BIODIVERSITAS
Volume 24, Number 10, October 2023
Pages: 5503-5512

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241032

Molecular screening of local Indonesian rice to identified resistant varieties against brown planthopper (*Nilaparvata lugens*) attacks

AGUNG NUGRROHO PUSPITO[1], NOOR ROZZITA[2], MELLFANI RHAMADINDA NENDRA TIGARA[2], SATRIA ISKANDAR DINATA PUTRA[2], IKA PURNAMASARI[2], SRI HARTATIK[2], MOHAMMAD UBAIDILLAH[2,♥]

[1]Program of Biotechnology, Graduate Program, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[2]Program of Agrotechnology, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia.
Tel.: +62-331-330224, ♥email: moh.ubaidillah.pasca@unej.ac.id

Manuscript received: 10 July 2023. Revision accepted: 22 October 2023.

Abstract. *Puspito AN, Rozzita N, Tigara MRN, Putra SID, Purnamasari I, Hartatik S, Ubaidillah M. 2023. Molecular screening of local Indonesian rice to identified resistant varieties against brown planthopper (*Nilaparvata lugens*) attacks. Biodiversitas 24: 5503-5512.* Brown planthopper (*Nilaparvata lugens* Stal) is a significant pest in rice (*Oryza sativa* L.), resulting in a yield loss of around 20-80%. Environmentally friendly control involves looking for potential resistance in local Indonesian rice, because it's naturally resistant to pests and diseases and has several tolerant mechanisms for dealing with stress. However, the resistance potential of local rice has not been widely studied, so it is necessary to carry out screening to identify the resistance possessed by local Indonesian rice and continue with molecular analysis to validate the presence of brown planthopper resistance genes. This study aimed to determine the possible resistance level of 24 Indonesian local rice varieties to brown planthopper pests. The research was conducted at the Agrotechnology Laboratory, Faculty of Agriculture, University of Jember. In June-November 2022. This research included screening using the third instar nymph brown planthopper, preference testing to analyze the preference level for each local rice variety and molecular analysis using six BPH markers (RM 247, RM 6869, RM 19291, g12140-2, RM 3331, and RM 5479) with the MAS technique. The results showed that in the preference test, Lahoten, Fatuk Masin, and Sukamandi had a higher preference value than other local Indonesian rice. In the screening, the average local rice with very resistant criteria is Leukat Medan, while those with moderately vulnerable criteria are Sukamandi. In the molecular analysis of local Indonesian rice, the Aek Sibundong variety had the most resistance, while the Kapuas and Sukamandi varieties had the least resistance.

Keywords: BPH, molecular assisted selection, resistance gene, rice

Abbreviations: BPH: Brown Planthopper; CTAB: Cetyl-trimethylammonium-bromide; MAS: Marker Assisted Selection; PCR: Polymerase Chain Reaction

INTRODUCTION

Agricultural cultivation activities carried out by farmers often need help with the implementation process. One of the problems that are often faced is related to pest attacks which can damage and hinder the productivity of agricultural products. According to Widiarta et al. (2020), pest attacks on plants are one of the limiting factors in increasing productivity in cultivated plants such as rice (*Oryza sativa* L.) plants. Rice is a staple food that almost half of the world's population (Hairmansis et al. 2017). Biotic and abiotic factors from the plant environment always affect the productivity of rice plants. Pest attacks on rice cultivation can affect yield production and interrupt the stability of demand and rice availability (Zhang et al. 2022). A major pest that attacks rice plants, especially in Asia, is the brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Naik et al. 2018). Indonesia is located in the tropics, so it has a high potential for pest attack, where the spread of brown planthopper pests in the tropics is one of the main yield loss factors (Ghobadifar et al. 2016). Attacks by brown planthopper pests on rice plants will cause yield losses of 20-80% through both direct and indirect damage (Balachiranjeevi et al. 2019). Brown planthopper damages rice plants by sucking fluids from the xylem and phloem tissues which will cause the plants to dry out like fires, causing yield loss. The brown planthoppers can also cause damage indirectly by transmitting the stunt virus to rice plants which can reduce yield losses (Kumar et al. 2020). The brown planthopper pest has attacks that fluctuate in the field, starting from low to its peak; an explosion causes rice plants to experience puso or death due to burning (hopper burn) (Kumar et al. 2020). The brown planthopper attack must be controlled, which is quite detrimental to farmers.

Control is often carried out using chemicals, which in the long term will cause pest resistance (Quan and Wu 2023). Proper control does not cause pest resistance and is environmentally friendly, using resistant varieties (Niones et al. 2022). Indonesia has introduced superior rice varieties resistant to planthoppers, such as varieties IR-24, IR-46, and IR-64 which have resistance genes to brown planthoppers. Efforts are to look for potential genetic sources, which will later be used in breeding programs to obtain new superior varieties that contain potential new genetics (Haliru et al. 2020). One of several efforts used to look for genetic sources was screening. Screening was a test to identify varieties with a higher resistance to their habitat. Each resistant variety has different resistance genes, so testing must be done through screening to identify the resistance level of each resistant variety. Establishing the screening method to identify resistance genes in rice can provide a source of information for rice improvement and be useful for rice breeding (Zhang et al. 2022).

Local rice is potential germplasm as a genetic source that controls several essential factors in rice plants (Karimah et al. 2020). Local rice has a unique advantage because it has been cultivated for generations so that the genotypes can adapt well to various biotic and abiotic stress conditions (Pathak et al. 2021). Before introducing high-yielding planthopper-resistant varieties, farmers in each area planted local rice varieties that had adapted to certain agroecosystems and then screened. Screening is a test to identify varieties with a higher level of resistance to their habitat. Each resistant variety has different resistance genes, so testing must be done through screening to identify the resistance level of each resistant variety. The condition of plant varieties that enter resistance to specific insect species is essential for breeding techniques to increase resistance (Heinrich et al. 1985). The results of the screening tests also need to be subjected to genomic analysis to obtain more accurate results. Screening tests for local rice varieties that have potential resistance to brown planthopper pests have not been widely carried out. Therefore, it is necessary to research screening the resistance level of local rice varieties to brown planthopper pests. In this study, a molecular analysis will also be carried out using Marker Assisted Selection (MAS) which has been validated to determine the level of resistance in local Indonesian rice.

MATERIALS AND METHODS

Time and place of research

This research was carried out for five months, from June to November 2022. The research was conducted at the Agrotechnology Laboratory and Greenhouse of the Faculty of Agriculture, University of Jember.

Plant materials

This study used a sampling method which was then scored based on the standard evaluation system from International Rice Research Institute (IRRI). This study consisted of 24 local rice varieties and three control varieties (Table 1). This research was conducted by planting local rice in planting tanks with a length of 75 cm, a width of 50 cm, and a height of 10 cm. Each rice variety was planted with ten rice clumps randomly according to the internal method (IRRI 1985). This study used TN1 as a susceptible variety, IR64 as a Bph1 resistant variety (Yang et al. 2019), IR36 as a Bph2 resistant variety (Kang et al. 2019), and local rice varieties to be tested. Planting control rice in containers was carried out in the test of local rice.

BPH resistance screening

The rice plant screening program for brown planthopper attack in this study used brown planthopper biotype three obtained from the Toxicology Laboratory, Faculty of Agriculture, Gadjah Mada University. The characteristics of the brown planthopper are shown in Table 2.

The brown planthoppers used in this study were biotype three brown planthoppers with third instar nymphs with a size of 1-2 mm (Figure 1). The number of instars three nymph populations of brown planthopper infested was 900 individuals. The overall morphological features of the body of the brown planthopper are light brown, and the abdominal segments are visible. Instar 3 brown planthoppers have developed wings and legs. The morphological characteristics of biotype 3 of the brown planthopper did not differ from the other biotypes. Based on morphological characters, brown planthopper biotypes cannot be distinguished, but based on a variety's malignancy level, they can be distinguished (Iamba and Dono 2021). Differences in brown planthoppers can be distinguished through genetic factors in brown planthoppers.

The brown planthopper biotype 3 is the result of a change in the brown planthopper from biotype two, which occurred due to the brown planthopper population explosion in Indonesia. The brown planthopper biotype 3 in this study is the most widely used brown planthopper in screening programs (Heinrich et al. 1985). The use of an instar three-nymph brown planthopper was determined based on the IRRI Genetic Evaluation for Insect Resistance in Rice method (Heinrich et al. 1985).

Table 1. List the varieties of local rice that were used in this research

No.	Varieties	No.	Varieties
1.	TN1 (Japonica - negative control)	15.	Bengawan Solo
2.	IR64 (Bph1, Bph2, Bph3)	16.	MS Pendek
3.	IR36 (Bph1, Bph2)	17.	Mentik
4.	Lahoten	18.	Super Manggis
5.	Jalawara	19.	Ciliwung
6.	Fatuk Masin	20.	Lamongan 1
7.	Bulu Putih	21.	Gajah Mungkur
8.	Kubiak Kusuik	22.	Cimelati
9.	Pontianak	23.	Cibodas
10.	Widnes	24.	Shinta
11.	Leukat Medan	25.	Cisadane
12.	Jatiluhur	26.	Aek Sibundonng
13.	Mahakam	27.	Sukamandi
14.	Kapuas		

Note: All the rice used in this table was taken from the Indonesian Center for Rice Research (ICRR)

Table 2. Characteristics of the infested brown planthoppers

Biotype	Stadia	Size	Population	From
Bph 3	Nymph instar 3	1-2 mm	900 head	University of Gadjah Mada

A **B**

Figure 1. A. Third instar brown planthopper side view. B. Third instar brown planthopper top view

Research implementation

The brown planthoppers (*Nilaparvata lugens* (Stål)) used in this study were obtained from the cultivation of brown planthoppers by the Toxicology Laboratory, Faculty of Agriculture, Gadjah Mada University. Maintenance of rice plants includes soaking rice seeds for 1x 24 hours and germination in a petri dish covered with cotton. Furthermore, the preparation of the planting media using dunes was then carried out by planting rice plants where each variety was planted in 10 clumps, and the last was watering. Brown planthopper nymphs instar 2-3 were infested on rice plants ten days after planting. After seven days of brown planthopper infestation, local Indonesian rice is scored. This scoring process assesses the resistance of local rice varieties to brown planthoppers based on the symptoms that occur in each local rice variety after being infested with brown planthoppers. The scoring results for all Indonesian local rice varieties are shown in (Table 4).

Molecular analysis

Materials used in the DNA isolation and amplification stages for molecular analysis, include young rice leaves, liquid nitrogen, Cetyl-trimethylammonium Bromide (CTAB) Buffer, PVP (polyvinylpyrrolidone), β Mercaptethenol, PCI (Phenol Chloroform Isoamyl Alcohol), Ammonium Acetate, Isopropanol, Ethanol, Aqudest, PCR Kit, EtBr, agarose powder, TAE, brown planthopper (Brown Planthopper) primer RM247, RM6869, RM19291, RM6217, RM3331, g12140-2, and RM5479. The markers chosen in this study were RM 247, RM 6869, RM 19291, g12140-2, RM 3331, and RM 5479, where each marker illustrated a resistant gene for BPH. RM 247 shows the Bph1 gene (Myint et al. 2012), RM 6869 for the Bph2 gene (Jena et al. 2006), RM 19291 for the Bph3 gene (Nguyen et al. 2019), g12140-2 for the Bph15 gene (Lv et al. 2014), RM 3331 for the Bph18 gene (Suh et al. 2011), and RM 5479 presents the Bph21 gene (Myint et al. 2012). The detection of markers on DNA rice sample would be an indicator for each variety that is classified as resistant to BPH attacks.

Observational variables

The first observation variable is the preference for brown planthoppers, which is done by counting the number of brown planthopper nymphs that live in 28 days after sowing (DAS) rice varieties in the 60 × 40 × 10 cm standard seedbox (Heinrich et al. 1985). The screening process was repeated three times at 2-day intervals. The level of preference of brown planthoppers for local rice varieties is used to determine the level of resistance of a rice variety to brown planthoppers. The preference test used as many as 24 local Indonesian rice varieties, which were selected based on the varieties widely planted by farmers in their regions. Some of these local rice varieties were obtained from the germplasm collections of the Sukamandi Rice Research Center (ICRR). The second variable is the scoring of the resistance level of rice varieties, where the scoring follows the evaluation standards at IRRI. The third variable is the analysis of brown planthopper resistance markers, observations based on the pattern of bands formed. The genomic analysis begins with DNA isolation using the CTAB buffer method. The Polymerase Chain Reaction (PCR) is performed, and the PCR results are electrophoresed on 1% agarose media, followed by DNA visualization.

Data analysis

The data obtained were analyzed in a quantitative descriptive and qualitative descriptive manner following the system evaluation standards from IRRI and presented in pictures and graphs.

RESULTS AND DISCUSSION

Characteristics of rice plants

In this study, we used some local Indonesian rice varieties from the Indonesian Center for Rice Research (ICRR). From Table 3, the observations of the 27 rice varieties conclude that each variety has different morphological variations from the others. Table 3. shows that the 27 rice varieties used have different characteristics, both from the size and pigment of the seeds as well as the morphology, which can be seen from the height of the plant and the resulting character in the form of the number of tillers. These differences are due to genetic factors.

The number of tillers of a rice variety is influenced by genetic traits and environmental factors, which play an essential role in determining the productivity of rice plants (Yanti et al. 2022). The number of productive tillers was divided into three groups, namely fewer tillers (less than 12 stems per clump), medium tillers (13-20 stems per clump), and many tillers (more than 20 stems per clump) (Ekamber 2014). The difference in producing the number of tillers for each variety is caused by the genetic factors of each variety. This leads to variations in the appearance of plants and the result of the adaptation of varieties to the environment in the production of the number of tillers. According to Gardner et al. (1991), the number of tillers will be maximized if the plant has good genetic traits and favorable environmental conditions. Differences in the number of tillers occur due to genetic or environmental factors such as high temperatures, which can cause high respiration and limited nutrients due to less fertile soil.

Table 3. Characteristics of Indonesian local rice plants

Variety	Plant height (cm)	Number of tillers	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Seed pigment
IR36	85.00+4.58	25.33±1.53	8.41+0.59	2.32+0.20	1.81+0.10	NP
IR64	110.00+3.61	23.67±2.08	10.71+0.32	2.34+0.19	1.95+0.06	NP
TN1	97.33+3.06	4.67±1.53	7.71+0.36	2.93+0.11	1.92+0.11	NP
Lahoten	112.33+4.04	11.33±2.08	9.98+0.44	3.40+0.15	2.05+0.08	P
Jalawara	168.00+2.65	8.67±1.53	10.27+0.52	2.35+0.16	1.83+0.09	NP
Fatuk Masin	101.96+4.93	7.67±3.06	9.59+0.33	2.81+0.06	1.93+0.08	NP
Bulu Putih	91.67+3.79	9.33±2.52	9.39+0.32	2.56+0.21	1.92+0.21	NP
Kuriak Kusuik	95.33+4.51	10.33±2.52	10.09+0.48	2.55+0.07	1.95+0.03	P
Pontianak	114.00+2.65	6.33±1.53	8.66+0.57	3.25+0.06	1.87+0.11	NP
Widnes	89.00+2.65	16.67±2.52	9.64+0.22	2.38+0.10	1.84+0.02	NP
Leukat Medan	116.33+4.51	7.67±2.52	9.45+0.26	2.42+0.17	1.78+0.07	NP
Jatiluhur	98.67+4.93	9.67±1.53	9.03+0.49	2.92+0.12	1.79+0.26	NP
Mahakam	102.00+3.00	11.33±2.08	9.46+0.47	3.08+0.20	2.01+0.07	NP
Kapuas	110.67+4.16	7.67±2.08	8.06+0.33	3.03+0.08	1.89+0.09	NP
Bengawan Solo	121.00+1.00	3.00±2.65	8.68+0.24	2.43+0.10	1.70+0.11	NP
MS Pendek	106.00±4.69	8.60±3.44	9.98±0.50	2.75±0.35	1.99±0.18	NP
Mentik	111.80±7.89	17.00±3.32	8.12±0.22	2.74±0.07	1.91±0.03	NP
Super Manggis	114.40±11.41	29.20±5.26	8.74±0.40	2.82±0.22	1.84±0.04	P
Ciliwung	112.60±10.69	13.80±4.02	9.49±0.91	2.53±0.08	1.94±0.09	P
Lamongan 1	108.20±3.90	16.20±3.63	9.32±0.83	2.46±0.18	1.83±0.10	NP
Gajah Mungkur	113.80±4.15	12.60±6.19	10.01±0.63	2.99±0.23	2.05±0.14	NP
Cimelati	94.80±2.17	14.40±5.37	10.30±0.24	2.12±0.11	1.61±0.06	NP
Cibodas	113.60±8.38	12.80±3.90	9.45±0.49	3.21±0.13	2.05±0.16	NP
Shinta	110.20±1.30	11.20±4.44	9.96±0.28	2.62±0.07	1.98±0.13	NP
Cisadane	86.60±2.70	17.20±3.35	9.48±0.43	3.13±0.15	2.08±0.12	NP
Aek Sibundong	72.40±2.88	15.00±3.16	9.22±0.33	2.95±0.13	1.78±0.37	P
Sukamandi	96.00±2.24	15.00±3.46	7.76±0.18	3.05±0.13	1.92±0.16	NP

Note: NP: Not pigmented, P: Pigmented

Morphological diversity among the 24 local Javanese rice varieties can also be seen from the appearance of plant characteristics, such as the color of plant seeds; the color of plant seeds is divided into two, namely pigmented seeds and non-pigmented seeds (Figure 2). The diversity of plant appearance characteristics can be influenced by two factors, namely genetic factors and environmental factors. Guritno and Sitompul (1995) stated that genetic and environmental factors could influence the appearance of plant characteristics. Environmental factors affecting plant morphology include temperature, soil type, soil conditions, altitude, and humidity. According to Qaderi et al. (2019), if environmental factors are more dominant than genetic factors, then plants that are in a place with different environmental conditions will have varied morphology, conversely if genetic factors are more dominant than environmental factors, the characteristics of the plant's appearance will be uniform according to the genetic carry even though the plants are grown in different environments.

Brown planthopper preference for Indonesian local rice varieties

Rosida et al. (2020) stated that the greater the number of green leafhoppers (*Nephotettix virescens* Distant) that land and eat on a variety, the higher the level of preference for planthoppers for that variety. Based on the preference results in Figure 3, the varieties that are avoided by the brown planthopper are the varieties of Kusuik, Kapuas, Pontianak, Mahakam, Bengawan Solo, Jatiluhur, Widas, Super Manggis, Ciliwung, Cimelati, Cibodas, and Cisadane. This shows that this variety has higher antixenosis resistance than other varieties. Sukumar et al. (2022), stated that brown planthoppers are not interested in settling in a rice variety that exhibits an antixenosis resistance mechanism. Antixenos (non-preference for the host) acts as a plant resistance mechanism to decrease the degree of pest damage (Palial et al. 2022). Antixenosis is a substance (chemical, physical, or morphological characteristics) produced by plants that affects negatively the way insects choose their hosts for activities like feeding, oviposition, and shelter (Santos et al. 2021). The level of initial preference for brown planthoppers to land on rice plants is shown by the behavior of planthoppers related to plant mechanisms. Nonpreferential or antixenosis resistance mechanisms are measured by perch ability or orientation, whereas antibiosis is measured by nymph survival, developmental period, imago life span, population increase, and feeding rate (Mishra et al. 2022).

Indonesian local rice plant screening

The results of observations made on the 27 Indonesian local rice varieties following the appearance of wounds on the stems caused by an infection in rice plants after infection in rice plants showed. Once the wound marks become yellow, the stems will eventually begin dried out, wilt, and die. This is due to the large amount of liquid in the rice plant transport network, which is sucked up by the brown planthopper.

Figure 2. Color characteristics of Javanese local rice seeds. 1: Ciliwung, 2: Mentik, 3: Cibodas, 4: Super Manggis, 5: TN1, 6: Lahoten, 7: Bulu Putih, 8: Jalawara, 9: Jatiluhur, 10: Sukamandi, 11: Lamongan1, 12: Gajah Mungkur, 13: MS Pendek, 14: IR36, 15: Kapuas, 16: Bengawan Solo, 17: Leukat Medan, 18: Pontianak, 19: Aek Sibundong, 20: Cimelati, 21: Shinta, 22: Cisadane, 23: IR64, 24: Widas, 25: Mahakam, 26: Fatuk Masin, 27: Kuriak Kusuik

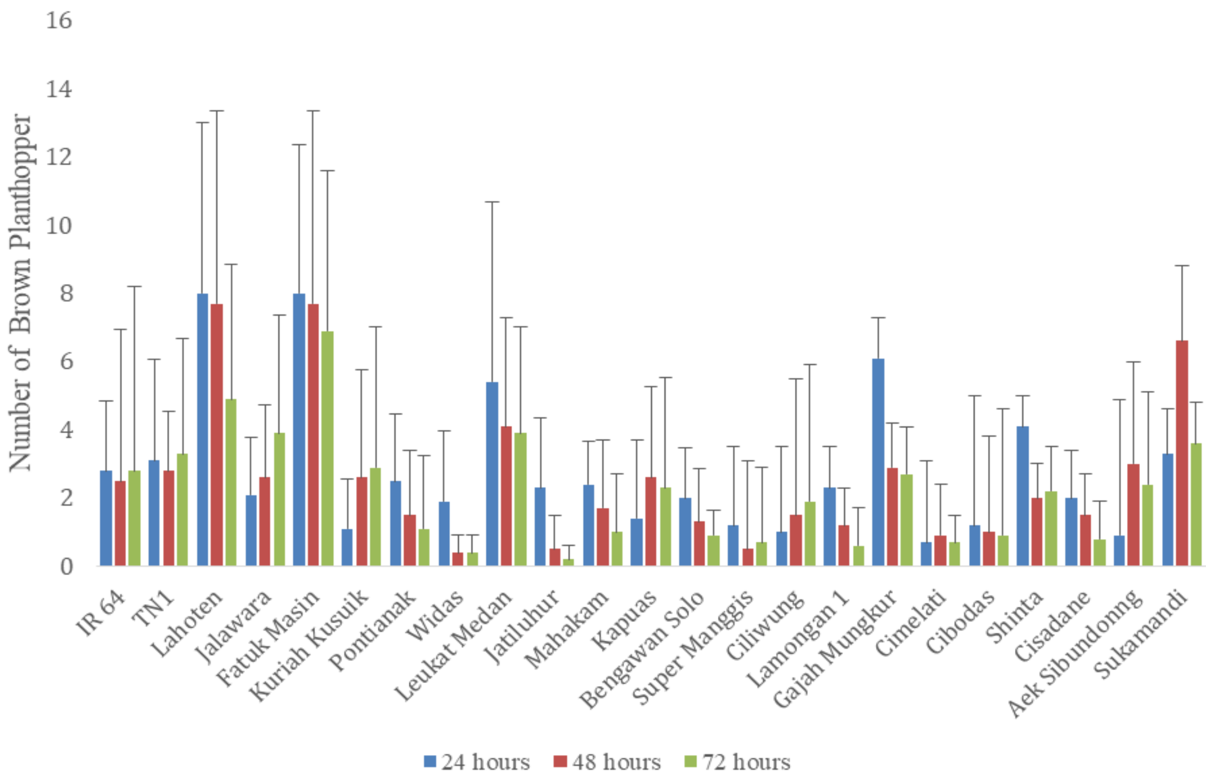

Figure 3. Brown planthopper preference in Indonesian local rice varieties during screening

Observation results after seven days of brown planthopper infestation on several local Indonesian rice varieties showed different morphological resistance responses (Figure 4). In Table 4, the scoring results show that local Indonesian rice varieties have a level of resistance to brown planthoppers, only the Sukamandi variety is likely to be suspected with a score above 5, while the variety with the highest level of resistance is Leukat Medan variety with a score below 1. The ability of local rice to be adaptable to brown planthopper attacks that grow in the geographical area of Indonesia is influenced by interactions between the variety and biotic components, pests and diseases that have occurred over a long period of time (Paiman et al. 2020). Other studies have also used local Indonesian rice varieties because of their potential for plant breeding programs, especially for pest-resistance genes (Karimah et al. 2020).

Symptoms of plant damage begin with a yellow or brown color on the plumules (tips) of the leaves. At a next stage of damage, the leaves appear wilted, curled, or the plant appears yellow. In severe damage, all parts of the plant wilt, droop, and dry out (hopper burn). The resistance of rice varieties is also influenced by the temperature factor, where increased temperatures can reduce the resistance of rice plants to BPH attacks (Morgan et al. 2021). On the seven day after brown planthopper infestation, morphologically it was known that the leaves of several resistant varieties were still in good condition with green leaves. However, some of the edges have started to turn yellow and curl. TN1 as a negative control showed shorter root development due to brown planthopper attack, while other varieties could maintain root condition. The determination of the diversity of resistance of each local rice variety is supported by the response shown by plants during the infestation process, where plants with resistance genes will have the properties to resist brown planthopper attacks (Rafiq et al. 2021). The resistant plant was presented in the IR64 variety (positive control), which has normal growth and less damage compared to others. Furthermore, Damayanti et al. (2022) explained that the symptoms that arise are caused by viral infections in plants that use plant proteins to replicate, thereby disrupting plant production. Meanwhile, in resistance rice, plants could reduce the damage caused by BPH through resistance mechanisms such as antixenosis, antibiosis, and tolerance to reduce economic and yield losses (Wang et al. 2015). In this study, we want to learn about the rice resistant variety based on their preference for the pest (Figure 3).

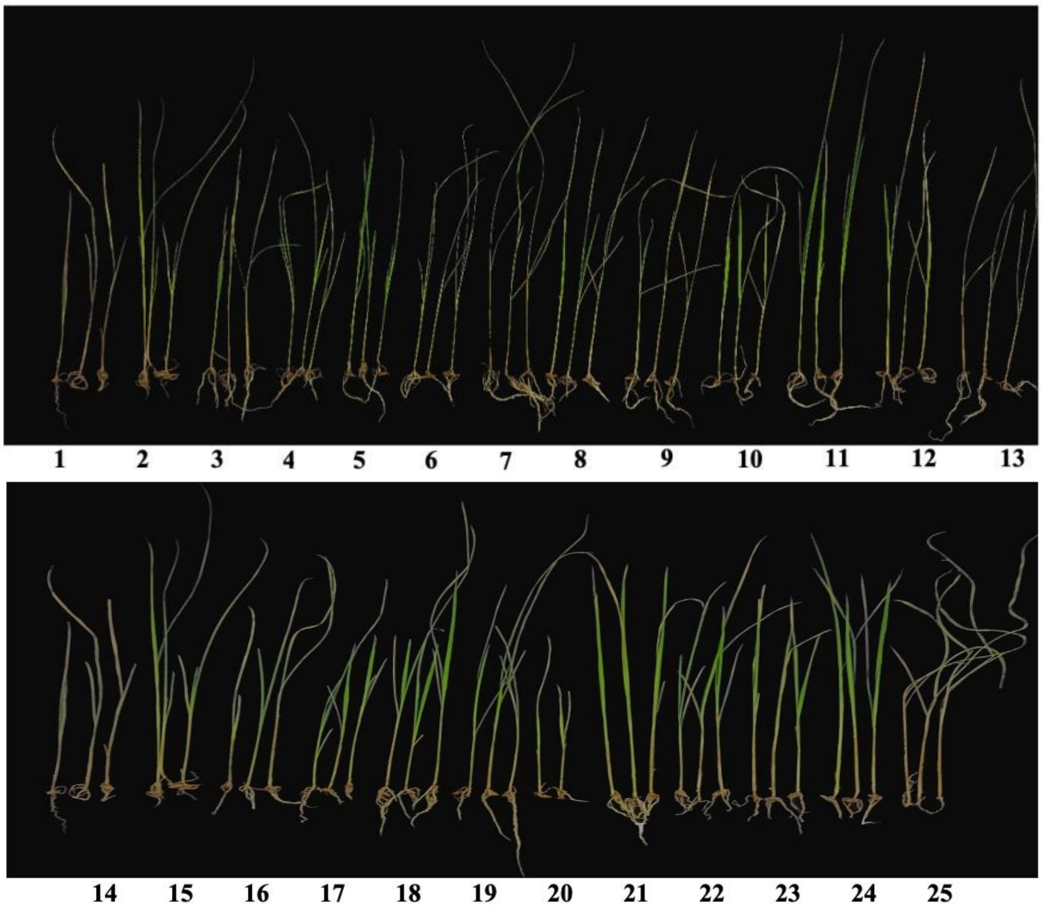

Figure 4. Symptoms of BPH attack on rice plants. 1. TN1, 2: IR 64, 3: Mahakam, 4: Bengawan Solo, 5: Leukat Medan, 6: Widas, 7: Lahoten, 8: Pontianak, 9: Fatuk Masin, 10: Kuriak Kusuik, 11: Jalawara, 12: Kapuas, 13: Jatiluhur, 14: TN1, 15: IR64, 16: Super Manggis, 17: Ciliwung, 18: Lamongan1, 19: Gajah Mungkur, 20: Cimelati, 21: Cibodas, 22: Shinta, 23: Cisadane, 24: Aek Sibundong, 25: Sukamandi

Table 4. Results of scoring the resistance of local Indonesian rice plants to attack by brown planthopper (*Nilaparvata lugens*) based on Standard Evalution System for Rice (SES)

Variety	Scoring results	Criteria
TN1 (negative control)	8.8±0.1	Highly susceptible
IR64 (Bph1, Bph2, Bph3)	1.7±0.3	Resistant
Mahakam	2.0±0.6	Resistant
Bengawan Solo	1.8±1.0	Resistant
Leukat Medan	0.7±0.3	Highly resistant
Widnes	3.4±0.3	Resistant
Lahoten	3.8±0.3	Resistant
Pontianak	2.3±1.2	Resistant
Fatuk Masin	3.6±0.2	Resistant
Kuriak Kusuik	2.1±1.0	Resistant
Jalawara	2.2±0.2	Resistant
Kapuas	3.4±0.2	Resistant
Jatiluhur	3.0±0.8	Resistant
Super Manggis	1.9±0.1	Resistant
Ciliwung	2.8±0.6	Resistant
Lamongan1	3.1±0.5	Resistant
Gajah Mungkur	3.0±0.1	Resistant
Cimelati	2.4±0.1	Resistant
Cibodas	1.8±0.3	Resistant
Shinta	3.2±1.0	Resistant
Cisadane	2.0±0.3	Resistant
Aek Sibundong	1.1±0.2	Resistant
Sukamandi	5.1±0.5	Moderately suspectible

Molecular analysis of Indonesian local rice plants

The research results in Table 5 above show that the local rice variety with the most resistance genes is the Aek Sibundong variety, with several positive resistance detections of 6 resistance. Meanwhile, the local rice varieties that had the fewest resistance genes were the Kapuas, Sukamandi and TN1 varieties as a negative control. The more Bph genes a rice variety has, the more resistance it has to other varieties. The resistance mechanism through Bph gene activation included physical barriers (callose deposition and accumulation of lignin, cellulose, and hemicellulose in cell walls) that inhibit BPH stylets from sucking sap and penetrating plant tissues, and by increasing the accumulation of secondary metabolites and activating the signaling pathways of cytokinin, salicylic acid, and jasmonic acid (Zheng et al. 2021). Since 1970, IRRI has been researching how to use Bph genes to develop BPH resistant plants. According to recent studies, combining two BPH genes, such as Bph6 and Bph9 or Bph14 and Bph15, can strengthen the ability of resistant rice varieties to control BPH (Yan et al. 2023).

The molecular analysis of all Indonesian local rice varieties using six resistance markers to brown planthopper showed different resistance levels. From the results of PCR visualization, if the band pattern formed has the same band pattern as TN1, then the variety is suspected of not having a gene for resistance to brown planthoppers (Figure 5).

Based on the results of electrophoretic visualization using primers RM247 (Bph1), RM6869 (Bph2), and RM19291 (Bph3), this following the results of research conducted by ICRR, which stated that the IR64 variety was proven to be resistant to brown planthopper biotypes 1, 2, and 3. Rice plants that contained Bph gene were resistant to BPH through several mechanisms, such as producing antibiotics, antixenosis (host plants become unattractive to BPH), and tolerance (plants can recover and survive after BPH infestations) (Haliru et al. 2020). There are more than 40 Bph genes that have been identified in wild and cultivated rice varieties, and were utilized as gene resources to produce commercial cultivars that are resistant to BPH infestations (Yang et al. 2023). Bph1 was the first Bph gene found and most identified in indica cultivars (Wan et al. 2019). The Bph2 gene has a function as an antibiosis defense mechanism, so it can cause harm or death to BPH, affect growth rate reduction, and decrease pest production (Haliru et al. 2020). The Bph3 gene can increase resistance levels in rice plant (Qing et al. 2019). Several Bph genes such as Bph1, Bph2, Bph3, and Bph4 have been studied to establish the possible allelic relationship (Bph1 with Bph2 and Bph3 with Bph4) and are located at five major loci on chromosomes 1, 3, 6, 8, and 12 (Muduli et al. 2021). Another study explained that rice cultivar carrying Bph15 gene was strongly resistant to BPH attacks from biotypes 1 and 2 (Chen et al. 2020). The other Bph resistant gene was Bph18, which was located at chromosome 12 and shares the same location as Bph21 and Bph26 (Ji et al. 2016; Mohanapriya et al. 2019; Haliru et al 2020). This Bph18 gene produced proteins that were widely localized on cell membranes, endoplasmic reticulum, golgi apparatus, and prevacuolar compartments (Ji et al. 2016). The Bph21 gene was reported as a dominant gene and showed high resistance performance to BPH attacks in the cross of *Oryza sativa* and *Oryza minuta* (Mohanapriya et al. 2019).

There are various mechanisms of plant resistance to brown planthopper attack, one of which is secondary metabolites. Resistance of varieties correlates with the content of secondary metabolites found in plants (Haliru et al. 2020). Each variety contains different secondary metabolite compounds, so each plant has different resistance mechanism. The resistance of a variety to brown planthopper comes from several secondary metabolite compounds with the contribution of each compound (Haliru et al. 2020). It can also be confirmed that Aek Sibundong has a resistance gene to the brown planthopper biotype 3; this result is also supported by Carsono et al. (2015), in their research results stated that the Aek Sibundong variety has a positive resistance gene detection of RM19291.

Table 5. Marker Assisted Selection (MAS) in local Indonesian rice

Variety	RM 247 (Bph1)	RM 6869 (Bph2)	RM 19291 (Bph3)	g12140-2 (Bph15)	RM 3331 (Bph18)	RM 5479 (Bph21)	Number of positive detects
IR 36 (Bph1, Bph2)	+	+	-	-	+	+	4
IR 64 (Bph1, Bph2, Bph3)	+	+	+	-	+	+	5
TN1	-	-	-	-	-	-	0
Lahoten	-	-	-	+	+	-	2
Jalawara	-	+	-	+	+	-	3
Fatuk Masin	-	-	+	-	-	+	2
Bulu Putih	+	+	+	nd	+	+	5
Kubiak Kusuik	+	+	+	+	-	-	4
Pontianak	+	+	nd	-	-	-	2
Widnes	-	-	+	-	+	+	3
Leukat Medan	-	+	+	+	+	-	4
Jatiluhur	-	+	-	+	-	-	2
Mahakam	+	+	-	+	-	-	3
Kapuas	-	-	nd	+	-	-	1
Bengawan Solo	+	-	-	+	-	+	3
MS Pendek	+	-	+	+	-	-	3
Mentik	+	-	+	-	-	-	2
Super Manggis	+	+	+	-	+	nd	4
Ciliwung	+	+	-	+	+	-	4
Lamongan1	+	-	+	-	-	-	2
Gajah Mungkur	+	+	-	-	-	-	2
Cimelati	-	+	+	+	-	-	3
Cibodas	+	+	-	+	+	+	5
Shinta	+	+	-	-	-	-	2
Cisadane	+	+	-	-	-	-	2
Aek Sibundong	+	+	+	+	+	+	6
Sukamandi	-	+	-	-	-	-	1

Note: (-): The band pattern has a similar size to TN1, which has no BPH resistance gene; (+): Opposite with the negative control (TN1) and suggested to have a BPH resistance gene; (nd): The band was not detected on the BPH gene and negative controls

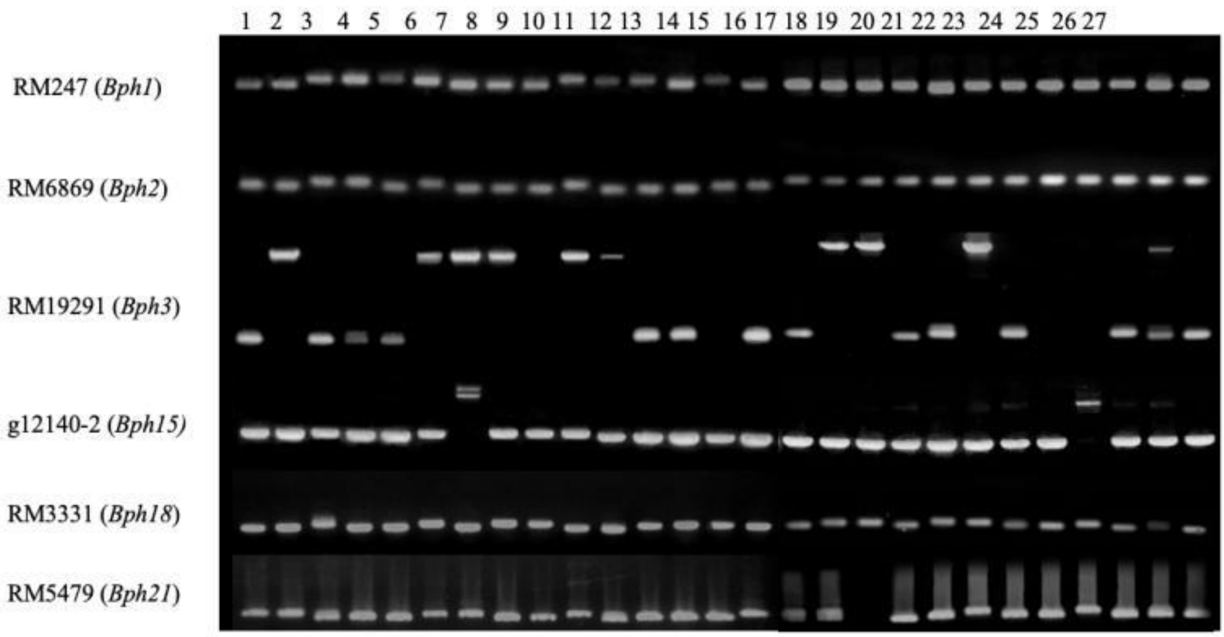

Figure 5. Visualization of PCR results using six resistance markers on 1% agarose gel. 1: IR36, 2: IR64, 3: TN1, 4: Lahoten, 5: Jalawara, 6: Fatuk Masin, 7: Bulu Putih, 8: Kubiak Kusuik, 9: Pontianak, 10: Widnes, 11: Leukat Medan, 12: Jatiluhur, 13: Mahakam, 14: Kapuas, 15: Bengawan Solo, 16: MS Pendek, 17: Mentik, 18: Super Manggis, 19: Ciliwung, 20: Lamongan1, 21: Gajah Mungkur, 22: Cimelati, 23: Cibodas, 24: Shinta, 25: Cisadane, 26: Aek Sibundong, 27: Sukamandi

This research concluded that Lahoten and Fatuk Masin have the highest population of brown planthoppers (*Nilaparvata lugens* (Stål)) compared to other varieties (Figure 3). Meanwhile, after screening, it was found that Leukat Medan became the most resistant variety against brown planthoppers compared to other varieties (Table 4). This study also found Aek Sibundong to be the variety with the most resistant genes after molecular analysis with six markers (Table 5). From this research, we found a correlation between resistant scoring (Table 4) and molecular analysis (Table 5). This correlation shows that Aek Sibundong and Leukat Medan contain a high number of Bph genes and have a low score based on SES. We also found that Sukamandi is highly susceptible compared to other varieties based on the Bph gene present and the high score caused by BPH infestations. Hopefully, this study can be useful as source of information for rice improvement and for agronomists to develop resistant plants against biotic stress, especially brown planthoppers which are major pest in rice cultivation. In addition, future research needs to use several biotypes of brown planthopper to understand the resistance ability of rice varieties to different biotypes of the pest. Using other varieties that are resistant to each type of Bph gene was also needed to find the significant differences between each variety if attacked with brown planthopper.

ACKNOWLEDGEMENTS

We want to thank the Head of the Agrotechnology Department, Faculty of Agriculture, University of Jember, Indonesia. Technicians who work with the researcher throughout the project's duration.

REFERENCES

Balachiranjeevi CH, Prahalada GD, Mahender A, Jamaloddin MD, Sevilla MAL, Marfori-Nazarea CM, Vinarao R, Sushanto U, Baehaki SE, Li ZK, Ali J. 2019. Identification of a novel locus, BPH38(t), conferring resistance to brown planthopper (*Nilaparvata lugens* Stal.) using early backcross population in rice (*Oryza sativa* L.). Euphytica 215 (11): 185. DOI: 10.1007/s10681-019-2506-2.

Carsono N, Barus YV, Sari S, Widarmi WD, Donor D, Sumekar Y, Murdaningsih HK. 2015. Identification of polymorphism on simple sequence repeats markers associated with brown planthopper resistance genes in twenty rice genotypes and their genetic relationship. KnE Life Sci 2 (1): 98. DOI: 10.18502/kls.v2i1.124.

Chen Q, Zeng G, Hao M, Jiang H, Xiao Y. 2020. Improvement of rice blast and brown planthopper resistance of PTGMS line C815S in two-line hybrid rice through marker-assisted selection. Mol Breed 40: 21. DOI: 10.1007/s11032-020-1098-9.

Damayanti TA, Nurjannah T, Listihani L, Hidayat SH, Wiyono S. 2022. Characterization of a variant isolate of Zucchini yellow mosaic virusinfecting green kabocha (*Cucurbita maxima* L.) in Bogor, Indonesia. Arch Phytopathol Plant Prot 55 (1): 121-128. DOI: 10.1080/03235408.2021.2003604 .

Ekamber K. 2014. Environmental factors undermine genetic expression of tiller dynamics in wild rice *Oryza nivara* and *Oryza rufipogon*. Am J Plant Sci 5 (18): 2617-2622. DOI: 10.4236/ajps.2014.518276.

Gardner F, Pearce RB, Mitchell RL. 1991. Physiology of Crop Plants. Field Crop Research, Amsterdam.

Ghobadifar F, Aimrun W, Jebur MN. 2016. Development of an early warning system for brown planthopper (BPH) (*Nilaparvata lugens*) in rice farming using multispectral remote sensing. Precision Agric 17 (4): 377-391. DOI: 10.1007/s11119-015-9422-9.

Guritno B, Sitompul SM. 1995. Analisis Pertumbuhan Tanaman. Gadjah Mada University Press, Yogyakarta. [Indonesian]

Hairmansis A, Yullianida, Supartopo, Jamil A, Suwarno. 2017. Variability of upland rice genotypes response to low light intensity. Biodiversitas 18 (3): 1122-1129. DOI: 10.13057/biodiv/d180333.

Haliru, BS, Rafli MY, Mazlan N, Ramlee SI, Muhammad I, Akos IS, Halidu J, Swaray S, Bashir YR. Recent strategies for detection and improvement of brown planthopper resistance genes in rice: A review. Plants 9: 1202. DOI: 10.3390/plants9091202.

Heinrich EA, Medrano EG, Rapusas HR. 1985. Genetic Evaluation for Insect Resistance in Rice. International Rice Research Institute, Philippines.

Iamba K, Dono D. 2021. A review on brown planthopper (*Nilaparvata lugens* Stal), a major pest of rice in Asia and Pacific. Asian J Res Crop Sci 6 (4): 7-19. DOI: 10.9734/AJRCS/2021/v6i430122.

Jena KK, Jeung JU, Lee JH, Choi HC, Brar DS. 2006. High-resolution mapping of a new brown planthopper (BPH) resistance gene, Bph 18(T), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 112: 1192-1194. DOI: 10.1007/s00122-005-0194-x.

Ji H, Kim SR, Kim YH, Suh JP, Park HM, Sreenivasulu N, Misra G, Kim SK, Hechanova SL, Kim H, Lee GS, Yoon UH, Kim TH, Lim H, Suh SC, Yang J, An G, Jena KK. 2016. Map-based cloning and characterization of the BPH18 gene from wild rice conferring resistance to brown planthopper (BPH) insect pest. Sci Rep 6: 34376. DOI: 10.1038/srep34376.

Kang K, Yue L, Xia X, Liu K, Zhang W. 2019. Comparative metabolomics analysis of diferent resistant rice varieties in response to the brown planthopper *Nilaparvata lugens* Hemiptera: Delphacidae. Metabolomics 15: 62. DOI: 10.1007/s11306-019-1523-4.

Karimah AZ, Siswoyo TA, Kim KM, Ubaidillah M. 2020. Genetic diversity of rice germplasm (*Oryza sativa* L.) of Java Island, Indonesia. J Crop Sci Biotechnol 24 (1): 1-9. DOI: 10.1007/s12892-020-00063-4.

Kumar K, Kaun P, Kishore A, Vikal Y, Singh K, Neelam K. 2020. Recent advances in genomics-assisted breeding of brown planthopper (*Nilaparvata lugens*) resistance in rice (*Oryza sativa*). Plant Breed 139 (6): 1052-1066. DOI: 10.1111/pbr.12851.

Lv W, Du B, Shangguan X, Zhao Y, Pan Y, Zhu L, He Y, He G. 2014. BAC and RNA sequencing reveal the brown planthopper resistance gene BPH15 in a recombination cold spot that mediates a unique defense mechanism. BMC Genomics 15: 674. DOI: 10.1186/1471-2164-15-674.

Mishra A, Barik SR, Pandit E, Yadav SS, Das SR, Pradhan SK. 2022. Genetics, mechanisms and deployment of brownt planthopper resistance genes in rice. Critical Rev Plant Sci 41 (2): 91-127. DOI: 10.1080/07352689.2022.2062906.

Mohanapriya B, Jeyaprakash P, Raveendran M, Soundararajan RP, Ramchander S, Subashini G, Prabhakaran P, Manonmani K, Robin S. 2019. Marker assisted introgression for brown planthopper resistance genes Bph20 and Bph21 in CO43Sub1 variety of rice. Electronic J Plant Breed 10 (2): 645-652. DOI: 10.5958/0975-928X.2019.00081.4.

Morgan FG, Arida A, Ardestani G, Almazan MLP. 2021. Elevated temperatures diminish the effect of a highly resistance rice variety on the brown planthopper. Sci Rep 11 (1): 262. DOI: 10.1038/s41598-020-80704-4.

Muduli L, Pradhan SK, Mishra A, Bastia DN, Samal KC, Agrawal PK, Dash M. 2021. Understanding brown planthopper resistance in rice: Genetics, biochemical and molecular breeding approaches. Rice Sci 28 (6): 532-546. DOI: 10.1016/j.rsci.2021.05.013.

Myint KKM, Fujita D, Matsumura M, Sonoda T, Yoshimura A, Yasui H. 2012. Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens* [Stal]) in the rice cultivar ADR52. Theor Appl Genet 124 (3): 495-584. DOI: 10.1007/s00122-011-1723-4.

Naik SB, Divya D, Sahu N, et al. 2018. A new gene Bph33(t) conferring resistance to brown planthopper (BPH), *Nilaparvata lugens* (Stål) in rice line RP2068-18-3-5. Euphytica 214 (3): 53. DOI: 10.1007/s10681-018-2131-5.

Nguyen CD, Verdeprado H, Zita D, Sanada-Morimura S, Matsumura M, Virk PS, Brar DS, Horgan FG, Yasui H, Fujita D. 2019. The development and characterization of near-isogenic and pyramided lines carrying resistance genes to brown planthopper with the genetic background of japonica rice (*Oryza sativa* L.). Plants 8: 11. DOI: 10.3390/plants8110498.

Niones JT, Sharp TR, Donayre DKM, Oreiro EGM, Milne AE, Oliva R. 2022. Dynamics of bacterial blight disease in resistant and suspectible rice varieties. Eur J Plant Pathol 163 (1): 1-17. DOI: 10.1007/s10658-021-02452-z.

Paiman, Ardiyanta, Ansar M, Effendy I, Sumbodo BT. 2020. Rice cultivation of superior variety in swamps to increase food security in Indonesia. Rev Agric Sci 8: 300-309. DOI: 10.7831/ras.8.0_300.

Palial S, Kumar S, Atri C, Sharma S, Banga SS. 2022. Antixenosis and antibiosis mechanisms of resistance to turnip aphid, *Lipaphis erysimi* (Kaltenbach) in *Brassica juncea-fruticulosa* introgression lines. J Pest Sci 95: 749-760. DOI: 10.1007/s10340-021-01418-8.

Pathak H, Kumar M, Molla K, Chakraborty, K. 2021. Abiotic stresses in rice production: Impacts and management. ORYZA Intl J Rice 58 (4): 103-125. DOI: 10.35709/ory.2021.58.spl.4.

Qaderi MM, Martel AB, Dixon S. 2019. Environmental factors influence plant vascular system and water regulation. Plants 8: 65. DOI: 10.3390/plants8030065.

Qing D, Dai G, Zhou W. Huang S, Liang H, Gao L, Gao J, Huang J, Zhou M, Chen R, Chen W, Huang F, Deng G. 2019. Development of molecular marker and introgression of Bph3 into elite rice cultivars by marker-assisted selection. Breed Sci 69 (1): 40-46. DOI: 10.1270/jsbbs.18080.

Quan Y, Wu K. 2023. Managing practical resistanceof lepidopteran pest to Bt cotton in China. Insects 14 (2): 1-13. DOI : 10.3390/insects14020179.

Rafiq CM, Rizwan M, Atta B, Sabir AM, Rizwan M, Arshad M, Zeeshan M, Latif H, Khalid UB, Iqbal S. 2021. Manipulating rice planting geometry and nutrient levels has an effect on brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) incidence. Pakistan J Zool 54: 1-7. DOI: 10.17582/journal.pjz/20210510160557.

Rosida N, Komalasari E, Praptana RH. 2020. Preference test of green leafhopper (*Nephotettix virescens* Distant) to some tungro-resistant promising lines. Agric 32 (2): 105-120. DOI: 10.24246/agric.2020.v32.i2.p105-120.

Santos TLB, Baldin E, Ribeiro LP, Souza CM, Soares MCE, Fanela TLM, Lourenção A. 2021. Resistance sources and antixenotic factors in Brazilian bean genotypes against *Bemisia tabaci*. Neotrop Entomol 50: 129-144. DOI: 10.1007/s13744-020-00821-7.

Suh JP, Yang SJ, Jeung JU, Pamplona A, Kim JJ, Lee JH, Hong HC, Yang CI, Kim YG, Jena KK. 2011. Development of elite breeding lines conferring Bph18 gene-derived resistance to brown planthopper (BPH) by marker-assisted selection and genome-wide background analysis in japonica rice (*Oryza sativa* L.). Field Crops Res 120 (2): 215-222. DOI: 10.1016/j.fcr.2010.10.004.

Sukumar S, Kennedy JS, Raveendran M, Balasubramani V, Pushpam R. 2022. Antixenosis and antibiosis mechanism of resistance in selected rice entries against brown planthopper, *Nilaparvata lugens* (Stal). Ecol Environ Conserv 28: S367-S372. DOI: 10.53550/EEC.2022.v28i06s.062.

Wan PJ, Zhou RN, Nanda S, He JC, Yuan SY, Wang WX, Lai FX, Fu Q. 2019. Phenotypic and transcriptomic responses of two *Nilaparvata lugens* populations to the mudgo rice containing Bph1. Sci Rep 9: 14049. DOI: 10.1038/s41598-019-50632-z.

Wang Y, Cao L, Zhang Y, Cao C, Liu F, Huang F, Qiu Y, Li R, Luo X. 2015. Map-based cloning and characterization of BPH29, A B3 domain-containing recessive gene conferring brown planthopper resistance in rice. J Exp Bot 66 (19): 6035-6045. DOI: 10.1093/jxb/erv318.

Widiarta DIN, Kiswanto, Rivaie AA. 2020. Major rice pest development after increasing rice planting index and acceleration of planting time in Lampung Province. IOP Conf Ser Earth Environ Sci 457 (1): 012064. DOI: 10.1088/1755-1315/457/1/012064.

Yan L, Luo T, Huang D, Wei M, Ma Z, Liu C, Qin Y, Zhou X, Lu Y, Li R, Qin G, Zhang Y. 2023. Recent advances in molecular mechanism and breeding utilization of brown planthopper resistance genes in rice: An integrated review. Intl J Mol Sci 24 (15): 1-21. DOI: 10.3390/ijms241512061.

Yang M, Cheng L, Yan L, Shu W, Wang X, Qiu Y. 2019. Mapping and characterization of a quantitative trait locus resistance to the brown planthopper in the rice variety IR64. Hereditas 156 (22): 1-9. DOI: 10.1186/s41065-019-0098-4.

Yang K, Liu H, Jiang W, Hu Y, Zhou Z, An X, Miao S, Qin Y, Du B, Zhu L, He G, Chen R. 2023. Large scale rice germplasm screening for identifcation of novel brown planthopper resistance sources. Mol Breed 43: 70. DOI: 10.1007/s11032-023-01416-x.

Yanti D, Safitri I, Rusnam, Stiyanto E. 2022. Rice productivity estimation using remote sensing method. J Agric Eng 11 (3): 451-465. DOI: 10.23960/jtep-l.v11i3.451-465.

Zhang Y, Zhou J, Xu P, Li J, Deng X, Deng W, Yang Y, Yu Y, Pu Q, Tao D. 2022. A genetic resource for rice improvement: Introgression library of agronomic traits for all AA genome *Oryza* species. Plant Breed 13: 856514. DOI: 10.3389/fpls.2022.856514.

Zheng X, Zhu L, He G. 2021. Genetic and molecular understanding of host rice resistance and *Nilaparvata lugens* adaptation. Curr Opinion Insect Sci 45: 14-20. DOI: 10.1016/j.cois.2020.11.005.