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Research Article
Genotypic Variation in Virulence Level of Several Brown Planthopper (*Nilaparvata lugens* Stål) Populations of Rice (*Oryza sativa* L.)

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Abstract
Background and Objective: The virulence level information of the Brown Planthopper (BPH) population is required to determine which populations will be used in the rice breeding program. This is to give a high pressure on the screening lines to obtain highly resistant lines. The purpose of this study was to determine the virulence level of 5 BPH populations and to determine resistant varieties to BPH populations that can be used as donor lines. **Materials and Methods:** The study used the Standard Seedbox Screening Test method on 5 BPH populations (Kediri, Subang, Banyuasin, Klaten and South Lampung). The genotype used in this study consisting of 9 BPH differential and 5 commercial rice varieties. The BPH population variance was analyzed and then grouped based on the level of virulence in each genotype using the UPGMA method. **Results:** Klaten's BPH population had the highest virulence (5.95), meanwhile, the BPH population from Subang (4.86) had the lowest virulence. Based on the clustering analysis, BPH populations from Kediri, Banyuasin and South Lampung are in the same group. Meanwhile, the BPH population from Klaten and Subang has been clustered in the different groups each. Balamawee identified as a high and stable resistance variety to all tested BPH populations followed by Rathu Heenati, PTB33, Swarnalata, Inpari13 and IR64 with medium resistance. **Conclusion:** The BPH population from Klaten is recommended to be used in the lines screening program because of its high virulence level and Balamawee is identified as a potential donor to develop BPH resistant variety.

Key words: Genotypic variation, brown planthopper, virulence level, screening test, biotic stress

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Currently, Brown Planthopper (BPH) is still considered the most destructive pest on rice because it causes a very significant reduction in yields[1,2], the ability to adapt from a resistant variety[3] and its ability to transmits rice ragged stunt virus[3,4]. These pests can reproduce and adapt quickly to new environments, forming new biotypes or colonies that can destroy several varieties that were previously considered resistant. Some brown planthopper populations have even been reported to have evolved because they were able to break the resistance of brown planthopper-resistant rice varieties in the past decade[5]. According to Jena and Kim[6] stated that the variability of the brown planthopper is high, so the specificity of resistance to brown planthopper by rice plants is important to study. Therefore, efforts to periodically monitor and evaluate the virulence level of the BPH population at rice plantations in Indonesia need to be carried out so that one can think about how to control it.

The use of BPH-resistant varieties is still considered the most economical, effective and environmentally friendly approach to control brown planthopper attacks[7]. The use of new improved commercial varieties[8] which are planted widely and continuously causes a population of brown planthoppers to adapt and reproduce quickly and has the potency to break its resistance. This is because the brown planthopper is a pest that has high genetic plasticity, so it will be easier and faster to form a new biotype[6,9]. This has been widely evidenced by a decrease in resistance to varieties carrying the Bph1, bph2, Bph3 and bph4 genes which have lost their resistance to brown planthopper due to the evolution of new biotype[1,2,7,9]. Therefore, efforts are needed to increase the diversity of varieties that have resistance to brown planthoppers from different sources of resistance to suppress the formation of new biotypes by identifying varieties that can be used as resistance donors.

Determination of resistance donors to brown planthopper is important to obtain varieties that have broad resistance to several brown planthopper populations. Several varieties were identified as having broad resistance to the brown planthopper population from Southeast Asia[2,3]. However, because the variability of the brown planthopper itself is wide, sometimes the use of varieties as a donor without validating the resistance to BPH populations in the targeted area will lead to an unexpected result. Although it has used well-known markers under Marker Assisted Selection (MAS) technology. This is due to differences in the use of the brown planthopper population in the process of gene discovery. For example, the use of the Bph18 gene was not effective in overcoming the brown planthopper in the Wuhan-China population because this gene was discovered using the BPH planthopper population from South Korea[10,11]. Likewise, the evaluation results of 6 rice plant resistance genes (Bph3, Bph14, Bph15, Bph18, Bph20 and Bph21) against brown planthopper in the Wuhan population (China) of the 6 genes evaluated; only the Bph14 and Bph15 genes had resistance to brown planthoppers that goes well against the brown planthopper of the Wuhan population. This is because the two genes were discovered using the BPH population from China[11,12]. Horgan *et al.*[3] added that certain genes are only effective for certain locations as well.

This study aimed to determine the virulence level of several BPH populations in Indonesia and also to evaluate several genotypes that can be used as donors of resistance to BPH. So that it can be used as initial information in compiling and determining the parents of the crosses in the brown planthopper-resistant rice breeding program in Indonesia.

MATERIALS AND METHODS

Brown planthopper populations: The test was carried out in February-August, 2020 in a greenhouse located at the Kambingan Farm of PT BISI International, Tbk, Pagu, Kediri Regency, East Java, Indonesia. The population of brown planthoppers used is a collection of PT BISI International, Tbk from 5 locations in 5 provinces (Banyuasin-South Sumatera, South Lampung-Lampung, Subang-West Java, Klaten-Central Java and Kediri-East Java) which were taken from endemic areas of brown planthoppers in 2016 and has been rearing in a greenhouse on the host plant Taichung Native 1 (TN1). The rearing method was carried out according to Heinrichs *et al.*[13].

Plant materials: The test used 14 genotypes of rice (*Oryza sativa* Lin.) consisting of 9 differential varieties and 5 commercial varieties. The selected differential varieties have specific resistance genes to brown planthopper, including Bph1, bph2, Bph3, Bph9, bph5, Bph6, bph8, Bph9, Bph17 and Bph27(t). The selection of this genotype was based on the aim of this study which was to determine the highest virulence response of the brown planthopper population not only in differential varieties but also in commercial varieties that have been widely grown by farmers. The seeds of differential varieties come from the germplasm collection of PT BISI International, Tbk, obtained from the International Rice Research Institute (IRRI) Philippines. Meanwhile, commercial rice seeds are obtained from the Indonesia Center for Rice Research (ICRR), Sukamandi, West Java, Indonesia.

Evaluation of resistance: Virulence testing used the Standard Seedbox Screening Test method[13], with a randomized block design and repeated three times. The test rice seeds were soaked for 18 hrs and then brooded for 24 hrs to obtain seeds with uniform growth. The seeds of each genotype that have been germinated are then sown in rows, each row consisting of 25 rice seeds sown and arranged in a seedbox. One seedling box represents one replicate with 14 genotypes in it. The rice seeds are then allowed to grow until 2 leaves (approximately 7 days after sowing). Thinning is done before infestation, leaving 20 plants for each genotype for each replication. Each seedbox containing the tested genotype was infested with 2-3 instar brown planthopper nymphs with 10 nymphs per plant. The infestation was carried out by taking 200 leafhopper nymphs (10 nymphs×20 seeds) and then infesting them in each test number sequentially. The observation of damage score is carried out 7-10 days after infestation, or when 90% of the TN1 susceptible check varieties have died. Scoring was carried out based on IRRI's Standard Evaluation System (SES)[14] with minor modification where a score of 1 = no damage until minor damage to several plants in a row, 3 = the first and second leaves of each plant partially yellowing, 5 = Yellowing or stunted plants between 10 and 25% of plants wilted in a row, 7 = more than 50% of plants wilted or died and remaining plant was severely stunted or dying and 9 = all plants wilted or died.

Data analyses: Data were analyzed using a combined ANOVA between brown planthopper populations to determine the virulence variance between genotype populations and the interactions between the two, if differences were found in the brown planthopper population, a further test was carried out to determine the most virulent population based on the 5% Tukey test. The brown planthopper population was then analyzed for its similarity and grouped according to the UPGMA (Unweighted Pair Group Method with Arithmetic) method using the SAHN (Sequential, Agglomerative, Hierarchical and Nested) program by changing the resistance scoring data to binary data (score<5 = 0 and score>5 = 1). Similarity matrices were analyzed using the DICE coefficient and the SIMQUAL program on the NTSYS 2.02 software[5,15].

RESULTS

Virulence: Based on Table 1, it can be seen that the differences of BPH populations from 5 locations are relatively moderate where there is only 1 BPH population which is significantly different based on the 5% Tukey test. The BPH population from Subang (4.86) had the lowest virulence level statistically significant compared to the other populations. This is presumably because this BPH population was unable to damage several genotypes, namely Mudgo, Ciherang and Inpari 33, where different things were seen in the other 4 BPH populations. It can be seen from the scoring results showing that the three varieties are still relatively resistant to the BPH population from Subang (score 3.00-5.67) compared to other BPH populations (score 4.33-9.00).

The BPH population from Klaten (score 5.95) had the highest virulence although it was not significantly different from the population of Kediri (5.76), Banyuasin (5.90) and South Lampung (5.86). However, it can be seen that the PTB33 and Rathu Heenati (resistant check) resistance scores to the

Table 1: Damage scoring result of 14 rice genotypes against 5 populations of brown planthoppers

Genotype	Gene	Kediri	Subang	Banyuasin	Klaten	South lampung	Mean of resistance
TN1	-	9.00^{c}	9.00^{e}	9.00^{c}	9.00^{f}	9.00^{d}	9.00^{a}
Mudgo	Bph1	7.67^{c}	5.00^{bcd}	7.00^{c}	6.33^{c-f}	8.33^{d}	6.87^{ab}
ASD7	bph2	8.33^{c}	8.33^{de}	9.00^{c}	9.00^{f}	9.00^{d}	8.73^{a}
PTB33	bph2; Bph3	2.33^{a}	3.00^{abc}	4.33^{b}	8.33^{f}	3.00^{ab}	4.20^{bc}
Rathu Heenati	Bph3; Bph17	1.67^{a}	1.67^{ab}	3.00^{ab}	5.00^{b-e}	4.33^{bc}	3.13^{cd}
ARC10550	bph5	7.67^{c}	6.33^{cde}	7.67^{c}	7.67^{ef}	8.33^{d}	7.53^{a}
Swarnalata	Bph6	5.00^{b}	3.67^{abc}	3.00^{ab}	3.00^{ab}	3.00^{ab}	3.53^{cd}
Pokali	Bph9	9.00^{c}	8.33^{de}	9.00^{c}	8.33^{f}	9.00^{d}	8.73^{a}
Balamawee	Bph9; Bph27(t)	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{d}
Ciherang	-	9.00^{c}	5.67^{cde}	9.00^{c}	7.00^{def}	8.33^{d}	7.80^{a}
IR64	Bph1; Bph37	3.00^{ab}	3.00^{abc}	3.67^{b}	3.67^{abc}	4.33^{bc}	3.53^{cd}
Mekongga	-	9.00^{c}	6.33^{cde}	9.00^{c}	7.67^{ef}	6.33^{cd}	7.67^{a}
Inpari13		3.00^{ab}	3.67^{abc}	3.67^{b}	3.00^{ab}	3.67^{abc}	3.40^{cd}
Inpari33		5.00^{b}	3.00^{abc}	4.33^{b}	4.33^{bcd}	4.33^{bc}	4.20^{bc}
Mean of virulence	5.76^{a}	4.86^{b}	5.90^{a}	5.95^{a}	5.86^{a}		
Mean square	27.17**	19.05**	24.34**	19.94**	22.64**		
Tukey 5%	2.94	3.76	2.29	3.38	2.82		

**Significant difference at p<0.01. The different alphabetical letter showed different significant level

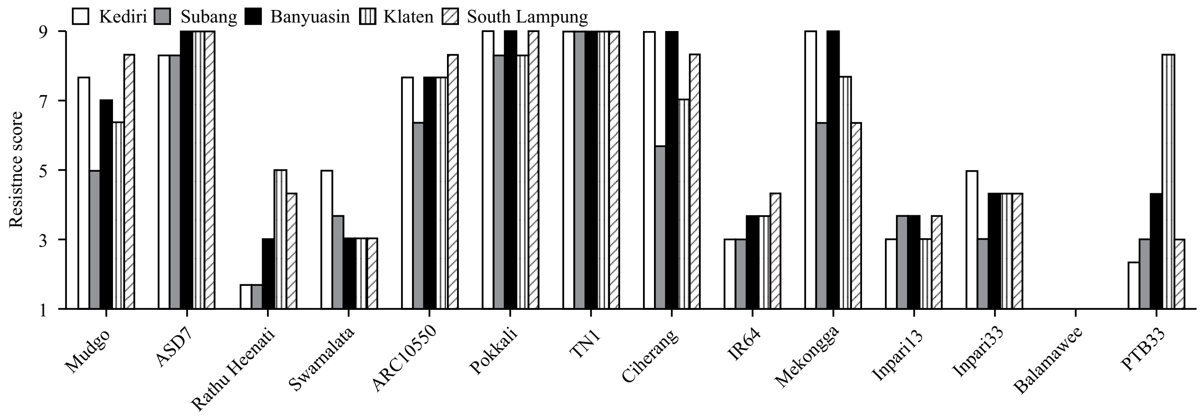

Fig. 1: Performance of the virulence level of each BPH population to the 14 test genotypes

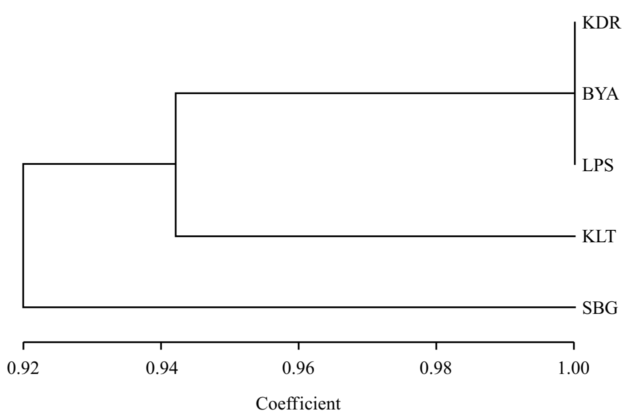

Fig. 2: Dendrogram indicating similarity among BPH populations from 5 different locations

KDR: Kediri, BYA: Banyuasin, LPS: South Lampung, KLT: Klaten, SBG: Subang

BPH of the Klaten population are at 8.33 and 5.00 respectively. Meanwhile, BPH populations from Kediri and Banyuasin had high virulence rates due to their high attack on Ciherang (9.00) and Mekongga (9.00) varieties. The high virulence of BPH from South Lampung was due to the high level of damage to the Mudgo and ARC10550 varieties with the level of damage at 8.33 each.

Genotypic variation: Each population showed different virulence in each genotype except for TN1 (susceptible check) with a score of 9 and Balamawee with a score of 1 in Fig. 1. Based on the test results, it is also seen that each BPH population has a different virulence based on the reaction on the differential varieties that have different resistance genes. Genotypes with high resistance to 5 BPH populations were Balamawee (score 1), while the genotypes with moderate resistance were Rathu Heenati (3.13), Inpari13 (3.40), Swarnalata (3.53) and IR64 (3.53). PTB33 showed an average resistance score of 4.22 with a score range of 2.33-8.33. The lowest resistance to PTB33 was found in the Klaten BPH population with a susceptible status.

BPH population clustering: Based on the UPGMA analysis, some BPH populations can be grouped into 3 main groups in Fig. 2. The results of grouping using the UPGMA method show that 3 out of 5 populations, namely the population of BPH Kediri, Banyuasin and South Lampung are in the same group. Meanwhile, the population of BPH Subang and Klaten are in different groups. The results of this grouping are also in line with the results of Tukey 5% tests where the Subang BPH population has the lowest virulence compared to the other four BPH populations. Meanwhile, the BPH population from Klaten which was still similar with a similarity coefficient of 0.94 with the populations of Kediri, Banyuasin and South Lampung was the BPH population with the highest virulence level.

DISCUSSION

Information of the BPH populations virulence level from 5 rice planting areas in Indonesia is needed to understand the similarity among them. This information is also used to obtain information on the rice genotype that could potentially be used as a donor for resistant parents. Generally, there are slight differences in virulence of the 5 BPH population in this study with the Subang BPH population is the only low in virulence. Similar results were obtained by Chaerani *et al.*[5] which showed that the virulence level of BPH populations from Central Java and East Java was still in the same range except for the BPH from West Java which shows lower virulence than the previous report. The difference in virulence of this study is due to the different sources of the BPH population. Chaerani *et al.*[5] use the BPH population from Bekasi and Karawang as a representative of West Java, while this study using Subang populations. However, there is no difference in virulence of the BPH population from Central Java and East Java. This shows that there has been no change in the virulence level of the BPH population for the past 4 years. This study also complements the previous study which added Lampung (South Lampung) and South Sumatra (Banyuasin) provinces to the location for evaluating the virulence of the brown planthopper population after the previous study of only conducted BPH virulence evaluations in the provinces of Banten, West Java, Central Java, East Java, South Kalimantan and South Sulawesi.

The high virulence level of the BPH population was from the Klaten location. This is due to the high damage score in the resistant varieties PTB33 (8.33) and Rathu Heenati (5.00) where this is not occurring in other BPH populations. The resistance of the two varieties in other BPH populations ranged from 2.33-4.33 in the PTB33 variety, while the Rathu Heenati variety ranged from 1.67-4.33. Both of these varieties have previously been reported to have broad-spectrum resistance to Brown planthopper[3,5,15], but in this study, these varieties show medium susceptible levels to the BPH population from Klaten. That means the BPH population from Klaten has a higher destructive ability than other BPH populations because it can break the resistance of BPH resistant varieties. This finding is in line with the previous study which reports that the BPH population from Klaten had broken the resistance of the Ciherang which was reported to have resistance to biotype 3[9]. Furthermore, the clustering analysis explained the dissimilarity between the BPH population from Klaten with BPH population from Kediri, Banyuasin and South Lampung, although statistically, the level of virulence is not significantly different. From this point of view, the BPH population from Klaten can be used to screen and select rice lines/varieties in the BPH-resistant rice breeding program. The expectation is that the lines selected using this BPH population will have high resistance to brown planthopper. The same thing was stated by Chaerani *et al.*[5] and Horgan *et al.*[3] that the use of the right BPH population will produce lines or rice varieties that have good resistance to brown planthoppers.

Genotypic variation can be seen in Fig. 1 as each genotype reaction to the 5 BPH population was different. TN1, Mudgo, ASD7, ARC10550, Pokali, Ciherang and Mekongga were showing susceptible reactions (score 6,87-8.73). Meanwhile, Balamawee, Rathu Heenati, PTB33, Swarnalata, IR64, Inpari33 and Inpari13 showing better reactions to the BPH population. Generally, each genotype has a different reaction to each of the BPH populations except for TN1 (susceptible check) and Balamawee that has a consistent reaction to all populations. Furthermore, the mean resistance between Balamawee and the 4 genotypes that showing good resistance to BPH populations was not significantly different. However, when looking at each population, the resistance of Balamawee to all BPH populations appears to be more stable than the four genotypes. Meanwhile, Rathu Heenati with the best resistance based on several reports is only in second place after Balamawee because it appears resistant only to 3 BPH populations, namely Kediri, Subang and Banyuasin. This finding showed that Balamawee has a broad-spectrum resistance to BPH populations from several locations in Indonesia. The same results were also obtained by Horgan *et al.*[3] who carried out the virulence test of BPH populations from South Asia and Southeast Asia that Balamawee and Rathu Heenati varieties were classified as varieties that had broad-spectrum resistance to BPH. As Balamawee has a stable resistance to 5 tested BPH populations, showing that this variety is a good donor that can be used to develop a broad spectrum of planthopper-resistant rice varieties. Further validation still needs to be done by testing this variety on BPH populations from other regions.

This study also found that there were differences in the effectiveness of gene action on Balamawee (score 1) and Pokali (score 8.73) where both varieties had the same gene namely Bph9[3,16-19], but Balamawee had the additional Bph27(t)[17] gene. This shows that Bph27(t) might have a bigger role in the resistance of brown planthoppers from the Indonesian population. Horgan *et al.*[3,18] in the previous study also explain that Bph9 is no longer effective against the BPH population from Asia. Compare to the previous study using the BPH population from other regions in South and Southeast Asia, the BPH population from Klaten is still less virulent than from India. This is because Balamawee has shown moderate to susceptible against the BPH population from India[19]. Therefore, there is a limitation used of Balamawee as a donor parent in regards to different targeted variety deployment areas. However, Balamawee is still a good donor parent when it comes to Indonesia's BPH populations.

The commercial variety that has been included in this study namely IR64 and Inpari 13 also showing a good reaction.

This information also should be used as a reference in disseminating varieties in the future. The varieties to be planted in Klaten particularly should have high resistance to BPH. At least the existing varieties IR64 and Inpari13, have better resistance than PTB33 and Rathu Heenati so that the two varieties are still feasible to be cultivated in the Klaten location, while the Ciherang, Mekongga and Inpari 33 varieties are not recommended to be planted in the Klaten location, because in the event of a BPH attack it can certainly reduce rice yield.

CONCLUSION

The BPH population from Klaten had a high virulence level and it was in the different groups compared to the other four BPH populations. Based on this virulence evaluation, it was found that the Balamawee variety had a broad spectrum of resistance to BPH. The BPH populations from Klaten as well as the Balamawee variety then recommended being used in the brown planthopper-resistant rice breeding programs.

SIGNIFICANCE STATEMENT

This study discovers the virulence level of 5 BPH populations and the resistant rice varieties to BPH populations which could be used as donor lines that can be beneficial for rice breeder and agronomist to increase the yield of rice. Therefore, this study will help the researcher to uncover the critical areas of low production of rice that many researchers were not able to explore.

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