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RESEARCH ARTICLE

STABILITY OF RESISTANCE RICE VARIETIES ON DIFFERENT LEVEL OF BROWN PLANTHOPPER BIOTYPES TO DETERMINE THE STANDARDIZATION RICE SCREENING

^{1,*}Baehaki, S.E. and ²Eko Hari Iswanto

¹Entomological Society of Indonesia, Bandung Branch, Faculty of Agriculture, Padjadjaran University, Bandung-Sumedang Street Km 21-Jatinangor, West Java-Indonesia

²Indonesian Center for Rice Research (ICRR), Jl. Raya No.9, Sukamandi, Subang 14256, West Java, Indonesia

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ABSTRACT

The research was conducted at the screen house of Indonesian Center for Rice Research (ICRR) to screen of 19 accessions by seedbox mass screening method. At 5 days old seedling of all varieties are thinned to remains 20 seedlings per row, then each set treatment infested by 5, 8 and 10 of 2-3 instar nymphs BPH/seedling from each biotypes 1, 2 and 3. The results showed that the seedbox screening method to obtain the stability of the resistance rice varieties to BPH must be infested 2-3 instar as much 8 or 10 BPH nymphs/seedling on 5 days old rice seedling. The stabilized varieties moderately resistant to all BPH biotype were Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, and IR 74. The population of BPH nymphs reduced height of rice seedling to cause stunting of rice seedling. Ability BPH nymphs to stunted rice seedling depend on to kind of BPH biotype, number BPH nymphs infested, and resistance rice varieties. Reduction seedling height by BPH biotypes 1 almost all stabilized varieties moderately resistant below 50%, but IR 74 and Kencana Bali 53 between 50-60%. Reduction seedling height by BPH biotypes 2 on stabilized varieties moderately resistant Rathu Heenati was smaller than 50%, but the other stabilized varieties moderately resistant were height reduction more than 50%. Reduction seedling height by BPH biotypes 3 on stabilized varieties moderately resistant Rathu Heenati, Swarnalata, Pokalli less than 50%, but the other stable varieties were reduction height more than 50%. The most stabilized rice variety was Rathu Heenati that stable on seedbox screening test, low reduction of plant height, and the little effect of population of BPH/row seedling. Kencana Bali 53 and Kencana Bali 54 can be used as differential varieties that were moderately resistant to BPH biotypes 1, 2, dan3. Likewise popular variety IR74 can be included as a comparison resistant in screening rice varieties to BPH. In the other hand screening methodology used 2-3 instar as much 5 BPH nymphs/seedling on 5 days old rice seedling resulted the unstable rice resistant.

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INTRODUCTION

Screening of rice accessions as lines, varieties, germplasms to brown plant hopper (BPH) *Nilaparvata lugens* Stal. has been conducted since the 1970s by International Rice Research Institute (IRRI) followed by other countries, including Indonesia that are members of International Network for Genetic Evaluation of Rice or INGER. Up to 1975, the screening system use 7 to 10 days old rice seedlings and each seedling was infested with 5 to 10 nymphs. The scoring system for rice damage has been used on 0 to 5 scale: 0 = no apparent damage, 1 = initiation of wilting or yellowing in one leaf, 2 = initiation of wilting or yellowing in all leaves, 3 = complete

wilting of 50 to 70% of the leaves, central leaf surviving, 4 = all leaves wilted, stem green, 5 = plant dead (Kalode and Khrishma, 1979). In 1976, IRRI had revised the scoring system to become 0 to 9. After the changes in scores, Seshu and Kauffman (1980) has reported the results of rice lines testing to BPH since 1975 to 1979 in various countries that coordinated by IRRI. IRRI (1980) released a standard evaluation system (SES) of resistance rice accessions to BPH, as a effort to make uniform results in internationally screening with the scale of damage from 0 to 9. The testing procedure at IRRI is always changing, it is necessary to improve the procedures and obtain lines/varieties durable resistant. In the initial screening test the resistance of the rice accessions was infested by BPH nymphs of 2-3 instar as much 10 nymphs/seedling at 7 days seedling after sowing (Heinrichs *et al.*, 1985). In the 1985 itself already began to change procedures that was infestation BPH nymphs of 2-3 instar as

*Corresponding author: Baehaki, S.E.,

Entomological Society of Indonesia, Bandung Branch, Faculty of Agriculture, Padjadjaran University, Bandung-Sumedang Street Km 21-Jatinangor, West Java-Indonesia.

much 5 nymphs/seedling at 7 days seedling after sowing (IRRI, 1985) and continued until 2000. In the 2000s the test procedure changed again, with infestations of the BPH nymphs 2-3 instar as much 5 BPH/seedling to 5 days old seedling after sowing, with scores based to SES 1996 (IRRI, 1996). This test procedure lasted on 2002. Starting from 2003 until now the testing procedure changes again with infestations of 2-3 instar as much 8 BPH nymphs /seedling on the 5 days old seedling after sowing (IRRI 2003; IRRI 2010), with scoring damage assessment using SES 2002 (IRRI, 2002). Development resistant rice lines/cultivars base on genetically is generally considered to be the most economic and the effective way for controlling BPH. Rice resistant to BPH is recognized as a qualitative and quantitative trait. The genetic basis of the qualitative and quantitative BPH resistance has been well studied and at least 21 major resistance genes have been discovered from cultivated varieties and wild relatives (Chen *et al.*, 2007; Jena *et al.*, 2006; Yang *et al.*, 2004), but now breeders have been discovered 26 single gen plus digenic resistant (Baehaki, 2012), and the last time was discovered Bph 32 (Ren *et al.*, 2016). The objective of this study to determine standardize the stability of varieties reaction on the level infestation of BPH, the ability of BPH biotype, and to obtain differential varieties of Indonesia. Varieties differential with different resistant genes is differentiator to determine level of BPH biotypes.

MATERIALS AND METHODS

Resistance test of the differential rice varieties to BPH was done in the screen house of Indonesian Center for Rice Research in Sukamandi use split plot design for each BPH biotype. The main plot were 19 rice varieties and the sub plot were level of BPH (5, 8, 10 nymphs/seedling). The rice varieties consisting of 11 differential varieties namely TN1 (without the gene resistant to BPH), Mudgo (Bph1), ASD 7 (bph2), Rathu Heenati (Bph3+Bph17), Babawee (bph4), ARC10550 (bph5), Swarnalata (Bph6), T12 (bph7), Chinsaba (bph8), Pokalli (Bph9), PTB33 (Bph3+bph2), and 2 candidate differential rice varieties of Kencana Bali 53 and Kencana Bali 54, and then the 6 popular varieties of IR 26 (Bph1), IR 42 (bph2), IR 64 (Bph1 + 7QTLs), IR 74 (Bph3), Ciherang, and Hipa 4. Research activities was began with mass rearing of three BPH biotype. BPH biotype 1 was establish and maintenance since 1976 reared on Pelita I/1 (non gene resistant) of 30 days old after transplanted (doat). BPH biotype 2 was establish and maintenance since 1992 reared on IR26 (Bph1) of 30 doat. BPH biotype 3 was establish and maintenance since 1994 reared on IR42 (bph2) of 30 doat. To obtained uniform age of BPH nymphs by infested the number of many BPH parents on rice in a cage, after 1-2 nights all BPH parent removed, and eggs were laid inside the plant and after hatched all nymphs maintenance up to 2-3 instar for infesting to rice varieties tested. All rice varieties planted in a box 200 cm x 60 cm x 20 cm size by containing Lembang's soil. Soil in a box divided to three parts (three replications) and bordered with wood. Each part divided to many row in furrow of 20 cm long and each accession sown 25-30 seeds at each row. In edges row placed susceptible rice varieties TN1 to prevent escape from the BPH attack. Differential resistant varieties placed in middle row as the focus of the BPH pressure. On the other hand the other varieties are placed randomly between the susceptible varieties.

At 5 days old seedling the tested varieties are thinned to remains 20 seedlings per row. Then the each seedling were infested by 5, 8 and 10 BPH nymphs of 2-3 instar of each biotypes 1, 2 or 3. The infested BPH nymphs must be evenly distributed to all of seedlings in box. Therefore, the number of nymphs were infested depending on the number of tiller of seedling varieties. Scoring damage is done at 7-10 days after infestation at the moment 90% of susceptible check variety TN1 dead. Scores based on the Standard Evaluation System for Rice (SES) (IRRI, 2002 and IRRI, 2008) (Table 1), and the rating based on Seshu and Kauffman (1980). Determination of the final score and resistance level of rice accessions to BPH was based on the value of the modus of three replications. The modus score was 0, the accession was highly resistant (HR) level. The modus score was 1, the accession was resistant (R). The modus score was 3, the accession was moderately resistant (MR). The modus score was 5, the accession was moderately susceptible (MS). The modus score was 7, the accession was susceptible (S), and The modus score was 9, the accession was highly susceptible (HS). The BPH population and plant height was observed from each accessions on row at 5 days after infestation. All data were analyzed by analysis of variance (ANOVA) and differences in the value was tested by Duncan's Multiple Range Test (DMRT) in 5% least significance different (LSD) level.

RESULTS AND DISCUSSIONS

Stability of rice resistance

Based on the guidelines of INGER 2003 (IRRI 2003) and based to SES 2002 (IRRI 2002), reaction varieties to BPH biotype 1 as much 5 nymphs/seedling showed that the differential varieties of Mudgo (Bph1), ASD 7 (bph2), Rathu Heenati (Bph3+Bph17), Babawee (bph4), ARC10550 (bph5), Swarnalata (Bph6), T12 (bph7), Chinsaba (bph8), Pokalli (Bph9), PTB33 (Bph3 + bph2) were score 3 with rating moderately resistant. Similarly, the candidate differential varieties of Kencana Bali 53 and Kencana Bali 54 as well as the popular varieties of IR 64 (Bph1+7QTL), IR 74 (Bph3), Ciherang were moderately resistant. In the other hand IR26, IR42, and hybrid Hipa 4 were moderately susceptible and TN1 was highly susceptible. On infestation of BPH biotype 1 as much 8 nymphs/seedling be able to eliminate resistant rice varieties that resulted 5 nymphs/seedling. Rathu Heenati, Pokalli, PTB33, Swarnalata, IR 74, Kencana Bali 53, and Kencana Bali 54 were moderately resistant, whereas Mudgo, Babawee, ARC10550, T12, Chinsaba, ASD7, IR 64, and Ciherang changes from moderately resistant to moderately susceptible.

In the other hand IR 26 and IR 42 still on moderately susceptible level, whereas Hipa 4 change from moderately susceptible to susceptible. TN1 variety was highly susceptible. Infestation of BPH biotype 1 as much 10 nymphs /seedling did not able to eliminate the rice varieties that were resistant to BPH biotype 1 on 8 BPH nymphs /seedling, therefore Rathu Heenati, Pokalli, PTB33, Swarnalata, IR 74, Kencana Bali 53, and Kencana Bali 54 were stable on moderately resistant. In the other hand Mudgo, Babawee, ARC10550, T12, Chinsaba, ASD7, IR26, IR 64, IR42, and Ciherang were moderately susceptible, whereas ASD 7 and Hipa 4 was moderately susceptible and TN1 was highly susceptible (Table 2).

Table 1. Scoring and rating of rice resistance to brown planthopper

Scale	Symptom of SES (IRRI, 2002)	Modus rating rice resistant of 3 replications
0	No damage	HR = highly resistant
1	Very slight damage of a few plants with yellowing on leaves tip less than 1%	R = resistant
3	First and 2nd leaves of most plants partially yellowing	MR = moderately resistant
5	Pronounced yellowing and stunting or about 10 to 25% of the plants wilting	MS = moderately susceptible
7	More than half of the plants wilting or dead and remaining plants severely stunted or dying	S = susceptible
9	All plants dead	HS = highly susceptible

Table 2. Reaction of varieties to some level of BPH nymphs of biotype 1

Varieties	Genotype	Level BPH nymphs of biotype 1					
		5 BPH/tiller		8 BPH/tiller		10 BPH/tiller	
		Score modus	Rating	Score modus	Rating	Score modus	Rating
Rathu Heenati	Bph3+Bph17	3	MR	3	MR	3	MR
Pokalli	Bph9	3	MR	3	MR	3	MR
PTB33	Bph3+bph2	3	MR	3	MR	3	MR
Swarnalata	Bph6	3	MR	3	MR	3	MR
IR 74	Bph3	3	MR	3	MR	3	MR
Kencana Bali 53	?	3	MR	3	MR	3	MR
Kencana Bali 54	?	3	MR	3	MR	3	MR
Mudgo	Bph1	3	MR	5	MS	5	MS
Babawee	bph4	3	MR	5	MS	5	MS
ARC10550	bph5	3	MR	5	MS	5	MS
T12	bph7	3	MR	5	MS	5	MS
Chinsaba	bph8	3	MR	5	MS	5	MS
IR 26	Bph1	5	MS	5	MS	5	MS
IR 42	bph2	5	MS	5	MS	5	MS
IR 64	Bph1+QTLs	3	MR	5	MS	5	MS
Ciherang	?	5	MR	5	MS	5	MS
ASD 7	bph2	3	MR	5	MS	7	S
Hipa 4	?	5	MS	7	S	7	S
TN1	non	9	HS	9	HS	9	HS

Remarks: 0= highly resistant (HR), 1= resistant (R), 3= moderately resistant (MR), 5= moderately susceptible (MS), 7= susceptible (S), 9= highly susceptible (HS).

Table 3. Reaction of varieties to some level of BPH nymphs of biotype 2

Varieties	Genotype	BPH population of biotype 2					
		5 BPH/tiller		8 BPH/tiller		10 BPH/tiller	
		Score modus	Rating	Score modus	Rating	Score modus	Rating
Rathu Heenati	Bph3+Bph17	3	MR	3	MR	3	MR
Swarnalata	Bph6	3	MR	3	MR	3	MR
Pokalli	Bph9	3	MR	3	MR	3	MR
PTB33	Bph3+bph2	3	MR	3	MR	3	MR
IR 74	Bph3	3	MR	3	MR	3	MR
Kencana Bali 53	?	3	MR	3	MR	3	MR
Kencana Bali 54	?	3	MR	3	MR	3	MR
Mudgo	Bph1	3	MR	5	MS	5	MS
T12	bph7	3	MR	5	MS	5	MS
IR 64	Bph1+QTLs	3	MR	5	MS	5	MS
Ciherang	?	3	MR	5	MS	5	MS
Babawee	bph4	5	MS	5	MS	5	MS
ARC10550	bph5	5	MS	5	MS	5	MS
ASD 7	bph2	5	MS	7	S	7	S
Chinsaba	bph8	5	MS	5	MS	7	S
IR 26	Bph1	5	MS	7	S	7	S
Hipa 4	?	7	S	7	S	7	S
IR 42	bph2	5	MS	7	S	9	HS
TN1	non	9	HS	9	HS	9	HS

Remarks: 0= highly resistant (HR), 1= resistant (R), 3= moderately resistant (MR), 5= moderately susceptible (MS), 7= susceptible (S), 9= highly susceptible (HS).

Infestation of BPH biotype 2 as much 5 nymphs /seedling showed that the differential varieties of Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, Mudgo, and T12 were moderately resistant. The popular rice varieties IR 64, IR 74, and Ciherang were moderately resistant reaction. Babawee, ARC10550, ASD 7, Chinsaba, IR 26, and IR 42 were moderately susceptible, whereas Hipa 4 was susceptible and TN1 was highly susceptible reaction. Infestation of BPH biotype 2 as much 8 nymphs /seedling

showed that the differential varieties Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, and IR 74 were moderately resistant, whereas Mudgo, T12 and popular varieties of IR 64 and Ciherang changes to moderately susceptible reaction, and Babawee, ARC10550, and Chinsaba still in moderately susceptible. The other varieties ASD 7, IR 26, and IR 42 changes from moderately susceptible (in BPH biotype 2 as much 5 nymphs /seedling) to susceptible, hybrid Hipa 4 was susceptible and TN1 was highly susceptible reaction.

Table 4. Reaction of varieties to some level of BPH nymphs of biotype 3

Varieties	Genotype	BPH population of biotype 3					
		5 BPH/tiller		8 BPH/tiller		10 BPH/tiller	
		Score modus	Rating	Score modus	Rating	Score modus	Rating
Rathu Heenati	Bph3+Bph17	3	MR	3	MR	3	MR
Swarnalata	Bph6	3	MR	3	MR	3	MR
Pokalli	Bph9	3	MR	3	MR	3	MR
PTB33	Bph3+bph2	3	MR	3	MR	3	MR
IR 74	Bph3	3	MR	3	MR	3	MR
Kencana Bali 53	?	3	MR	3	MR	3	MR
Kencana Bali 54	?	3	MR	3	MR	3	MR
Mudgo	Bph1	3	MR	5	MS	5	MS
Babawee	bph4	5	MS	5	MS	5	MS
T12	bph7	5	MS	5	MS	5	MS
IR 64	Bph1+QTLs	3	MR	5	MS	5	MS
Ciherang	?	3	MR	5	MS	5	MS
ASD 7	bph2	5	MS	7	S	7	S
ARC10550	bph5	5	MS	5	MS	7	S
Chinsaba	bph8	5	MS	7	S	7	S
IR 26	Bph1	5	MS	7	S	9	HS
IR 42	bph2	7	S	7	S	9	HS
Hipa 4	?	7	S	7	S	9	HS
TN1	non	9	HS	9	HS	9	HS

Remarks: 0= highly resistant (HR), 1= resistant (R), 3= moderately resistant (MR), 5= moderately susceptible (MS), 7= susceptible (S), 9= highly susceptible (HS).

Infestation of BPH biotype 2 as much 10 nymphs /seedling did not able to eliminate the rice varieties that were resistant to BPH biotype 2 on 8 BPH/seedling, therefore Rathu Heenati, Pokalli, PTB33, Swarnalata, IR 74, Kencana Bali 53, and Kencana Bali 54 were stable on moderately resistant. In the other hand Mudgo, T12, Babawee, ARC10550 and popular rice varieties IR 64 and Ciherang were moderately susceptible. Rice varieties ASD 7, Chinsaba, IR 26, and Hipa 4 were susceptible, whereas IR 42 and TN1 were highly susceptible reaction (Table 3). Infestation of BPH biotype 3 as much 5 nymphs /seedling showed that the differential varieties of Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, Mudgo, IR 74, IR 64, and Ciherang were moderately resistant (score 3), whereas T12, Babawee, ASD 7, ARC10550, Chinsaba and popular IR 26 were moderately susceptible. Rice varieties IR 42 and Hipa 4 were susceptible, and TN1 was highly susceptible reaction. Infestation of BPH biotype 3 as much 8 nymphs /seedling showed that the differential varieties of Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, and IR 74 were moderately resistant, whereas Mudgo, IR 64, and Ciherang changes to moderately susceptible together group of T12, Babawee, and ARC10550. In the other hand ASD 7, Chinsaba, IR 26, IR42 and Hipa 4 were susceptible, and TN1 was highly susceptible. Infestation of BPH biotype 3 as much 10 nymphs /seedling showed that the differential varieties of Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, and IR 74 were stable in moderately resistant level. In the other hand Mudgo, T12, Babawee, IR 64, Ciherang were moderately susceptible. Rice varieties of ASD 7, ARC10550, and Chinsaba were susceptible, whereas IR 26, IR 42, Hipa 4, and TN1 were highly susceptible reaction (Table 4). Screening varieties to BPH biotype 1 with the infestation level 5, 8, and 10 BPH nymphs/seedling, obtain moderately resistant varieties were 78.9, 36.8 and 36.8% respectively. Screening varieties to BPH biotype 1 with the infestation level 5, 8, and 10 BPH nymphs/seedling obtain highly susceptible varieties were 5.3, 5.3, and 5.3% respectively (Figure 1).

From Figure 1 indicates that screening varieties with 5 BPH nymphs biotype 1/seedling will be obtained unstable rice resistant. In the other hand, suggested the screening varieties by 8 or 10 BPH biotype 1 nymphs/seedling that infested on 5 days old seedling were obtained 36.8% stabilize rice resistant.

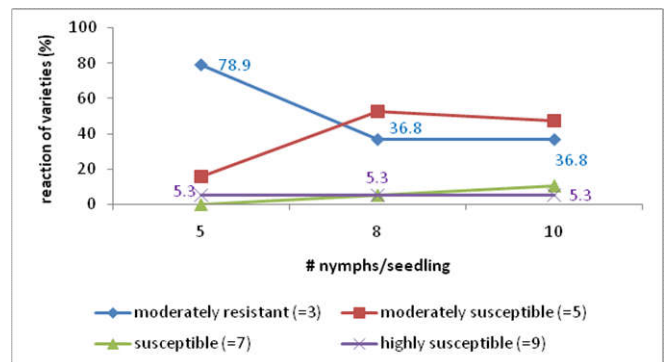


Figure 1. Decreasingly rice resistance on pressure of increasing level of BPH nymphs biotype 1

Screening varieties to BPH biotype 2 with the infestation level 5, 8, and 10 BPH nymphs/seedling obtain moderately resistant varieties were 57.9, 36.8 and 36.8% respectively. Screening varieties to BPH nymphs biotype 2 with the infestation level 5, 8, and 10 BPH nymphs/seedling obtain highly susceptible varieties were 5.3, 5.3, and 10.5% respectively (Figure 2).

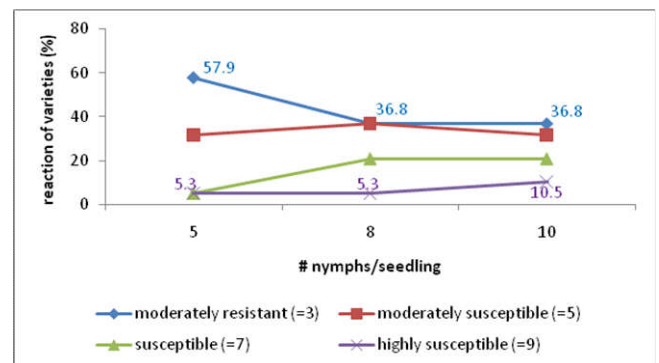


Figure 2. Decreasingly rice resistance on pressure of increasing level of BPH nymphs biotype 2

From Figure 2 indicates that screening varieties with 5 BPH nymphs biotype 2/seedling will be obtained unstable rice resistant. In the other hand, suggested the screening varieties by 8 or 10 BPH biotype 2 nymphs/seedling that infested on 5 days old seedling were obtained 36.8% stabilize rice resistant. Screening varieties to BPH biotype 3 with the infestation level 5, 8, and 10 BPH nymphs/seedling had obtained moderately resistant varieties as much 52.6, 36.8 and 36.8% respectively. Screening varieties to BPH nymphs biotype 3 with the infestation level 5, 8, and 10 BPH nymphs/seedling had obtained highly susceptible varieties as much 5.3, 5.3, and 21.1% respectively (Figure 3). From Figure 3 indicates that screening varieties with 5 BPH nymphs biotype 3/seedling will be obtained unstable rice resistant. In the other hand, suggested the screening varieties by 8 or 10 BPH biotype 3 nymphs/seedling that infested on 5 days old seedling were obtained 36.8% stabilize rice resistant.

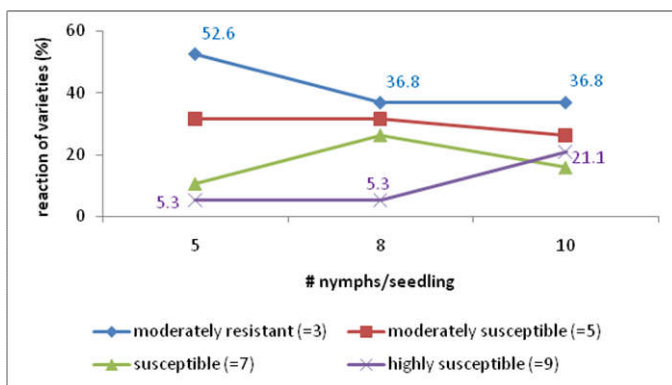


Figure 3. Decreasingly rice resistance on pressure of increasing level of BPH nymphs biotype 3

The above result did not gotten highly resistant or resistant varieties to all BPH biotype, although infested by 5 BPH nymphs/seedling on 5 days old seedling. In this research were obtained only moderately resistant against to all BPH biotype. These results differ from Seshu and Kauffman (1980), which reported from IRRI that Mudgo, ASD7, and PTB resistant to BPH biotype 1, while ASD7 and PTB resistant biotype 2, and PTB33 resistant to BPH biotype 3. This is due to the infestation of BPH in this study on 5 days old seedling as much 5, 8, and 10 BPH nymphs/seedling, while Seshu and Kauffman (1980) infestation BPH on 7 days old seedling approximately 5-10 BPH nymphs/seedling.

Also differ from Bhanu *et al.* (2014) that infested 2-3 instar approximately 8-10 BPH nymphs/seedling at 7 days old seedling, and also different in rating 0=immune, 1= highly resistant, 3 =resistant, 5= moderately resistant, 7= moderately susceptible, and 9= highly susceptible. Jairin *et al.* (2007) reported that the seedling and tillering stage, Rathu Heenati and PTB33 expressed strong resistance to BPH in both the standard seedbox screening (SSBS) and modified mass tiller screening (MMTS), but Rathu Heenati and PTB33 showed high resistance to BPH in the vegetative stage (seedling to tillering stages) of heavy BPH infestation, they showed susceptibility during the reproductive stage (flowering to grain filling stage) when the remaining BPH in the field moved to feed on the panicles and panicle necks until plants died. Resistance of rice variety is determined by research methodology. On scale 0 to 9 the PTB 33 was resistance to BPH, because the seedlings were 7 days old (at 3 leaf stage),

they were infested with 2nd instars nymphs at the rate of 3 nymphs/seedling (Madurangi *et al.*, 2010). IR26 highly susceptible with Bangladesh BPH population at infested 3.5 to 4 leaf seedling stage with the selected nymphs at a density of 10 nymphs per seedling (Yang *et al.*, 2011). Alagar and Suresh (2007) reported that 30 and 60 day old plants of ARC10550, KAU1661 and ARC6650 take significantly longer period for wilting than TN1. Similarly, Qiu *et al.* (2014) submitted bph7 in rice variety T12 to mainly account for tolerance component of resistance against BPH. Since tolerant trait is believed to exert less selection pressure on the insect, such gene may contribute to durable resistance. Genetic basis of antixenosis is just emerging. Only several QTLs linked to different parameters of antixenosis against both BPH and WBPH are reported in rice (Fujita *et al.*, 2013). Qiu *et al.* (2013) reported a QTL Qbph8 along with the major gene Bph6 in rice variety Swarnalata accounting for antixenosis in BPH. Further, less BPH insects were observed on Bph6+Qbph8 plants compared to the Bph6 or Qbph8 plants alone, indicating a stronger antixenotic effect in pyramided plants. A gene coding for sesquiterpene synthase (STPS) in Rathu Heenati is reported to influence antixenosis during the first 120 h of BPH interaction with the rice (Kamolsukyonyong *et al.*, 2013).

BPH resistance in rice cultivars carrying Bph3 was reported to govern an antixenotic reaction to BPH (Sexena and Okech, 1985). Rathu Heenati has no repellent chemical against planthoppers and only has common volatiles as released by susceptible cultivars. The other research showed that Rathu Heenati, PTB33, and Swarnalata had antibiosis and tolerance resistant mechanism to BPH (Baehaki dan Abdullah, 2008).

Differences malignancy of BPH, beside differences by biotype also determined by mitochondrial DNA sequences. In BPH, three haplotypes were found and all populations sampled shared a dominant haplotype. Localities in Korea contained two haplotypes AC and GA and localities in China and the Philippines contained three AC, GA, and AA, but in Bangladesh, Thailand, Vietnam, and Malaysia only one haploid type AC (Mun *et al.*, 1999). In one region some varieties and lines with the same resistance gene give differently reaction, even contradictory when tested in various locations of BPH origin. Variety IR72 (Bph3) was moderately susceptible to BPH from Kudus and Klaten, and then susceptible reaction to BPH from Pati. On the other hand, IR74 (Bph 3) was resistant to BPH from Kudus and moderately resistant to BPH from Pati and Klaten (Baehaki and Munawar, 2009). Likewise restless behavior of BPH on PTB33 as insects moved all over the leaf sheath to find suitable feeding site. It suggested that the test plants presented some mechanical barrier to penetration for probing or plant sap was not palatable to the insects (Sarao and Bentur, 2016).

The screening methodology to obtain the stability of the resistance rice varieties to BPH must be infested 2-3 instar as much 8 or 10 BPH nymphs/seedling on 5 days old rice seedling. This result similarity with SES-IRRI (IRRI, 2002) that infested 2-3 instar as much 8 BPH nymphs/seedling on 5 days old rice seedling. From our research showed that differential rice varieties of Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, and IR 74 was stable moderately resistant to BPH biotypes 1, 2, and 3. On the other hand that Kencana Bali 53 and Kencana Bali 54 as a local varieties of Indonesia can be used as differential varieties that

Table 5. Effect of BPH nymphs level that infested on rice seedling to reduced seedling height

Varieties	Rice genes	Seedling height (cm) on 5 days after BPH nymphs treatments		
		Biotipe 1	Biotipe 2	Biotipe 3
Population				
Population 5 BPH/seedling		9.67 a	7.24 a	8.43 a
Population 8 BPH/seedling		7.54 b	6.37 b	6.98 b
Population 10 BPH/seedling		6.78 c	6.58 b	6.32 c
Variety				
Rathu Heenati	Bph3+Bph17	6.66 h	7.05 ef	7.63 e
Pokalli	Bph9	10.16 bc	7.29 ef	8.22 d
PTB33	Bph3+bph2	6.19 hij	4.38 i	5.76 ijk
Swarnalata	Bph6	11.81 a	7.92 bcde	8.26 d
IR 74	Bph3	8.44f g	8.89 ab	8.41 cd
Kencana Bali 53	?	6.04 hij	5.04 hi	6.00 ghi
Kencana Bali 54	?	9.16 def	8.03 bcde	8.26 d
Mudgo	Bph1	9.80 cd	8.48 bcd	8.77 bc
Babawee	bph4	6.29 hi	5.58 gh	6.32 gh
ARC10550	bph5	10.71 b	9.78 a	9.47 a
T12	bph7	7.95 g	7.59 de	7.23 f
Chinsaba	bph8	9.29 de	8.68 bc	8.89 b
IR 26	Bph1	5.78 ij	4.60 hi	5.39 k
IR 42	bph2	6.27 hi	5.06 hi	5.91 hij
IR 64	Bph1+QTLs	8.56 efg	7.71 cde	7.14 f
Ciherang	?	6.82 h	5.04 hi	6.39 g
ASD 7	bph2	10.31 bc	6.54 fg	8.00 de
Hipa 4	?	6.25 hi	5.31 hi	6.08 ghi
TN1	-	5.45 j	4.96 hi	5.52 jk
Interaction Population x variety		F Value = 30.74 Pr > F = <.0001	F Value = 8.00 Pr > F = <.0001	F Value = 26.13 Pr > F = <.0001

were moderately resistant to BPH biotypes 1, 2, dan3. Likewise popular variety IR74 can be included as a comparison resistant in screening rice varieties to BPH.

Reducing plant height

The BPH populations can reduce height of rice seedling at 5 days after seedling infested and significantly different among treatments 5, 8, and 10 BPH nymphs/seedling. Reduction height of rice seedling when compared to height of untreated seedling, showed that the population of BPH biotype 1 as much as 5, 8, and 10 BPH nymphs/seedling reduced plant height were 33.8, 48.4, and 53.6%. The population of BPH biotype 2 as 5, 8, and 10 BPH nymphs/seedling reduced plant height were 50.4, 56.4, and 54.9% respectively. The population of BPH biotype 3 as much 5, 8, and 10 BPH nymphs/seedling reduced plant height were 42.3, 53.4, and 56.7% respectively (Table 5). BPH of all biotype were significantly reducing seedling height at every varieties and different to each other. All varieties become stunted in every attack by BPH biotype 1, 2, and 3. The level of reduction depends on the type of BPH nymphs population that infested to seedling, kind of resistant varieties that caused interaction between BPH population against rice variety on probability $Pr > F = <.0001$ (Table 5). BPH biotype 1, 2, 3 reduced height of rice seedling according to the number BPH that infested, height reduction is even greater in BPH infestation increasingly, although there are some varieties that the reduction was higher in the low population of BPH infested. Reduction seedling height in row by BPH biotypes 1 is almost entirely below 50%, but there are several varieties of IR 74, Kencana Bali 53, Ciherang, Hipa 4 between 50-60%, even reduction of T12 was 63% with population of 116 BPH nymphs/seedling row. On the other hand the reduction of PTB33 seedling reached 44.3% although the population reached 134 BPH nymphs/row seedling.

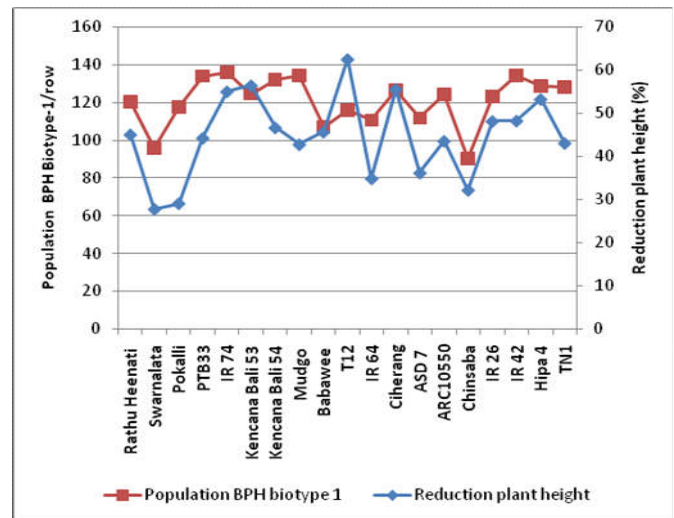


Figure 4. Relationship between BPH biotype 1 population in row rice seedling and plant height reduction

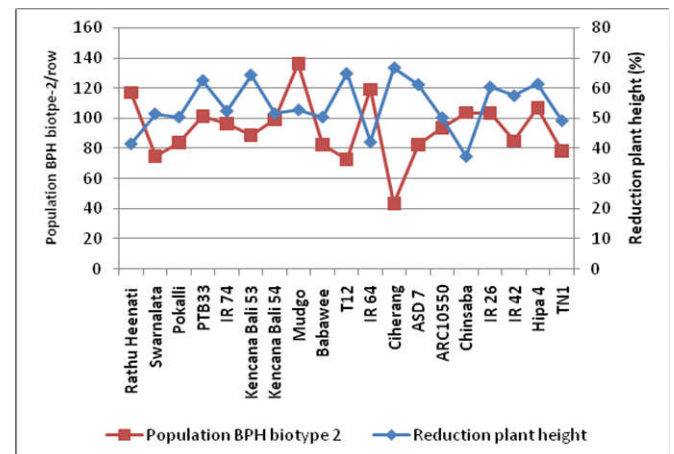


Figure 5. Relationship between BPH biotype 2 population in row rice seedling and plant height reduction

Reduction seedling height in the row by BPH biotype 2 between 50-60%, but there are several varieties PTB33, Kencana Bali 53, T12, ASD 7, IR 26, and Hipa 4 between 60-70%, including the very high reduction of Ciherang was 70%, although only 44 BPH biotype 2 nymphs/row seedling. On the other hand Rathu Heenati, IR 64, Chinsaba, and TN1 achieve its reduction less than 50% by population between 80-119 BPH/row seedlings (Figure 5). Reduction seedling height in the row by BPH biotype 3 mostly between 50-60% with BPH population between 117-134 BPH biotype-3 nymphs/row seedlings. In the other hand Rathu Heenati, Swarnalata, Pokalli, Babawee, IR 64, Chinsaba, IR 42, and TN1 reached less than 50% by population between 112-133 BPH/row seedlings (Table 6).

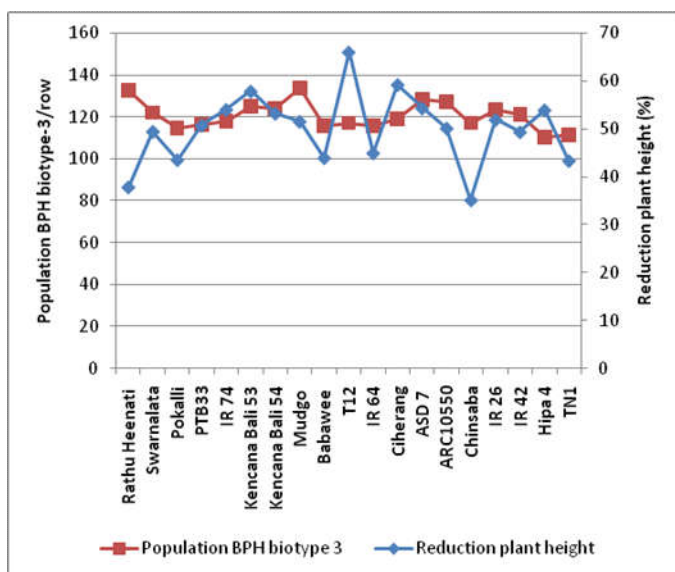


Figure 6. Relationship between BPH biotype 3 population in row rice seedling and plant height reduction

The stabilized varieties moderately resistant to all BPH biotype on screening varieties use 8 or 10 BPH nymphs/seedling those are Rathu Heenati, Swarnalata, Pokalli, PTB33, Kencana Bali 53, Kencana Bali 54, and IR 74, although did not showed consistency with the BPH population/row seedling or reduction seedling height. Reduction seedling height by BPH biotypes 1 almost all stabilized varieties moderately resistant below 50%, but IR 74 and Kencana Bali 53 between 50-60%. Reduction seedling height by BPH biotypes 2 on stabilized varieties moderately resistant Rathu Heenati was smaller than 50%, but the other stabilized varieties moderately resistant were height reduction more than 50%. Reduction seedling height by BPH biotypes 3 on stabilized varieties moderately resistant Rathu Heenati, Swarnalata, Pokalli less than 50%, but the other stable varieties were reduction height more than 50%.

The above analyses shows that Rathu Heenati was the most stabilized varieties moderately resistant in seedbox screening test, reduction of plant height, and the population of BPH/row seedling. The fact was urged by the stability of BPH resistance in Rathu Heenati, a traditional Sri Lankan rice cultivar containing Bph 3, has made this strain one of the most popular hopper resistance donors in the Mekong subregion, where rice production is highly intensive (Kamolsukyung *et al.*, 2013). The superiority variety of Rathu Heenati resulting polygenic resistant that recently progress that Rathu Heenati

carrying Bph3+Bph17 to BPH (Hu *et al.*, 2016). Bph17 is actually a cluster of three genes encoding plasma membrane-localized lectin receptor kinases (OsLecRK1—OsLecRK3), which collectively function to confer broad-spectrum, durable resistance and provide an important gene source for MAS and transgenic breeding for BPH resistance (Liu *et al.*, 2015). According to the rules of genetic nomenclature for rice, it is necessary for the authors of different reports to rename duplicated genes to avoid confusion to readers. Bph3 and Bph17 each described as single Mendelian factors in the resistant cultivar Rathu Heenati (RH) by different research groups. The rice scientific community has accepted the findings as Bph17 on chromosome 4 (Rahman *et al.*, 2009, Qiu *et al.*, 2012) and Bph3 on chromosome 6 (Jairin *et al.*, 2010). Rathu Heenati widely used as donor of resistant gene as Thai jasmine rice KDML105 (KD) is one of the most sensitive cultivars to BPH, and its BPH resistance has recently been improved by backcross introgression of the critical Bph3 region linked to RM589 on chromosome 6 from Rathu Heenati (Jairin *et al.*, 2009). Rathu Heenati shows the resistance against all of the BPH biotype, but three genes bph5, Bph6 and bph7 are resistant to biotype 4 but susceptible to biotypes 1, 2 and 3 (Kumari, 2009).

Conclusions

The seedbox mass screening method to obtain the stability of the resistance rice varieties to BPH must be infested 2-3 instar as much 8 or 10 BPH nymphs/seedling on 5 days old rice seedling. The stabilized varieties moderately resistant to all BPH biotype were Rathu Heenati (Bph3 + Bph17), Swarnalata (Bph6), Pokalli (Bph9), PTB33 (Bph3+bph2), Kencana Bali 53, Kencana Bali 54, and IR 74 (Bph3). The population of BPH nymphs reduced rice seedling height to cause stunting of rice seedling. Ability BPH nymphs to stunted rice seedling depend on to kind of BPH biotype, number BPH nymphs infested, and resistance rice varieties. Reduction seedling height by BPH biotypes 1 almost all stabilized varieties moderately resistant below 50%, but IR 74 and Kencana Bali 53 between 50-60%. Reduction seedling height by BPH biotypes 2 on stabilized varieties moderately resistant Rathu Heenati was smaller than 50%, but the other stabilized varieties moderately resistant were height reduction more than 50%. Reduction seedling height by BPH biotypes 3 on stabilized varieties moderately resistant Rathu Heenati, Swarnalata, Pokalli less than 50%, but the other stable varieties were reduction height more than 50%. The most stabilized rice variety was Rathu Heenati that stable on seedbox screening test, low reduction of plant height, and the little effect of population of BPH/row seedling. Kencana Bali 53 and Kencana Bali 54 can be used as differential varieties that were moderately resistant to BPH biotypes 1, 2, dan3. Likewise popular variety IR74 can be included as a comparison resistant in screening rice varieties to BPH. In the other hand screening methodology used 2-3 instar as much 5 BPH nymphs/seedling on 5 days old rice seedling resulted the unstable rice resistant.

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