for DNA Extraction

a) *TE buffer:* 1M Tris HCl (pH 8.0), 1.0 ml 0.5M EDTA and 0.2 ml. Final volume was made to 100 ml with appropriate amount of sterile water and autoclaved.

b) *EDTA (1M pH 8.0):* Disodium EDTA (disodium ethylene diamine tetra acetic acid, MW 372.2 g mole[1]) of 37.22 g was dissolved in 80 ml of distilled water. The pH was set to 8.0 by using concentrated sodium hydroxide (NaOH) and final volume was adjusted to 100 ml with distilled water and sterilized by autoclave.

c) *5M NaCl:* 23.36 g of NaCl was dissolved in 80 ml of distilled water. Final volume is adjusted to 100 ml and sterilized using autoclave.

d) *1M Tris HCl (pH 8.0 at 25 °C):* Tris base of 121.1 g was dissolved in 800 ml of distilled water. The pH was set to 8.0 using concentrated HCl. Before making a final adjustment of pH 8.0, the solution was allowed to cool at room temperature. The final volume was adjusted to 1000 ml with distilled water and sterilized by autoclaving.

e) *70% Ethanol:* 70 ml of absolute ethanol is added to double distilled water (30 ml) to make 100ml.

2.4 Procedure of DNA Isolation

1) About 5-10 cm of leaf sample was taken, cut it into small pieces and placed in the eppendorf tubes with an iron ball. 200 µl of CTAB buffer (Table 1) was added and placed in tissue lyser for 3 min.

2) Later 400 µl of CTAB buffer and 600 µl of chloroform was added and mixed well by inversion for about 5 min and centrifuged at 13000 rpm for 15min at room temperature. 3) Then 300-400 µl of the supernatant was transferred in to a fresh centrifuge tube without disturbing the intermediate layer of insoluble proteins. 4) Then equal volume of chilled isopropanol was added to the supernatant and kept undisturbed for overnight. The contents were mixed and centrifuged at 10000 rpm for 10 min at room temperature. 5) The supernatant was discarded and the DNA pellet was washed with 200 µl of 70% ethanol by tapping gently so that the pellet was not-disturbed. The contents were centrifuged at 7000 rpm for 7 minutes at room temperature.

6) The supernatant was discarded and the DNA pellet was air-dried at room temperature for 2-3 hours.

7) The DNA pellet was dissolved in 50-100 µl of TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH 8.0) prepared using sterile double distilled water. The DNA solution was then stored at 4°C for further analysis.

2.5 Quantification of DNA

The purity and concentration of isolated genomic DNA was estimated by Nanodrop method by running 0.8% agarose gel electrophoresis unit.

2.6 Running 0.8% Agarose Gel Electrophoresis

- 0.3g of agarose was weighed and added into a 250 ml conical flask. 30 ml of 0.5X TBE was added to a 250 ml beaker and boiled gently in a microwave oven. 10X TBE was used for preparation of 0.5X TBE, which was prepared by diluting 10X TBE twenty times with sterile distilled water.
- When the boiled agarose cooled down (to about 45°C), 2 µl of ethidium bromide solution (10 mg/ml of distilled water) was added and mixed gently.
- The melted agarose was then slowly poured in gel casting tray and allowed to solidify (~20-30 min). Care was taken so that air bubbles are not formed in gel.
- After solidification, the gel was removed from its casting tray and put into a gel tank, which was then filled with 0.5X TBE buffer till the buffer reaches 0.5 cm above the gel surface (~100 ml). 5 µl of DNA solution was then mixed with ~1-2 µl of loading dye (0.0025% bromophenol blue in 40% sucrose), loaded into the wells and the electrodes were connected to power pack.
- DNA solutions of known concentration (50 ng/µl, 100 ng/µl, 150 ng/µl and 200 ng/µl) were also loaded along with the samples for comparison of the concentration of the DNA samples.
- The samples were then subjected to electrophoresis at constant voltage (75 V) for 20-30 minutes till the dye front reached 2/3rd of the running length of the gel. The

Table 1. Components used for CTAB (2%) extraction buffer

Component	Stock Concentration	Working concentration	Volume/quantity of stock
Tris-HCL (pH8.0)	1M	100M	10ml
EDTA (pH 8.0)	0.5M	20M	4ml
NaCl	5M	1.4M	28
CTAB	-	2%	2g
PVP	-	1%	1g
Double distilledwater Total volume		To make up the volume to 100ml	

Table 2. Master mix used for one sample in a PCR reaction

S. No	Components	Volume
1.	Master mix	
	10 × Buffer	1.0 µl
	dNTPs	0.5 µl
	Forward primer	0.5 µl
	Reverse primer	0.5 µl
	Taq Polymerase	0.5 µl
2.	Sterile distilled water	4.0 µl
3.	Template DNA	3 µl
	Total volume	**10 µl**

- gel was then visualized under UV light in gel documentation system to check the quality and quantity of DNA.
- Based on comparison with intensity of DNA solution of known concentration, the unknown samples were diluted to a final concentration of 50 ng/µl using 1X TE buffer.

2.7 Polymerase Chain Reaction (PCR) Analysis for SSRs

Procedure: PCR reactions were carried out for samples consists of 50-100 ng of template DNA, 0.5 µl of each primer, 0.5 µl of Taq polymerase, dNTPs of 0.5 µl and 1 µl 10× PCR buffer per solution to check the presence of resistance genes. The SSR markers were used for assessing the polymorphism between the donor and recipient parents following PCR conditions described by Chen et al. [12]. The PCR components were mixed as given below to give a final reaction volume of 10 µl per sample shown in Table 2. The PCR tubes were then set up in a programmable thermal cycler for DNA amplification.

2.8 Agarose Gel Electrophoresis

- PCR samples were prepared for loading by mixing with 3x loading dye and loaded in the wells.
- Electrophoresis was carried out in 0.5X tetra acetic acid (TAE) buffer, at 150 volts for 2 hours. For sizing, the fragments 100bp ladder was run along with the samples.

2.9 Gel Documentation

Gels were photographed in gel documentation system and polymorphic marker scoring was done manually. To do the profiling for each BPH resistant gene, known resistant source (BM 71) was used as positive control, whereas susceptible backcross derived line TN1 was used as negative control. Scoring was done based on the presence of amplicon similar to resistant or susceptible type.

3. RESULTS AND DISCUSSION

Molecular confirmation was done for the presence of Bph3 and Bph4 genes for the lines which were found phenotypically resistant (three Siddhi backcross derived lines were found to be resistant and nine Siddhi backcross derived lines were found to be moderately resistant). Two Simple Sequence Repeats (SSR) markers viz., RM 3766 and RM 586 linked to each BPH genes Bph3 and Bph4 were used. Earlier, SSR markers have been extensively used for molecular confirmation of presence or absence of pests & disease resistance genes [13,14,15].

Table 3. Phenotypic and genotypic reaction of resistant and moderately resistantlines of Siddhi

S. No	Backcross derived lines	Phenotypic reaction	Bph3	Bph4	Remarks
1.	Siddhi-BC_2F_6 BPH-BL-11	MR	RR	Rr	MR
2.	Siddhi-BC_2F_6 BPH-BL-12	MR	RR	RR	MR
3.	Siddhi-BC_2F_6 BPH-BL-19	MR	RR	RR	MR
4.	Siddhi-BC_2F_6 BPH-BL-24	MR	Rr	RR	MR
5.	Siddhi-BC_2F_6 BPH-BL-30	R	RR	RR	R
6.	Siddhi-BC_2F_6 BPH-BL-43	R	RR	RR	R
7.	Siddhi-BC_2F_6 BPH-BL-52	MR	RR	Rr	MR
8.	Siddhi-BC_2F_6 BPH-BL-56	MR	RR	RR	MR
9.	Siddhi-BC_2F_6 BPH-BL-57	MR	RR	RR	MR
10.	Siddhi-BC_2F_6 BPH-BL-60	MR	RR	RR	MR
11.	Siddhi-BC_2F_6 BPH-BL-61	MR	RR	RR	MR
12.	Siddhi-BC_2F_6 BPH-BL-64	R	RR	RR	R
13.	TN1(S)	S	rr	rr	S
14.	BM71(R)	R	RR	RR	R

RR= Resistant; rr= susceptible; Rr= Partial resistance; R= Resistant; MR= Moderatelyresistant; S= Susceptible

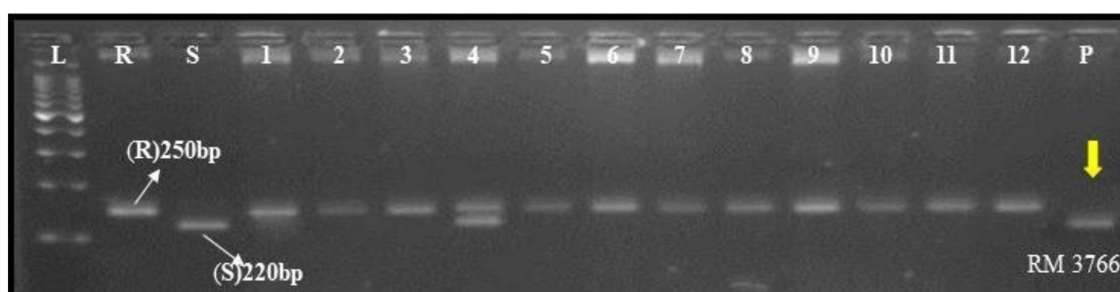

Fig. 1. PCR analysis of resistant backcross derived lines for *Bph3* gene using RM 3766 marker

L= DNA Ladder (100bp), R= Resistant (BM71), S= Susceptible (TN1), 1-12 test lines: 1. Siddhi-BC_2F_6 BPH-BL-11, 2. Siddhi-BC_2F_6 BPH-BL-12, 3. Siddhi-BC_2F_6 BPH-BL- 19, 4. Siddhi-BC_2F_6 BPH-BL-24, 5. Siddhi-BC_2F_6 BPH-BL-30, 6. Siddhi-BC_2F_6 BPH- BL-43, 7. Siddhi-BC_2F_6 BPH-BL-52, 8. Siddhi-BC_2F_6 BPH-BL-56, 9. Siddhi-BC_2F_6 BPH-BL-57, 10. Siddhi-BC_2F_6 BPH-BL-60, 11. Siddhi-BC_2F_6 BPH-BL-61, 12. Siddhi- BC_2F_6 BPH-BL-64, P= Siddhi. Yellow arrow indicates parent line Siddhi

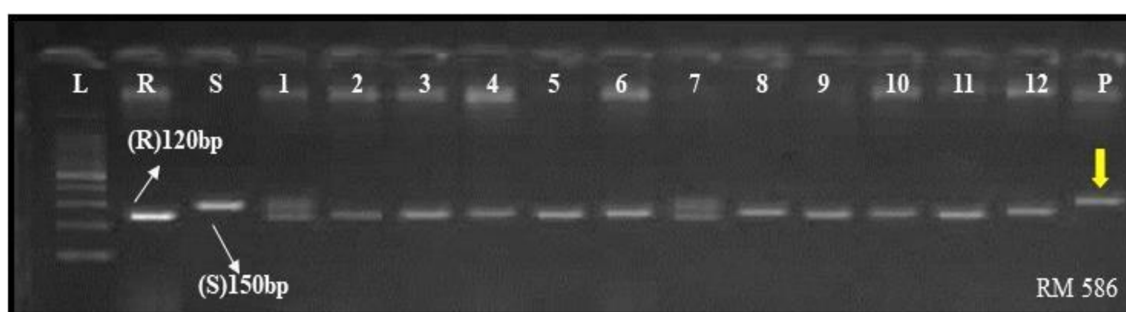

Fig. 2. PCR analysis of resistant backcross derived lines for *Bph4* gene using RM 586 marker

L= DNA Ladder (100bp), R= Resistant (BM71), S= Susceptible (TN1), 1-12 test lines: 1. Siddhi-BC_2F_6 BPH-BL-11, 2. Siddhi-BC_2F_6 BPH-BL-12, 3. Siddhi-BC_2F_6 BPH-BL- 19, 4. Siddhi-BC_2F_6 BPH-BL-24, 5. Siddhi-BC_2F_6 BPH-BL-30, 6. Siddhi-BC_2F_6 BPH- BL-43, 7. Siddhi-BC_2F_6 BPH-BL-52, 8. Siddhi-BC_2F_6 BPH-BL-56, 9. Siddhi-BC_2F_6 BPH-BL-57, 10. Siddhi-BC_2F_6 BPH-BL-60, 11. Siddhi-BC_2F_6 BPH-BL-61, 12. Siddhi- BC_2F_6 BPH-BL-64, P= Siddhi. Yellow arrow indicates parent line Siddhi

Molecular confirmation for Bph3 gene: In the present study, RM 3766 marker was used to know the presence or absence Bph3 gene. Out of 12 advanced backcross derived rice lines, 11 backcross derived lines viz., Siddhi-BC2F6 BPH BL- 11, Siddhi-BC2F6 BPH BL-12, Siddhi-BC2F6 BPH BL-19, Siddhi-BC2F6 BPH BL-24, Siddhi-BC2F6 BPH BL-30, Siddhi-BC2F6 BPH BL-43, Siddhi-BC2F6 BPH BL-52, Siddhi-BC2F6 BPH BL-56, Siddhi-BC2F6 BPH BL-57, Siddhi-BC2F6 BPH BL-60, Siddhi-BC2F6 BPH BL-61 and Siddhi-BC2F6 BPH BL-64 were found as homozygous for resistant allele while Siddhi-BC2F6 BPH BL-24 was found as heterozygous (partial resistance) for the Bph3 gene (Table 3; Fig. 1).

Earlier, Liu et al. [16] mapped BPH resistance gene namely Bph3, in Rathu Heenati and developed a molecular marker for Bph3 and introgressed it into susceptible rice restorer backcross lines.

Molecular confirmation for Bph4 gene: In the present study, RM 586 marker was used to know the presence or absence Bph4 gene. Out of 12 advanced backcross derived lines of Siddhi,10 backcross derived lines viz., Siddhi-BC2F6 BPH BL-12, Siddhi-BC2F6 BPH BL-19, Siddhi-BC2F6 BPH BL-24, Siddhi-BC2F6 BPH BL-30, Siddhi-BC2F6 BPH BL-43, Siddhi-BC2F6 BPH BL-52, Siddhi-BC2F6 BPH BL-56, Siddhi-BC2F6 BPH BL-57, Siddhi-BC2F6 BPH BL-60, Siddhi-BC2F6 BPH BL-61 and Siddhi-BC2F6 BPH BL-64 were found homozygous for Bph4 gene, while 2 rice backcross derived lines namely Siddhi-BC2F6 BPH BL-11 and Siddhi-BC2F6 BPH BL-52 were found as heterozygous (partial resistance) for Bph4 gene (Table 3; Fig. 2).

The present results were in accordance with Sai *et al.* (2013) who screened 28 rice genotypes for BPH resistant loci spread through the genome using closely linked simple sequence repeat (SSR) markers establishing clear genotypic relationship between BPH resistance and BPH resistance linked markers. Earlier, Jairin et al. [17] stated that one SSR marker, RM586 was associated with BPH resistance and additional markers surrounding the RM586 locus were examined to define the location of Bph4. He also reported that the Bph3 and Bph4 were reported to be closely linked and have been mapped on to chromosome 6 in a region flanked by SSR markers [18].

4. CONCLUSION

Advances in plant molecular biology have made it simpler to identify individuals carrying the desired gene using molecular markers and integrate it into susceptible cultivars through marker-assisted backcrossing. Simple sequence repeats (SSRs) are a popular molecular marker employed to identify polymorphisms in parental lines specific to BPH resistance genes. Molecular studies by using two SSR markers (RM 3766 for Bph3 gene and RM 586 for Bph4 gene), revealed the presence of resistant genes in all the resistant and moderately resistant lines of Siddhi except Siddhi-BC2F6 BPH-BL-24 that had shown heterozygous (partial resistance) condition for Bph3 gene whereas Siddhi-BC2F6 BPH-BL-11 and Siddhi-BC2F6 BPH-BL-52 shown heterozygous condition (partial resistance) for Bph4 gene.

5. FUTURE SCOPE

The backcross derived lines homozygous for both Bph3 and Bph 4 genes can be further used to develop high yielding BPH resistant rice cultivars for use by farmers. These lines help in sustainable pest management by reducing the pesticide usage.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Molla KA, Karmakar S, Molla J, Bajaj P, Varshney RK, Datta SK, Datta K. Understanding sheath blight resistance in rice: The road behind and the road ahead. Plant Biotechnology Journal. 2020;18 (4):895-915.
2. Food and Agricultural organization. FAO STAT database FAO; 2021. Available:WWW.Faostat.Org
3. USDA-International Production Assessment Division; 2023.

Available:https:// ipad.fas.usda.gov/ cropexplorer/util/new_get_psd_data.aspx? regio nid=sasia

4. Bhogadhi SC, Bentur JS, Rani CVD, Thappeta G, Yamini KN, Kumar NAP, Satynarayana PV. Screening of rice genotypes for resistance to brown plant hopper biotype 4 and detection of BPH resistance genes. International Journal of Life Sciences Biotechnology and Pharma Research. 2015;4(2):90.
5. Atwal A, Dhaliwal G. Agricultural Pest of South–East Asia & Their Management; 2002.
6. Heong KL, Hardy B. (Eds.). Planthoppers: New threats to the sustainability of intensive rice production systems in Asia. Int. Rice Res. Inst; 2009.
7. Vanitha K, Suresh S, Gunathilagaraj K. Influence of brown planthopper *Nilaparvata lugens* (Stal.) feeding on nutritional biochemistry of rice plant. Oryza. 2011;48(2):2249-5266.
8. Kumar K, Kaur P, Kishore A, Vikal Y, Singh K, Neelam K. Recent advances in genomics-assisted breeding of brown planthopper (*Nilaparvata lugens*) resistance in rice (*Oryza sativa*). Plant Breeding. 2020;139(6):1052-1066.
9. Tanaka K, Endo S, Kazano H. Toxicity of insecticides to predators of rice planthoppers: Spiders, the mirid bug and the dryinid wasp. Applied Entomology and Zoology. 2000;35(1):177-187.
10. Kumari RP, Anusha C, Kumar RS, Prasad KR, Hari Y. Screening of rice genotypes for their resistance against brown plant hopper, *Nilaparvata lugens* (Stal) under Field and Glasshouse conditions; 2022.
11. Zheng K. Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genet. Newsl. 1995;12:255-258.
12. Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theoretical and applied genetics. 1997; 95:553-567.
13. Chen JW, Wang L, Pang XF, Pan QH. Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene bph19 (t). Molecular Genetics and Genomics. 2006; 275:321-329.
14. 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.
15. 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.
16. Liu Y, Wu H, Chen H, Liu Y, He J, Kang H, Wan J. A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nature Biotechnology. 2015;33(3):301-305.
17. Jairin J, Sansen K, Wongboon W, Kothcharerk J. Detection of a brown planthopper resistance gene bph4 at the same chromosomal position of Bph3 using two different genetic backgrounds of rice. Breeding Science. 2010;60(1):71-75.
18. Harini AS, Kumar SS, Balaravi P, Sharma R, Dass MA, Shenoy V. Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. African Journal of Biotechnology. 2013;12(19).

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3803