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SSR MARKERS REVEALED GENETIC DIVERGENCE OF RICE BROWN PLANTHOPPER POPULATIONS MAINTAINED ON TWO SETS OF DIFFERENTIAL HOST VARIETIES

Penanda SSR Mengungkap Perbedaan Genetik Populasi Wereng Batang Cokelat Padi yang Dipelihara pada Dua Set Varietas Inang Diferensial

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ABSTRACT

Resistance screening of promising rice lines in Indonesia requires the use of brown planthopper (BPH) biotypes 1, 2, and 3. Three BPH populations have been raised as biotypes 1, 2, and 3 on differential rice host of improved varieties Pelita I-1 (no *Bph* gene), IR26 (*Bph1*), and IR42 (*bph2*), respectively. Three alternative populations have also been developed on the respective traditional varieties TN1 (no *Bph* gene), Mudgo (*Bph1*), and ASD7 (*bph2*). Although these populations displayed two virulence patterns other than biotype 1 to 3 phenotypes, they were expected to be discriminated into two virulence groups by SSR analysis. The study aimed to investigate the level of genetic variation among the six BPH populations using SSR markers and to relate it with the observed virulence patterns. Genotyping of 30 females with 29 polymorphic SSR markers revealed higher genetic parameter values in populations reared on improved varieties than those on traditional varieties. This difference was marked as two population clusters in PCoA plots corresponding to the host variety type, in contrast to the previous assumption that clustering would be based on virulence patterns. The presence of individuals with unwanted virulence allele, either resulting from contamination during the long period of rearing or lack of host adaptation period, is suspected. The result of this study indicates that the six populations are not suitable for resistance screening. Virulence selection must be performed until they attain biotype 1 to 3 phenotypes which can be genetically separated by DNA markers.

[Keywords: brown planthopper, rice, virulence adaptation, DNA marker, genetic differentiation]

ABSTRAK

*Penapisan ketahanan padi calon galur unggul harapan di Indonesia terhadap wereng batang cokelat (WBC) mensyaratkan penggunaan biotipe 1, 2, dan 3. Tiga populasi WBC telah dipelihara sebagai biotipe 1, 2, dan 3 pada inang varietas padi diferensial hasil perbaikan, yaitu berturut-turut Pelita I-1 (tanpa gen ketahanan Bph), IR26 (*Bph1*), dan IR42 (*bph2*). Tiga populasi alternatif juga telah diadaptasikan berturut-turut pada varietas tradisional TN1 (tanpa gen ketahanan* Bph*), Mudgo (*Bph1*), dan ASD7 (*bph2*). Keenam populasi masih memperlihatkan dua pola virulensi selain fenotipe biotipe 1 sampai 3, tetapi diharapkan dapat dipisahkan menjadi dua kelompok virulensi berdasarkan genotipe penanda SSR. Penelitian ini bertujuan menganalisis keragaman genetik enam populasi WBC menggunakan penanda SSR dan menghubungkannya dengan pola virulensi yang teramati. Analisis genotipe 30 ekor betina menggunakan 29 penanda SSR polimorfik mengungkap nilai parameter genetik yang lebih tinggi pada populasi asal varietas hasil perbaikan dibandingkan dengan populasi asal varietas tradisional. Perbedaan ini ditandai oleh terbentuknya dua kelompok populasi pada plot PCoA sesuai tipe varietas inangnya, sehingga berbeda dari anggapan semula bahwa pengelompokan genotipe SSR akan sesuai dengan fenotipe virulensi. Diduga keenam populasi masih mengandung individu pembawa alel virulensi yang tidak diinginkan, akibat terkontaminasi selama jangka waktu pemeliharaan yang lama atau kurangnya periode adaptasi pada inang. Hasil penelitian menunjukkan bahwa keenam populasi belum memenuhi syarat untuk digunakan dalam penapisan ketahanan padi. Seleksi individu harus dilakukan hingga keenamnya memperlihatkan pola virulensi biotipe 1 hingga 3 yang dapat dipisahkan secara genetik antarbiotipe menggunakan penanda DNA.*

[Kata Kunci: wereng batang cokelat, padi, adaptasi virulensi, penanda DNA, perbedaan genetik]

INTRODUCTION

Brown planthopper (*Nilaparvata lugens* Stål; Hemiptera: Delphacidae) exhibits phenotypic plasticity, or flexible change of phenotype over time, in response to environmental changes (Ferrater 2016; Saxena and Barrion 1983). When exposed to a monoculture of resistant rice varieties having a single major resistance (R) *Bph* gene, field brown planthopper (BPH) populations are capable of evolving new virulence characters or biotypes (Cheng et al. 2013). Various alleles of virulence genes contained in a field population facilitated the population to adapt to any

rice resistance gene (Kobayashi 2016). This phenotypic plasticity along with BPH's short life cycle, high fecundity, and high reproduction rate is a threat to the rice resistance stability and has impeded successful rice breeding and deployment of resistant varieties (Haliru et al. 2020; Horgan 2018; Saxena and Barrion 1983).

BPH biotypes differ in virulent allele frequencies of genes associated with host adaptation (Claridge and Den Hollander 1983; Kobayashi 2016). Among biotypes, there are exist minor variations in electrophoretic, morphological, and cytological features, as well as acoustic behavior (Saxena and Barrion 1983). BPH biotype numbering system is based on virulence to *Bph* R gene and is generally limited to four. Biotype 1 is virulent only to varieties void of *Bph* R genes, biotype 2 and 3 are virulent to *Bph1* and *bph2* gene, respectively, and biotype 4 is virulent to both resistance genes (Cheng et al. 2013). Biotype 5, rarely mentioned in the literature, is virulent to *bph4* (Claridge and Morgan 1987). The absence of mating barriers among biotypes permits cross-hybridization with individuals from other biotypes and causes the shift in virulence characteristic of the population (Claridge et al. 1984). Therefore, virulence change of biotype stocks during continuous rearing on their respective hosts can occur (Baehaki and Munawar 2008; Manzila et al. 2000).

Genetic analysis by Jing et al. (2012) revealed that BPH biotypes differ in the level of genetic variation. The use of 72 simple sequence repeat (SSR) markers derived from the BPH expressed sequence tag (EST) database successfully clustered BPH individuals into their respective biotypes (biotypes 1, 2, 3, or Y) (Jing et al. 2012). The study also revealed a positive correlation between the levels of genetic variation in each biotype with the resistance level of the respective host. In addition to EST-SSR markers (e-SSR), genomic SSR (g-SSR) markers have been isolated from the BPH genome (Jing et al. n.d.; Qiu et al. 2016) and shown to be more polymorphic than e-SSR (Jairin et al. 2013). Both SSR types have been used in the genetic diversity analysis of natural BPH populations (Jing et al. n.d.; Liu and Hou n.d.; Qiu et al. 2016; Sun et al. 2011), experimental populations (Jing et al. 2012), and mapping populations (Jairin et al. 2013; Jing et al. 2014). Among several SSR advantages, high reproducibility and repeatability are important features that amenable comparison among laboratories and experiments (Zalapa et al. 2012).

Although field BPH populations in Indonesia have adapted to *Bph1* and *bph2* genes (Baehaki 2012) and also to multiple *Bph* genes (Chaerani et al. 2016), resistance to biotype 1, 2, or 3 is still required for promising rice lines to be registered and released (Sasmita et al. 2019). In our previous studies, two sets of three BPH populations have been adapted on differential host varieties. One population set was BPH cultures derived from stocks maintained as biotype 1, 2, and 3 on improved rice varieties Pelita I-1 (no *Bph* genes), IR26 (*Bph1*), and IR42 (*bph2*), respectively (Chaerani et al. 2015). The other set was the results of virulence selection of individuals from field populations followed by adaptation on traditional varieties (TN1, Mudgo, and ASD7) carrying the equivalent *Bph* genes to that contained in the improved varieties (Chaerani et al. 2021). Although raised on the respective host for a while, these populations are still segregated into two virulence phenotypes other than the desired biotype 1 to 3 phenotypes (Chaerani et al. 2021; 2015), making it unsuitable for use in resistance screening. Nevertheless, their genetic variations in SSR loci are presumably can be clustered according to the observed virulence patterns.

The objective of this study was to investigate the level of genetic variations among the six BPH populations using SSR markers. This study is expected to provide suggestions for ensuring the purity of BPH biotypes used in rice resistance screening.

MATERIALS AND METHODS

BPH Population

This study used two sets of BPH populations reported in previous studies (Chaerani et al. 2021; 2015) and an unpublished study. The first three populations were derived from stocks maintained as biotype 1, 2, and 3 on the respective improved varieties Pelita I-1 (no *Bph* gene), IR26 (*Bph1*), and IR42 (*bph2*) under the glasshouse environment of the Indonesian Center for Rice Research, Indonesian Agency for Agricultural Research and Development (IAARD). When used in this study these populations, hereinafter referred to as P, 26, and 42 to denote the host varieties, were at the 4th, 5th, and 12th generations of culture on their original hosts in the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD (Chaerani et al. 2015). Because these populations have been long adapted on their hosts since the late 1970s and early 1990s, virulence selections were not attempted. The other populations were T, M, and A resulted from adaptation on a differential host of traditional varieties TN1 (no *Bph* gene), Mudgo (*Bph1*), and ASD7 (*bph2*), respectively. These populations were at the 2nd, 4th, and 3rd generation after four virulence selection cycles combined with one selfing of females derived from three field populations (Chaerani et al. 2021). The virulence of these six populations deviated from the expected biotype 3 virulence pattern, i.e. population P, 42, T, and M had biotype 4 virulence characteristics, whereas the remaining

were highly virulent by damaging multiple genes *Bph3* and *Bph17* carried by variety Rathu Heenathi (Chaerani et al. 2021; 2015).

DNA Isolation and PCR Amplification

Except for population 42, genomic DNAs and PCR amplicons for the remaining populations have been available from previous studies (Chaerani et al. 2021; 2015). The SDS buffer extraction protocol (Latif et al. 2012) was used for DNA isolation of five females randomly selected from population 42. A total of 38 SSR primers previously used for genetic analysis of BPH field populations and samples of biotype 1 and 2 cultures (Chaerani et al. 2015), were tested for polymorphisms on DNA pools of the six populations consisting of five individuals each.

DNA amplification was performed in a total volume of 10 µl containing 1× PCR buffer containing 1.5 mM $MgCl_2$ (DreamTaq), 0.2 mM dNTPs (Vivantis), 0.3 µM of each primer, 0.5 U of *Taq* DNA polymerase (DreamTaq), and 10 ng DNA. PCR cycling was performed on a Bio-Rad thermal cycler using the following cycling profile: a 5-min denaturation step at 94 °C followed by 39 cycles of 30 s denaturation at 94 °C, 30 s annealing at 55 °C, and 60 s extension at 72 °C. The PCR cycle was terminated with a 7 min final extension at 72 °C. For easy SSR allele scoring, amplicons were separated by electrophoresis on 4% agarose gels in 0.5× TBE at a constant electric current of 150 V for 2–2.5 hours. Gels were stained with ethidium bromide (5 µg l^{-1}) and visualized under UV light (Chemidoc XRS Bio-Rad).

Allele sizes were determined by comparing them with a 100-bp DNA ladder (Vivantis) which was run together with samples. Primers yielding polymorphic bands among the six populations were further used for SSR amplification of DNA of five samples from population 42 and of one to two samples randomly selected from each of the remaining five populations. These representative samples were included in the PCR amplification and run together in agarose gel electrophoresis to serve as controls for gel scoring consistencies among experiments (Chaerani et al. 2021; 2015).

SSR Analysis

The PCR products were scored manually in a codominant fashion based on the allele size and then converted into binary data (score "1" and "0" for the presence and absence of an allele size, respectively). The binary data matrix of a total of 30 females (five females per population) was submitted to the software PowerMarker ver. 3.25 (Liu and Muse 2005) for calculation of the allele number produced by each marker and the polymorphic information content (PIC). Estimates of BPH population genetic diversity as measured by the percentage of polymorphic loci, number of different alleles (*Na*), number of effective alleles (*Ne*); Shannon's Information Index (*I*), expected heterozygosity (*He*), observed heterozygosity (*Ho*), unbiased heterozygosity (*UHe*), fixation index (F), and genetic differentiation between population calculated as pairwise values of Phi statistics (PhiPT) were calculated using the population genetic software GenAlex 6.41 (Peakall and Smouse 2012). Hierarchical analysis using analysis of molecular variance (AMOVA) at 999 permutations and multivariate analysis using principal coordinate analysis (PCoA) based on a standardized covariance distance matrix was also performed by GenAlEx 6.4.1.

RESULTS AND DISCUSSION

Marker Polymorphism

SSR amplification by e-SSR or g-SSR primers yielded clear and simple DNA bands when size-fractionated on 4% of agarose gels (Figure 1). The inclusion of five samples from population 42 in polymorphism tests of 38 primers resulted in only 9 primers which did not yield polymorphic bands (Table 1). The use of the remaining 29 primers yielded an average of 4 alleles, with the highest number of alleles (7) was produced by primer NLES33 and NLES34, and the least (2) was yielded by primer BM1277 and NLES35. Four markers (BM140, BM432, DB845048, and NLES 35) were not informative because the PIC values were less than 0.5 (Botstein et al. 1980). With the average PIC values over 0.5 (Table 1) the remaining markers are assumed efficient for genotype discrimination and useful for measuring polymorphism (Alzahib et al. 2021).

We used both types of SSR markers because e-SSR markers are known to be less polymorphic than g-SSR markers as a consequence of greater DNA sequence conservation in transcribed regions (Jairin et al. 2013). Therefore, the marker polymorphism rate in our studied populations was higher (29 out of 38 primers or 76%) than that obtained in the genetic analysis of four BPH biotypes (155 out of 351 e-SSR primers or 44%) (Jing et al. 2012).

Population Genetic Diversity

The mean percentage of polymorphic loci in all populations was high (84%) and ranged from 72% (population M) to 100% (population 42; Table 2). The number of alleles

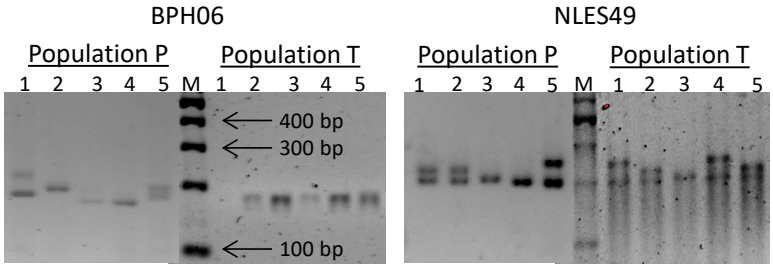

Fig. 1. Examples of DNA amplification of brown planthopper individuals (1 to 5) from population P and T raised on rice host variety Pelita I-1 and TN-1, respectively, by e-SSR primer BPH06 and g-SSR primer NLES49. Amplicons were size-fractionated by electrophoresis on 4% agarose gel for 2.5 hours at a constant electric current of 150 V and run together with a 100 bp DNA ladder (M).

Table 1. Performance of 29 polymorphic SSR markers in six rice brown planthopper (*Nilaparvata lugens* Stål) populations raised on differential rice host varieties.

SSR name and type[a]	Reference[b]	Linkage group[c]	Na[d]	Allele size (bp)	PIC[e]
NLES33 (e)	1	ND	7	150, 165, 175, 180, 226, 266, 280	0.84
NLES11 (e)	1	ND	5	195, 222, 238, 267, 277	0.80
NLES22 (e)	1	1	5	172, 178, 180, 191, 201	0.75
BM1262 (g)	3	ND	6	157, 168, 175, 190, 195, 216	0.75
BM265 (e)	2	ND	4	177, 186, 195, 200	0.74
NLES24 (e)	1	8	5	195, 216, 224, 234, 244	0.72
BM25 (e)	2	11	5	277, 279, 301, 320, 330	0.72
BM194 (e)	2	ND	5	159, 165,170, 175, 180	0.71
NLES8 (e)	1	7	5	100, 120, 130, 140, 156	0.70
NLES34 (e)	1	ND	7	120, 130, 135, 140, 185, 200, 210	0.69
BM76 (e)	2	ND	5	230, 240, 284, 290, 294	0.69
DB839391	5	ND	3	173, 187, 195	0.68
NLES2 (e)	1	15	5	116, 121, 125, 140, 146	0.67
BM395 (e)	2	9	4	146, 150, 161, 172	0.67
BPH06 (e)	4	ND	5	152, 173, 181, 195, 200	0.67
BM298 (e)	2	3	5	186, 215, 220, 230, 240	0.65
BM68 (e)	2	ND	4	285, 290, 301, 325	0.65
NLES15 (e)	1	2	4	190, 196, 205, 228	0.65
BM339 (e)	2	ND	3	230, 250, 280	0.64
BM435 (e)	2	ND	3	120, 124, 132	0.64
NLES49 (e)	1	2	4	300, 330, 340	0.57
BM499 (e)	2	11	4	136, 143, 154, 162	0.56
BM375 (e)	2	7	5	190, 200, 202, 208, 228	0.54
BM1277 (g)	3	ND	2	175, 185	0.53
BM246 (e)	2	ND	4	290, 300, 310, 350	0.53
DB845048	5	ND	3	107, 109, 115	0.49
BM432 (e)	2	ND	3	275, 282, 300	0.44
NLES35 (e)	1	ND	2	155, 166	0.43
BM140 (e)	2	6	3	244, 252, 258	0.26
Mean			4.3		0.60

[a]e: derived from expressed sequence tag; g: isolated from genomic DNA.

[b]1 (Jairin et al. 2013); 2 (Jing et al. n.d.); 3 (Jing et al. 2012); 4 (Liu and Hou n.d.); 5 (Sun et al. 2011).

[c]ND = not determined.

[d]Na = number of the alleles.

[e]PIC = polymorphic information content.

was not too different among populations (Na 2.07–2.83 and Ne 1.71–2.20). The number of private alleles in all populations was negligible (0.07–0.17), implying that essentially no specific alleles could refer to a certain population (Alzahib et al. 2021). Non-varying number of total alleles and private alleles across populations in our

study could be the consequence of the presence of only two virulence variations among the six populations. This argument is supported by Jing et al. (2012) study who found a positive correlation between the degree of biotype virulence and SSR allele number, as well as between the level of BPH genetic variation with the level of host resistance. They observed that BPH biotype 1 adapted on TN1 displayed the least number of alleles (2.3 alleles in biotype 1 vs 4.5 alleles in biotype Y), consistent with the high susceptibility of the rice host.

Contrary to the previous assumption that pairs of populations feeding on differential hosts carrying the same *Bph* gene would display similar genetic parameter values, this study revealed higher heterozygosity values in populations raised on improved varieties than that shown by their counterparts raised on traditional varieties. Interestingly, population P, which fed on a variety void of *Bph* genes, showed the highest heterozygosity as shown by the genetic parameter values *I* (0.835); *Ho* (0.537); *He* (0.490); and *Uhe* (0.554). This population also displayed excess heterozygosity as indicated by the negative value of fixation index (F = -0.073).

Inter-mating among biotype populations may explain the higher genetic variation in populations that originated from improved varieties. Stocks of biotype 1 have been reared on Pelita I-1 since 1976, biotype 2 on IR26 since 1992, and biotype 3 on IR42 since 1994 (Baehaki and Munawar 2008). Accidental contamination may have been occurred during the long adaptation under greenhouse environment and resulted in mixtures of biotype individuals. Additionally, the variety Pelita I-1 has been known to exert a weak selection pressure (coefficient 0.016) (Baehaki 2012) which can increase its resistance to BPH (Slamet and Warsun 2016). Even under weak selection pressure, virulent individuals are selected, reproduced and predominated the ensuing generation leading to a change in genotypic frequency. Experiments have shown that virulent individuals remain virulent even when maintained on the susceptible host for years, indicating that virulence has little fitness cost (Kobayashi 2016)

Population Genetic Structure by AMOVA

Partitioning of the genetic variances by AMOVA indicated that the largest part of variations was accounted for within (76%) rather than among (24%) populations (Table 3). A similar rate of within and among four biotype populations genetic variation (74.5% and 25.5%, respectively) was reported by Jing et al. (2012). Higher molecular variation within populations is a common phenomenon in outcrossing species to sustain the population (Jing *et al*. 2012; Reiker *et al*. 2015).

Although among population variation contributed little to total genetic variation, pairwise analysis of PhiPT population values detected the presence of significant genetic differentiation between pairs of

Table 2. Genetic diversity of 30 genotypes of six rice brown planthopper (*Nilaparvata lugens* Stål) populations raised on differential host varieties based on 29 polymorphic SSR markers.

BPH population (host variety and *Bph* resistance gene)	P (%)	Na	Ne	I	Ho	He	uHe	F	Pa
P (Pelita I, no *Bph* gene)	89.7	2.83	2.20	0.835	0.537	0.490	0.554	-0.073	0.17
26 (IR26, *Bph1*)	82.8	2.35	2.07	0.703	0.432	0.441	0.516	0.021	0.07
42 (IR42, *bph2*)	100.0	2.52	1.99	0.755	0.432	0.473	0.533	0.171	0.10
T (TN1, no *Bph* gene)	82.8	2.10	1.71	0.570	0.275	0.385	0.464	0.207	0.07
M (Mudgo, *Bph1*)	72.4	2.07	1.80	0.572	0.368	0.362	0.499	-0.018	0.14
A (ASD7, *bph2*)	75.9	2.28	1.90	0.614	0.264	0.376	0.469	0.244	0.17
Mean	83.9	2.36	1.95	0.67	0.385	0.421	0.506	0.109	0.12

P = polymorphic loci, Na = number of different alleles with a frequency ≥5%, Ne = number of effective alleles, I = Shannon's information index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased expected heterozygosity, F = fixation index, and Pa = number of private alleles (allele unique to a single population)

Table 3. Analysis of molecular variance (AMOVA) results for 30 genotypes from six rice brown planthopper (*Nilaparvata lugens* Stål) populations based on 29 SSR markers.

Source of variation	df	SS	MS	Estimates of variance components	Molecular variation (%)
Among populations	5	323.300	64.660	7.915	24
Within populations	24	602.000	25.083	25.083	76
Total	29	925.300	89.743	32.999	

populations (Table 4). By referring to the threshold that a PhiPT value greater than 0.15 indicates a significant genetic differentiation between a pair of populations (Frankham et al. 2002), all populations were genetically separated except for a pair of populations raised on improved varieties (P–26, PhiPT value 0.094) and all possible pair of populations between those adapted on traditional varieties (PhiPT values 0.023–0.118). Lack of host adaptation period and the use of three common field populations as a source of individual virulence selection (Chaerani et al. 2021) could contribute to the close genetic relationship among the artificially developed populations.

Genetic separation among the two population sets was unexpected because a pair of populations raised on a differential host carrying the same *Bph* gene is presumably would experience the same rate of selection pressure. Differences in host genetic background may explain this discrepancy. Pelita I-1, IR26, and IR42 are improved varieties resulting from cross-breeding with traditional varieties with resistance to BPH (Brar et al. 2009), and hence they may carry additional minor *Bph* resistance genes which can put additional selection pressure.

Principal Component Analysis (PCoA)

Plots of PCoA revealed that the first and the second axis accounted for the largest total molecular variations (52.17%), followed by the first and the third axis (51.34%) (Figure 2). Bi-plot of axis 1 vs 2 and 1 vs 3 (Figure 2a and b) show the clear separation of BPH populations maintained on different types of differential host varieties, and thus confirms the result of pairwise analysis of PhiPT population values (Table 4). Aggregation of individuals was exhibited by population 42 in plots of axis 1 vs 2 and axis 2 vs 3 (Figure 2a and c) and by population T in plots of axis 2 vs 3 (Figure 2c), indicating a genetic similarity among individuals within the respective population.

Two unexpected results were obtained in this study: (1) a higher genetic variation in a population that has been long maintained on varieties without *Bph* genes, and (2) differential rate of genetic variation between a pair of populations raised on rice hosts carrying the same *Bph* gene, and this resulted in two clusters based on host variety type instead of virulence variation. The presence of various genotypes in each population, either caused by presumably accidental contamination in population P, 26, and 42, or lack of host adaptation period for population

Table 4. Pairwise PhiPT values based on 29 SSR markers of six rice brown planthopper (*Nilaparvata lugens* Stål) reared on differential host varieties.

BPH culture/population (host variety and Bph resistance genes[a]	P	26	42	T	M
P (Pelita I-1, no *Bph* genes)	-				
26 (IR26, *Bph*1)	0.094	-			
42 (IR42, *bph*2),	0.160	0.174	-		
T (TN1, no *Bph* gene),	0.289	0.319	0.326	-	
M (Mudgo, *Bph*1)	0.310	0.330	0.341	0.087	-
A (ASD7, *bph*2)	0.286	0.281	0.281	0.118	0.023

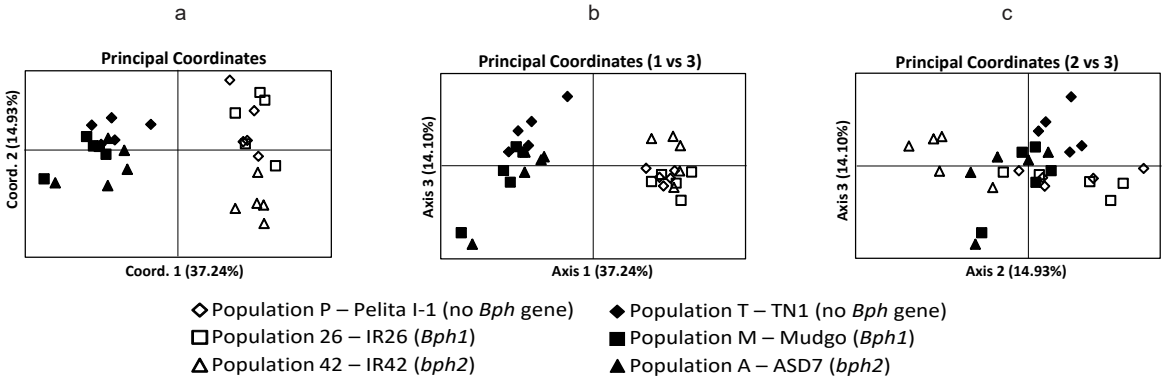

Figure 2. Bi-plots of the genetic distance of rice brown planthopper (*Nilaparvata lugens* Stål) maintained on differential host varieties based on 29 SSR markers. The total molecular variation explained in plot of axis 1 vs 2, 1 vs 3, and 2 vs 3 was 52.17% (a), 51.34% (b), and 29.03% (c), respectively.

T, M and A, may explain those discrepancies. Virulence selection cycles must be performed to remove individuals carrying unwanted virulence alleles. Virulence selection may be less complicated for populations raised on the improved varieties as biotypes 1, 2, and 3. A previous study showed that purification of contaminated biotype 1 and 2 populations took less than a year (Manzila et al. 2000) to result in two biotype groups in phylogenetic analysis by RAPD markers (Bahagiawati and Rijzaani 2005). The artificially developed populations were still at the early host adaptation and developed from three field populations (Chaerani et al. 2021) which potentially contain various genotypes with high virulence allele frequency (Kobayashi 2016). More selection cycles would be necessary for these populations to attain the desired biotype 1 to 3 virulence patterns.

The purity of BPH insects is the most essential part in rice resistance evaluation for the identification of resistant lines (Haliru et al. 2020) or genetic mapping of BPH virulence genes (Jairin et al. 2013; Jing et al. 2014; Kobayashi 2014) to obtain reliable resistance scores. The result of this study implies that populations raised on differential host varieties with no *Bph* gene, *Bph1,* and *bph2* for some time could not always be referred to as biotype 1, 2, and 3, respectively. Therefore, the claimed virulence phenotypes and clear genetic differentiation among them must be first demonstrated.

CONCLUSION

SSR analysis of six BPH populations indicated higher genetic parameter values in populations raised on improved varieties than those adapted on traditional varieties. Although among population genetic variations accounted for only 24% of the total genetic variations, significant genetic differentiation between the majority of population pairwise was detected by pairwise analysis of PhiPT population values. A genetic divergence based on host variety type (improved vs traditional) instead of virulence phenotype shown in PCoA plots indicated that each population still consists of a mixture of genotypes. Before application in rice resistance screening, these populations must undergo virulence selection cycles until the desired biotype 1 to 3 virulence phenotype with clear genetic separation to one another is attained.

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