Virulence of Brown Planthopper and Development of Core Collection of the Pest

Virulensi Wereng Batang Cokelat dan Pembentukan Koleksi Intinya

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ABSTRAK

Wereng batang cokelat (*Nilaparvata lugens* Stål) *merupakan hama utama tanaman padi di Indonesia. Kemampuan adaptasi dan reproduksi WBC pada varietas tahan cukup tinggi, menjadi biotipe yang lebih virulen dan menyebabkan terjadinya ledakan populasi dengan gejala tanaman "terbakar" dan puso. Pemuliaan untuk ketahanan padi terhadap wereng batang cokelat (WBC) memerlukan informasi tingkat virulensi lapang strain WBC untuk mengantisipasi berkembangnya WBC yang lebih ganas. Tujuan penelitian ini ialah mengetahui virulensi populasi WBC lapang dan mengelompokkan tingkat virulensi dalam koleksi WBC inti. Tiga belas populasi WBC yang dikumpulkan dari enam provinsi (Banten, Jawa Barat, Jawa Tengah, Jawa Timur, Kalimantan Selatan, dan Sulawesi Selatan) pada tahun 2011 dan 2013, diuji terhadap 10 varietas diferensial dan tujuh varietas inang populasi WBC, menggunakan teknik skrining baku. Berdasarkan respon ketahanan empat varietas diferensial utama (TN1, Mudgo, ASD7, dan Rathu Heenathi), sebagian besar populasi memiliki virulensi yang lebih ganas daripada biotipe 4 (T1, Banten; PG, Jawa Barat; BY, Jawa Timur; B2 dan B3, Kalimantan Selatan, X1 dan X3 (Sulawesi Selatan). Empat populasi termasuk biotipe 4 (JWDL, Jawa Tengah; SD, Jawa Timur; serta X2 dan X4, Sulawesi Selatan), dan masing-masing satu populasi diidentifikasi memiliki virulensi seperti biotipe 3 (T2, Banten) dan biotipe 2 (S1, Jawa Barat). Populasi B3 dan X1 virulen terhadap semua varietas uji, sedangkan T2 paling rendah tingkat virulensinya. Populasi WBC yang lebih virulen dari biotipe 4 telah berkembang di lapangan, sehingga skrining ketahanan galur-galur padi terhadap WBC sebaiknya menggunakan WBC dengan tipe virulensi tersebut. Lima kelompok WBC yang terbagi lagi menjadi 10 subkelompok yang mewakili kelompok virulensi terhadap 10 varietas differensial teridentifikasi dalam koleksi WBC yang diuji. Setiap kelompok virulensi dapat dibedakan berdasarkan kemampuannya untuk mengatasi 4-8 gen ketahanan tunggal maupun ganda pada tanaman padi. Koleksi virulensi inti ini dapat digunakan untuk krakterisasi ketahanan calon varietas dan galur padi isogenik, atau untuk studi genetik interaksi WBC dan tanaman padi.*

Kata kunci: Padi, wereng batang cokelat, virulensi, biotipe.

ABSTRACT

Brown planthopper is the most important rice pest in Indonesia. Its high adaptability to feed and reproduce on previously introduced resistant varieties to form more virulent population often causes BPH outbreak and hopperburn that lead to total crop yield loss. Rice breeding for resistant to BPH requires information on the current status of BPH virulences in the fields to anticipate the virulence adaptation on new varieties. The objectives of this study were to investigate the degree of virulence of BPH populations and to cluster the BPH virulence to form BPH core collection. Thirteen BPH populations collected from paddy fields in six provinces (Banten, West Java, Central Java, East Java, South Kalimantan, and South Sulawesi) in 2011 and 2013 were tested on 10 differential rice varieties and seven host varieties of BPH populations, using the standard seedbox screening technique. Based on resistance reaction of four differential varieties (TN1, Mudgo, ASD7, and Rathu Heenathi), most BPH populations were identified as more virulent than biotype 4 (T1, Banten, PG, West Java; BY, East Java; B2 and B3, South Kalimantan; X1 and X3, South Sulawesi), four populations were biotype 4 (JWDL, Central Java; SD, East Java; X2 and X4, South Sulawesi), and one population each was biotype 3 (T2, Banten) and biotype 2 (S1, West Java). Populations X1 and B3 showed broad virulences to all varieties, whereas T2 was the least virulent. BPH field's population had evolved into more virulence than biotype 4. Genotype resistance screening should use the BPH of this virulence population. Five BPH clusters which were further divided into 10 subclusters representing differential virulence toward 10 differential varieties were present in the tested BPH. Each virulence cluster was characterized by its ability to overcome four to eight single or double resistant genes. This BPH virulence core collection can be used in the characterization studies of candidate for resistant varieties or to form near-isogenic lines, or to study the insect and rice plant interaction.

Keywords: Rice, brown planthopper, virulence, biotype.

PENDAHULUAN

The brown planthopper (BPH) (*Nilaparvata lugens* Stål), is currently the most destructive insect pests of rice in Indonesia. Heavy infestation can cause hopperburn and may result in total crop loss. The highest BPH attack occurred in 1976/1977 which rendered 450,000 ha rice crop failed (Bahagiawati 2012). During 2005 to 2011 BPH infested area increased yearly and reached the highest in 2011 where 223,606 ha were infested and caused total loss of crop damage at about 36,000 ha (Tanindo 2011). After this period BPH infestation area were maintained between 30,000 to 40,000 ha (BBPOPT 2014). In addition to directly damage the crop, BPH can also indirectly damage plant by transmitting grassy stunt and ragged stunt viruses (Baehaki 2012a).

Variation in BPH virulence or the ability to adapt to resistant rice varieties has been recorded. According to the International Rice Research Institute (IRRI) system, BPH populations with distinct patterns of virulence as revealed by mass screening of test varieties carrying specific *Bph* (*Brown planthopper*) resistance genes, are termed as 'biotypes' and labeled with numbers (Brar *et al*. 2009). BPH population that can damage rice variety carrying *Bph1* gene is termed as biotype 2, population that can defeat *bph2* gene is biotype 3, whereas population that can overcome both genes is biotype 4. Among the biotypes there were minor morphological and chemical differences, and lack of significant breeding barriers (Claridge and den Hollander 1983, Claridge and Morgan 1987).

The emergence of new BPH biotypes that are able to damage rice varieties previously resistant to it is induced by selection pressure exerted by intensive cultivation of modern improved rice varieties carrying a single major *Bph* resistance gene. BPH biotypes in tropical Asia where resistant rice varieties were introduced from IRRI have been adopted massively, evolved faster than in the northern temperate Asia. In Indonesia biotype 1 appeared in 1971 in North Sumatera after the release of varieties IR5 and IR8 from the International Rice Research Institute (IRRI) in 1967 (Baehaki 2012a). After the introduction of IR26 (*Bph1* gene) in 1973 and 1974, biotype 2 developed in Indonesia and also in the Philippines, and Vietnam in 1976-1977 (Oka and Bahagiawati 1984, Brar *et al.* 2009, Baehaki 2012a). In 1980 IR36 and IR42 with the *bph2* gene were released and widely planted in the three countries. Only in one year after introduced, IR42 became susceptible in North Sumatera, Indonesia, to the infesting BPH population that was later identified as biotype 3 (Oka and Bahagiawati 1984, Baehaki 2012a). In northern temperate Asia, where BPH outbreaks caused by immigrating population from southerly tropical and subtropical regions, biotype 1-4 appeared later. Biotype 1-3 gradually increased in 1980 and 1988 in Korea and in 1988-1990 in Japan; BPH population which could survive on ASD7 (*bph2* gene) increased in 1997 in Japan and in 1998 in Southern China (Seo *et al*. 2009).

Baehaki (2012a) reviewed the temporal and spatial distribution of BPH biotypes in Indonesia. In 1985 biotype 1 and 2 predominated all rice growing areas, whereas biotype 3 population was minor. The distribution of biotype 3 increased in Sumatera, Java, and South Sulawesi in 1995, 1998, and 2006; biotype 4 was detected in 2006 in North Sumatera and 2010 in Lampung. Mixtures of two or three biotypes among biotype 2-4 were often found in the same area, but biotype 1 was no longer detected. The delay in the virulence adaptation of biotype 3 to biotype 4 in Indonesia was contributed by the introduction of IR64 in 1986 (Baehaki 2012a). The resistance durability of IR64 in the field was assumed due to *Bph1* gene and quantitative trait loci (QTLs)/other minor genes (Cohen *et al*. 1997).

Resistant rice varieties have been advocated as the most economical control measure of BPH outbreaks, and therefore breeders continuously seek for high yielding varieties with acceptable resistance level to BPH. Recent screening tests recommended the use of BPH biotype 3 as selection agent for resistance of promising rice lines (Baehaki and Munawar 2013). Given the highly virulence adaptability of the pest, field BPH might now have shifted to the more virulent ones, and therefore the current status of field BPH virulence should be investigated.

The main objective of this study was to determine the virulence of BPH population collected from six provinces in Indonesia. From the studied populations we developed core set of BPH populations with differential virulence toward varieties carrying defined *Bph* resistance genes. The core collection will provide breeders wide choice of BPH virulence for screening and characterization of their candidate varieties rather than using laboratory BPH biotype only.

MATERIALS AND METHODS

BPH population

This study was carried out from March 2013 to September 2015 under a glasshouse condition at Cikeumeuh Glasshouse Compound of ICABIOGRAD. Twelve BPH populations were collected from BPH endemic rice fields in Banten, West Java, South Kalimantan, and South Sulawesi Provinces in 2013. Sampling location was chosen under consultation with the local agricultural office. Adult BPH were collected by using a hand-made aspirator and then released into open top screen-

covered transparent plastic bottles containing trimmed plants of their respective host varieties. Hundreds of gravid females could be collected, but in some cases where the field had just received insecticidal spray or had just harvested, only as few as 20 females could be obtained. Nine populations produced sufficient gravid females for virulence studies. Four additional populations were also included in the test; three of them were selected from 16 populations collected from Central and East Java in 2011 by Habib Rijzaani (ICABIOGRAD) and one population obtained from Baehaki S.E. (ICRR). In total, 13 BPH populations from six provinces were tested in this study (Table 1).

Mass Rearing of BPH

BPH were mass reared in cages with glass door and top; and wire mesh sidewalls, under glasshouse condition following the procedure of Baehaki (2012b). The insects were reared on 45 to 50 days old (d.o.) plants of their respective rice host cultivars (Table 1). Feeding plants were replaced twice a week or as necessary. Each BPH population was maintained on each host variety to preserve their genetic make-up. Nymphal culture used in the virulence test was prepared by releasing 300 gravid females into cages containing 12 pre-cleaned 45-50 d.o. potted plants which were raised under a glasshouse condition. After three nights, the ovipositing females were removed from the cages. The emerged nymphs were maintained until molted into their 2nd and 3rd instars.

Virulence Tests of BPH

Tests were done using the standard seedbox screening technique following the procedure of Baehaki (2012b). Seeds of 10 differential varieties with known BPH resistance genes and six host and popular varieties were dried in an oven (50°C) for overnight and pre-germinated on moistened filter paper. Twenty germinating seeds from each variety were planted in 2.5 cm-distant apart rows in wooden boxes (60 cm × 45 cm × 10 cm) with holes at the bottom and containing mud soil. TN-1 (susceptible check, carrying no *Bph* resistance genes) was planted in two rows per box at the center and the left or right side of the box. The 2nd and 3rd nymphs were evenly spread on the seedlings at 5 to 7 days after planting at the rate of 8 nymphs per seedlings using tapping method. After infestation the boxes were covered with cages having glass top, front, and back; and wire mesh at the other sidewalls. Tests were done sequentially according to the first population having the most abundant gravid females, but in some cases two populations could be tested simultaneously. BPH populations were tested at about 3 to 21 generations of the insects after collected from the field.

Plant damage was evaluated when ≥ 90% plants of the susceptible variety were dead using per plant basis scoring following the method of Sun *et al*. (2005) and Kumari *et al.* (2010) (Table 2). The plant resistance were divided into 6 categories according to Baehaki and Munawar (2008), where a variety with score 0-1 is categorized as highly resistant, >1-3 resistant, >3-5 moderately resistant, >5-7 moderately susceptible, >7-8 susceptible, and score >8 is highly susceptible. For the purpose of BPH biotype designation, plant resistance criteria were simplified into two classes: resistant (damage score ≤5) and susceptible (score >5). BPH population was designated as biotype 1 if it attacks TN-1; biotype 2 if it severely damages both TN-1 and Mudgo (*Bph1* gene); biotype 3 if it devastates both TN-1 and ASD7 (*bph2* gene); and biotype 4 if it attacks the three varieties (Brar *et al.* 2009). The use of 'biotype' term is problematic and there has been no consensus on the

Table 1. Brown planthopper tested in virulence study.

No.	Population	Year collected	Host variety	Province	Regency
1.	T1	2013	Inpari 13	Banten	Serang
2.	T2	2013	Inpari 13	Banten	Serang
3.	S1	2013	Manohara	West Java	Bekasi
4.	PG[1]	2011	Ciherang	West Java	Karawang
5.	JWDL[1]	2011	Ciherang	Central Java	Klaten
6.	SD[1]	2011	Ciherang	East Java	Lamongan
7.	BY[2]	2011	IR64	East Java	Banyuwangi
8.	B2	2013	Karang Dukuh Kuning	South Kalimantan	Barito Kuala
9.	B3	2013	Inpari 13	South Kalimantan	Hulu Sungai Selatan
10.	X1	2013	Ciliwung	South Sulawesi	Maros
11.	X2	2013	Ciliwung	South Sulawesi	Gowa
12.	X3	2013	Ciliwung	South Sulawesi	Takalar
13.	X4	2013	Memberamo	South Sulawesi	Jeneponto

[1]Obtained from Habib Rijzaani (ICABIOGRAD)
[2]Obtained from Baehaki S.E. (ICRR)

numbering system (Claridge and Morgan 1987). Therefore, we restrict the "biotype" numbering to 4 and BPH population that damages Rathu Heenathi (*Bph3* gene) was labeled as more virulent than biotype 4.

Development of BPH Core Collection Based on Their Virulence

To develop BPH core collection with specific virulence on different *Bph* genes, cluster analysis was performed based on BPH virulence data on 10 differential varieties (TN1 [none], Mudgo [*Bph1*], ASD7 [*bph2*], Rathu Heenathi [*Bph3*], Babawee [*bph4*], ARC10550 [*bph5*], Swarnalata [*Bph6*], T12 [*bph7*], Pokkali [*Bph9*], and PTB33 [*bph2*, *Bph3*]). BPH virulence data were converted into binary values: virulent BPH (damage score d"5) and avirulent (score >5) were scored as '0' and '1', respectively. The binary data was then used to generate similarity data matrix among BPH population using the Simple Matching (SM) coefficient of similarity available in the SimQual module of NTSYS 2.02 (Applied Biostatistics) using the following formula (Wikipedia 2015):

$$\text{SMC} = \frac{\text{Number of Matching Attributes}}{\text{Number of Attributes}}$$

$$= \frac{M_{00} + M_{11}}{M_{00} + M_{01} + M_{10} + M_{11}}$$

where SMC is simple matching coefficient; M_{00} is the total number of attributes where *x* and *y* both have a value of 0; M_{01} is the total number of attributes where *x* is 0 and *y* is 1; M_{10} is the total number of attributes where *x* is 1 and *y* is 0; and M_{11} is the total number of attributes where *x* and *y* both have a value of 1. The resulting similarity matrix was subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm in the Sequential, Agglomerative, Hierarchical and Nested (SAHN) module of NTSYS 2.02. The UPGMA algorithm constructs a dendrogram that reflects the structure present in a pairwise similarity matrix. At each step of clustering, the nearest two clusters are combined into a higher-level cluster. The distance between any two clusters A and B is taken to be the average of all distances between pairs of objects *x* in A and *y* in B (Wikipedia, 2015). The mean distance between elements of each cluster is:

$$\frac{1}{|\mathcal{A}| \cdot |\mathcal{B}|} \sum_{x \in \mathcal{A}} \sum_{y \in \mathcal{B}} d(x, y)$$

Table 2. Criteria for evaluation of damage per plant caused by brown planthopper (Sun *et al*. 2005, Kumari *et al*. 2010).

Score	Plant status
0	None of the leaves shrank and the plant was healthy
1	Very slight damage or one leaf showed yellowing
3	Partial yellowing of the 1st and 2nd leaf or 1 leaf shrank
5	Pronounced yellowing of half of the plant or 1-2 leaves shrank or one leaf shriveled
7	Wilting of more than half of the plant or leaves shriveled but plant was still alive
9	Whole plant dead

RESULTS AND DISCUSSION

BPH Virulence

The timing for scoring of plant damage, which was at the time when ≥90% seedlings of TN-1 were dead, occurred at 3 to 10 days after the nymphal infestation. Virulence data on four differential varieties (TN-1, Mudgo, ASD7, and Rathu Heenathi), showed that BPH population with virulence level higher than biotype 4 (T1, Banten; PG, West Java; BY, East Java; B2 and B3, South Kalimantan; X1 and X3, South Sulawesi) was dominant than biotype 4 (JWDL, Central Java; SD, East Java; X2 and X4, South Sulawesi) (Table 3). Contrary to our expectation, BPH populations with biotype 2 virulence (S1, West Java) and 3 (T2, Banten) still existed. Across all varieties, X1 was the most virulent population (average virulence score 8.1) followed by B3 (average virulence score .9), whereas T2 was the least virulent (average virulence score 4.9).

Virulence of field BPH across four differential varieties (TN1, Mudgo, ASD7, and Rathu Heenathi) in this current study was higher than that observed less than a decade ago. Baehaki and Munawar (2008) reported that, without addressing the cultivated varieties, BPH populations with biotype 3 virulence were dominant in 2006 in rice fields, whereas biotypes 2 and 4 were rare. Later, biotype 4 was detected in Central Java, East Java, and Lampung (Baehaki 2012a). Biotype 2 populations were still found in North Sumatera, East Java, Lampung, and South Sulawesi, but mixed with biotype 3 or biotype 4. Because of its dominance in the field, BPH biotype 3 was recommended for screening of promising lines (Baehaki and Munawar 2013). The current situation shows that field BPH virulence has evolved to the more virulence ones, and therefore rice resistance test should use population that can defeat all *Bph* resistance genes such as B3 and X1. Otherwise, the resistant rice variety to be released might succumb to BPH infestation in case the field population had already been adapted to the gene for resistance in the candidate variety. Among the resistance genes, *Bph6* and the multiple resistance genes

Table 3. Plant damage scores of 17 rice varieties infested with 13 brown planthopper population (BPH, *Nilaparvata lugens*) collected in Java, South Kalimantan, and South Sulawesi.

1	2	Rice variety and brown planthopper resistance gene																			
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22[b]
T1	Mean[a]	8.7	8.5	8.0	6.0	7.1	8.2	3.4	8.4	5.6	6.1	5.8	8.4	8.0	4.5	8.5	7.6	7.5	7.0	7.1	>4
	S.D.	1.5	1.9	2.4	3.7	3.5	2.3	3.6	1.6	2.9	3.7	3.9	1.7	2.5	3.8	1.8	2.9	2.6			
T2	Mean	9.0	3.7	8.3	1.3	8.3	9.0	1.0	NT[c]	5.0	1.7	3.0	6.3	5.7	1.0	7.7	6.3	1.7	5.3	4.9	3
	S.D.	0.0	3.1	1.2	1.5	1.2	0.0	0.0	NT	3.5	1.2	3.5	2.3	3.1	0.0	2.3	3.1	1.2			
S1	Mean	8.3	7.7	4.3	3.7	7.0	9.0	1.3	NT	5.7	2.3	3.7	7.0	7.0	3.7	8.3	4.3	3.7	5.5	5.4	2
	S.D.	1.6	2.3	4.2	4.6	2.0	0.0	1.5	NT	3.1	2.3	4.6	3.5	2.0	4.6	1.2	4.2	4.6			
PG	Mean	8.8	8.9	8.7	8.1	8.7	8.7	3.9	8.9	5.5	6.7	7.1	5.5	8.5	6.5	8.4	8.6	7.4	7.7	7.6	>4
	S.D.	1.2	0.4	1.2	1.8	1.1	1.2	3.1	0.9	2.7	2.3	2.6	3.8	1.8	2.8	1.6	1.2	2.7			
JWDL	Mean	8.8	8.3	9.0	4.3	9.0	2.8	5.7	8.6	5.5	3.8	5.5	6.8	8.3	4.3	8.6	6.5	5.2	6.6	6.5	4
	S.D.	1.2	1.1	0.3	3.8	0.3	2.9	3.0	1.2	3.2	3.5	3.2	2.7	1.9	3.7	1.8	3.0	3.3			
SD	Mean	8.2	8.0	8.3	3.0	7.0	8.6	3.4	8.9	5.3	2.0	4.9	7.0	7.3	5.2	8.6	7.1	4.4	6.3	6.3	4
	S.D.	2.4	1.7	1.6	3.0	2.7	1.4	2.8	0.8	2.7	2.7	3.3	2.6	2.8	3.4	1.5	2.7	3.4			
BY	Mean	9.0	9.0	8.7	6.2	8.7	9.0	1.4	8.8	4.9	3.6	6.0	7.9	7.3	2.6	8.5	7.2	4.4	6.9	6.7	>4
	S.D.	0.0	0.3	1.1	3.6	1.1	0.0	2.7	0.9	2.8	3.7	3.1	2.3	2.8	3.1	1.8	3.0	3.3			
B2	Mean	8.9	8.2	8.8	6.2	8.6	8.8	1.4	7.7	8.0	4.2	7.8	6.9	8.0	6.1	7.8	7.3	6.9	7.1	7.2	>4
	S.D.	1.0	1.4	0.6	2.3	1.1	0.6	2.5	1.8	1.2	3.2	1.8	2.1	1.7	2.3	2.1	2.1	2.5			
B3	Mean	9.0	8.8	9.0	5.3	8.4	9.0	5.7	8.9	7.6	7.1	7.6	8.4	8.7	6.6	8.8	7.7	7.5	7.9	7.9	>4
	S.D.	0.2	0.7	0.0	4.0	1.9	0.5	3.9	0.4	2.7	3.5	2.9	1.7	1.3	3.1	1.1	3.0	3.0			
X1	Mean	9.0	9.0	9.0	8.1	8.9	9.0	6.0	9.0	8.0	6.3	7.9	8.6	7.3	6.4	8.2	8.2	8.5	8.2	8.1	>4
	S.D.	0.0	0.0	0.0	2.3	0.4	0.0	3.4	0.3	2.3	3.3	2.3	1.3	3.1	3.5	2.2	2.3	1.2			
X2	Mean	8.8	7.8	8.8	3.8	6.6	4.9	2.9	6.6	3.9	1.9	3.8	2.4	7.5	6.7	6.9	5.8	4.7	5.6	5.5	4
	S.D.	1.1	2.4	1.1	4.3	3.6	4.2	4.0	3.7	3.0	3.4	4.0	3.3	3.2	3.7	3.4	3.2	4.2			
X3	Mean	8.9	7.2	8.9	6.1	8.2	8.7	3.9	8.1	6.0	4.2	6.7	8.1	7.9	5.5	8.2	6.2	5.5	7.0	7.0	>4
	S.D.	0.5	2.2	0.6	3.4	2.1	1.2	3.1	1.6	3.0	3.3	3.0	2.3	2.3	3.2	2.1	3.4	3.6			
X4	Mean	9.0	7.6	9.0	4.7	9.0	9.0	3.7	8.8	3.8	5.4	5.0	7.1	5.9	5.2	9.0	6.1	6.1	7.0	6.7	4
	S.D.	0.0	3.0	0.0	4.1	0.3	0.0	4.3	1.1	4.2	4.0	4.3	3.4	3.8	4.2	0.3	3.9	3.7			
Mean variety (resistance)[d]		8.8 (HS)	7.9 (S)	8.4 (HS)	5.1 (MS)	8.1 (HS)	8.1 (HS)	3.4 (MR)	8.4 (HS)	5.8 (MS)	4.3 (MR)	5.8 (MS)	7.0 (S)	7.5 (S)	4.9 (MR)	8.3 (HS)	6.8 (S)	5.7 (MS)			

[a] Average of 60 scores (3 replicates of 20 seedlings each)
[b] Based on virulence on TN1 (carrying no *Bph* resistance gene), Mudgo (*Bph1* gene), ASD7 (*bph2* gene), and Rathu Heenathi (*Bph3, bph17* genes)
[c] NT = not tested
[d] 0-1 = highly resistant (HR), >1-3 = resistant (R), >3-5 = moderately resistant (MR), >5-7 = moderately susceptible (MS), >7-8 = susceptible (S), >8 = highly susceptible (HS)

1 = BPH population
2 = Mean ± standard deviation) (S.D.)
3 = TN-1 (none)
4 = Mudgo (*Bph1*)
5 = ASD7 (*bph2*)
6 = Rathu Heenati (*Bph3*)
7 = Babawee (*bph4*)
8 = ARC10550 (*bph5*)
9 = Swarnalata (*Bph6*)
10 = T12 (*bph7*)
11 = Pokkali (*Bph9*)
12 = PTB 33 (*bph2*, *Bph3*)
13 = IR 64 (*Bph1*+)
14 = Ciherang (unknown)
15 = Ciliwung (unknown)
16 = Inpari 13 (unknown)
17 = Karang Dukuh Kuning (unknown)
18 = Memberamo (unknown)
19 = Manohara (unknown)
20 = Mean virulence on 10 differential varieties
21 = Mean virulence on all varieties
22 = Biotype designation

bph2+*Bph3* were still effective to most BPH populations. Incorporation of these genes into our commercial varieties is expected to prolong resistance durability in the field before emergence of new virulent BPH.

The virulence level of field BPH in Indonesia is higher than that of BPH population in Thailand. Out of 17 BPH populations selected from 45 populations collected from 31 provinces in Thailand, 13 populations showed biotype 4 virulence pattern and the remaining populations were of biotype 2 and 3 virulence. All populations were non-virulent to *bph2* + *Bph3* genes and 15 populations were non-virulent to *Bph6*. These resistance genes also showed resistance to biotypes 1 to 4 in the Phillipines, Bangladesh, China, and Japan (Jairin *et al*. 2007).

The mechanism by which field BPH is capable of rapid adaptation to new rice varieties can be explained by its complex genotypes for virulence. BPH is a very unstable species in virulence characteristics because of overlapped and considerable individual variations in virulence characteristics among individuals within a population, and the proportion of virulent individual makes up the virulence level of that population (Claridge and den Hollander 1983). Long-term planting of one variety in large areas would be expected to put strong selection pressure on virulent individuals leading to the formation of new BPH virulent that potentially cause serious outbreaks and hopperburn. Experiments in laboratory and small scale field trial showed that BPH virulence can totally change in at least five generations after successive selection on the same variety (Nemoto and Yokoo 1994, Jing *et al.* 2011). We assumed that the wide and continuous planting of varieties resistant to biotype 3 has selected virulent individuals that later developed virulence into biotype 4 and higher. In the six studied provinces, 20 to 84% of the total area in each province was planted with Ciherang, followed by Ciliwung, IR64, and other varieties including local varieties (Puslitbangtan 2014). Ciherang and Ciliwung were released in 2000 and 1989, respectively, to replace IR64; both of them had IR64 in their pedigree and had similar resistance level to biotype 3 (Suprihatno *et al*. 2010).

We do not preclude the possibility that the detection of field BPH with biotype 2 (S1) and 3 (T2) as few as one population and the presence of differential virulence among populations collected from the same variety within neighborhood areas (T1 and T2 from variety Inpari 13 in Banten, and X1-X3 from variety Ciliwung in South Sulawesi) may be due to the artifact of sampling process in the field. When BPH culture is established from a relatively small number of individuals with low proportion of virulent ones, the population would develop virulence level much slower than BPH culture that is started from hundreds of individual which possibly contained higher proportions of virulent individuals. Therefore, the virulence level showed by its offsprings in the test may not reflect the actual virulence in the field. The low virulence level of population S1 may also due to its slow adaptability to its host, Manohara, a locally bred variety released in Bekasi, West Java, in 2010 (Jamaludin 2011).

BPH Core Collection Based on Its Virulence

Based on UPGMA clustering analysis, the virulence of 13 BPH populations toward 10 differential varieties can be divided into five virulence clusters at the similarity coefficient of 0.86 (Figure 1). The main clusters were further divided into 10 subclusters based on their avirulence toward one to four single or double resistance genes in the host. Three pairs of BPH populations shared identical virulence level and therefore they occupied the same branches in the dendrogram. These populations were B2-X3 in cluster I, B3-X1 and T1-PG in cluster II.

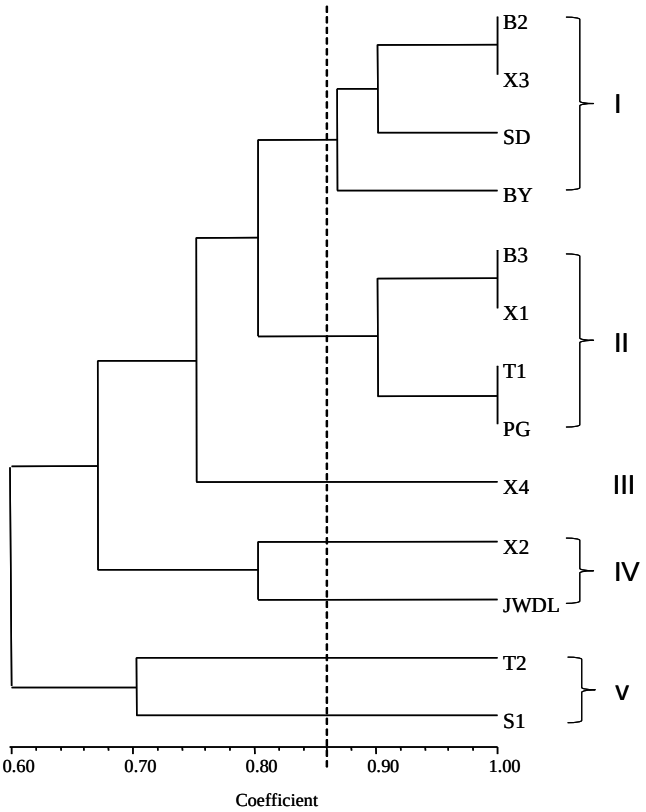

Figure 1. UPGMA clustering of 13 brown planthopper populations collected from Java, South Kalimantan, and South Sulawesi based on Simple Matching (SM) coefficient of plant damage scores of ten differential varieties (TN1 [none], Mudgo [*Bph1*], ASD7 [*bph2*], Rathu Heenathi [*Bph3*], Babawee [*bph4*], ARC10550 [*bph5*], Swarnalata [*Bph6*], T12 [*bph7*], Pokkali [*Bph9*], and PTB33 [*bph2*, *Bph3*]).

Cluster II contained most virulent populations: B3 and X1 which were virulent to all resistance genes, and T1 and PG that were non-virulent to one resistance gene (*Bph6*) only (Table 3). Four populations in cluster I (B2, X3, SD, and BY) were incapable of defeating *Bph6* and *bph2+Bph3* genes; member of this cluster could be discriminated to each other by lack of the ability to defeat *Bph3* (population SD) or *Bph9* gene (population BY; Table 1). Cluster III contained population X4 which were non-virulent to *Bph3*, *Bph6*, and *Bph9*. Member of cluster IV were populations X2 and JW which could not overcome *Bph3*, *bph5*, and *bph2+Bph3*; X2 can be differentiated from JWDL by inability to defeat *Bph6* and *Bph9* genes. The last cluster which contained populations T2 and S1 were non-virulent to *Bph3*, *Bph6*, and *bph2+Bph3* genes; both populations is discriminated by their inability to damage the resistance genes *Bph1* (population T2) or *bph2* (population S1).

To our knowledge, a large collection of BPH populations showing differential virulence has not been maintained in Indonesia despite extensive virulence studies in the past (Baehaki 2012a). We have not obtained all BPH virulence variations toward the differential varieties. Given the considerable virulence variability among BPH individuals, the chance of finding new virulence variations in the field is great. Therefore, studies on temporal and spatial distribution of BPH should be continued. Once other virulence variation is detected, the list of BPH virulence group could be extended.

The BPH virulence core collection we developed will be useful for characterizing resistance of candidate rice variety and rice near-isogenic lines (NILs) which are being developed in our institute. Before it can be utilized, purification of each population to form inbred population is necessary. The most laborious task in BPH collection maintenance is preparation of feeding plants because it is prone to mishandling, i.e. mismatch between host variety and BPH population. It may be possible to culture BPH on a universally susceptible variety lacking no *Bph* genes. Mint *et al*. (2009) reported that BPH populations retained their virulence level as when they were collected from the field even though they have been long-laboratory cultured on Reiho, a variety with no BPH resistance genes.

We use "biotype" terminology in a non-specific sense to denote similar phenotypic virulence patterns of BPH population and we limit the biotype numbering to 4 according to the IRRI standard. When the observed virulence patterns on 10 differential varieties were compared to those of biotype 1 to 4 on the complete 10 differential varieties described by Brar *et al*. (2009), none of our biotype 2-4 designation conform perfectly the virulence patterns in the list. As described by Brar *et al.* (2009), biotypes 1 to 3 are also virulent to ARC10550 (carrying *bph*5 gene), Swarnalata (*Bph6*), T12 (*bph7*), and Pokkali (*Bph9*); whereas biotype 4 is virulent to Pokkali. If any variation in the virulence patterns were designated as "new biotypes", then the potential number of BPH biotypes would be unmanageable, while "biotype" terminology is potentially misleading in BPH-rice system. According to Diehl and Bush (1984), if each insect biotype differed in at least one gene specifically matching one of the host's resistance genes, there are potentially 2^n possible biotypes. Because of the exponential relationship between the number of resistance genes and possible biotypes, the potential number of biotypes becomes infinite and the utility or feasibility of biotype designation becomes questionable. Therefore, Claridge and Morgan (1987) advised that the application of this term to BPH virulence should be temporary and the numbering should not be extended. They argued that biotypes in BPH refer to selected populations rather than genetically distinct and diverging host races. The term "biotype" for BPH population should be provisional until a clear gene for gene relationship between virulence in BPH and rice resistance in the rice plant have been established like the case for Hessian fly (*Mayetiola destructor*)–wheat interaction for which 16 biotypes have been identified on the basis of their differential response to four resistance genes in the host (Carrera 2013).

Classical genetic studies concluded that BPH virulence was under polygenic control with at least two genes operating (Cheng 1985, Tanaka 1999), but recent genetic mapping studies using molecular marker found a gene-for-gene relationship between biotype 2 virulence and *Bph1* gene (Kobayashi *et al*. 2014). Further genetic mapping studies in the insect should be carried out to ascertain the genetic basis of 'biotype' differences in BPH. To this end, the BPH virulence core collection we developed will serve as a valuable resource for genetic studies in the insect and the host.

CONCLUSIONS

Virulence studies of 13 BPH populations collected from paddy fields in six provinces (Banten, Central Java, East Java, West Java, South Kalimantan, and South Sulawesi) showed that the field populations was dominated by BPH virulence higher than biotype 4 (7 populations) followed by populations with biotype 4 virulence (4 populations). Populations with biotype 3 and 2 virulence were still detected in Bekasi (West Java and Banten). Because of its current dominance in the field, BPH with virulence higher than biotype 4 should be used in

resistance screening of candidate rice varieties to anticipate rapid virulence adaptation of BPH. Cluster analysis obtained five BPH clusters which were further divided into 10 subclusters representing differential virulences toward 10 differential varieties. The 10 virulence groups will be maintained as BPH virulence core collection that will be useful for characterizing candidate resistant rice varieties and rice NILs and also genetic studies of the insect and rice plant.

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