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LOW ASSOCIATION OF *Bph17* ALLELE IN LANDRACES AND IMPROVED VARIETIES OF RICE RESISTANT TO BROWN PLANTHOPPER

Asosiasi Rendah Alel Bph17 *pada Varietas Lokal dan Varietas Unggul Padi Tahan Wereng Batang Cokelat*

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ABSTRACT

Resistance traits to brown planthopper on rice varieties are controlled by dominant and recessive genes called *Bph/bph. Bph17* is one of dominant genes that control rice resistance to brown planthopper. Marker of *Bph17* allele can be used as a tool of marker assisted selection (MAS) in breeding activity. Association of *Bph17* allele and resistance to brown planthopper in Indonesian landraces and new-improved varieties of rice is not clearly known. The study aimed to determine the association of *Bph17* allele in landraces and new-improved varieties of rice resistant to brown planthopper. Twenty-one rice genotypes were used in the study, consisting of 13 landraces, 5 improved varieties, 3 popular varieties and a check variety Rathu Heenati. Two simple sequence repeat markers linked to *Bph17* allele were used, i.e. RM8213 and RM5953. The results showed that association of *Bph17* allele in landraces and new-improved varieties of rice resistant to brown planthopper resistance was very low (r = -0.019 and -0.023, respectively). The presence of *Bph17* allele did not constantly express resistance to brown planthopper. The study suggests that *Bph17* allele cannot be used as a tool of MAS for evaluating resistance of landraces and new-improved varieties of rice to brown planthopper. Further research is needed to obtain a specific gene marker that can be used as a tool of MAS and applicable for Indonesian differential rice varieties.

[**Keywords**: association, brown planthopper, *Bph17* allele, improved varieties, landrace, plant resistance, rice]

ABSTRAK

Sifat ketahanan terhadap wereng batang cokelat (WBC) pada varietas padi dikendalikan oleh gen dominan dan gen resesif yang disebut Bph/bph. Bph17 merupakan salah satu gen dominan yang mengendalikan sifat ketahanan tanaman padi terhadap WBC. Marka alel Bph17 *dapat menjadi alat bantu seleksi (*marker assisted selection*, MAS) pada kegiatan pemuliaan. Hubungan antara kehadiran alel* Bph17 *dan sifat ketahanan terhadap WBC pada varietas padi lokal Indonesia dan beberapa varietas unggul baru (VUB) belum diketahui secara jelas. Penelitian ini bertujuan untuk mengetahui asosiasi antara kehadiran alel* Bph17 *dan karakter ketahanan terhadap WBC pada padi varietas lokal dan VUB. Sebanyak 21 genotipe digunakan dalam penelitian ini, terdiri atas 13 varietas lokal, 5 VUB, 3 varietas populer, dan Rathu Heenati (cek postif). Dua penanda spesifik alel* Bph17 *digunakan, yaitu RM8213 dan RM5953. Hasil penelitian membuktikan bahwa asosiasi antara keberadaan alel* Bph17 *dan sifat ketahanan terhadap WBC pada padi lokal dan VUB sangat rendah (r = -0,019 dan -0,023). Kehadiran alel* Bph17 *tidak mengekspresikan ketahanan terhadap WBC pada varietas lokal dan VUB. Hasil penelitian ini menunjukkan bahwa alel tidak dapat digunakan sebagai alat bantu seleksi untuk mengevaluasi ketahanan padi varietas lokal dan VUB terhadap WBC. Diperlukan penelitian lebih lanjut untuk mendapatkan penanda gen spesifik yang dapat digunakan sebagai alat bantu seleksi untuk varietas diferensial padi Indonesia.*

[***Kata kunci***: alel *Bph17*, hubungan, padi, sifat ketahanan, varietas lokal, varietas unggul baru, wereng cokelat]

INTRODUCTION

Brown planthopper (BPH; *Nilaparvata lugens*) is a major pest of rice crop around the world, including Indonesia. The pest is cosmopolitan, potentially reducing rice production even causing crop failure (Watanabe et al. 2009; Direktorat PTP 2016). Rice plants stricken by BPH show symptoms of leaf yellowing and dry, stunted growth, and eventually die (Baehaki 2012). The 100-140-day old rice plant had higher number of BPHs per hill compared to 80-90-day old crop (Prashant et al. 2012). Current technology that effectively controls BPH is a resistant variety (Baehaki and Mejaya 2014).

Resistance traits to BPH on rice varieties are controlled by major and minor genes called *Bph/bph* (Brar et al. 2009). The genes were mapped on chromosomes 2, 3, 4, 6, 7 and 9 (Liu et al. 2001; Liu et al, 2009). *Bph17* is one of dominant genes that control resistance trait to BPH (Brar et al. 2009). The gene is located on chromosome 4 (Rahman et al. 2009) and derived from BPH donor resistance gene of Rathu Heenati (Sun et al. 2005).

Bph17 gene has been used as a donor in breeding program of BPH-resistant rice varieties (Iswanto et al.

2015). Sun et al. (2005) and Jena et al. (2006) revealed that RM8213 and RM5953 DNA markers are closely linked to *Bph17* gene that controls the expression of resistance trait. These markers can be used as a tool of marker assisted selection (MAS) with marker alleles at 177 bp on RM8213 PCR products and 140 bp on RM5953 PCR products (Sun et al. 2005; Jena et al. 2006). DNA products of RM8213 follow the Mendelian inheritance pattern of 1:2:1 (susceptible: heterozygous segregation: resistant) (Pertiwi et al. 2014; Carsono et al. 2016).

The success of MAS such as *Bph17* marker depends on several factors, including genetic base of trait, degree of association between molecular marker and target gene, number of individuals analyzed, and genetic background of the target gene to be transferred (Francia et al. 2005; Wang et al. 2008). DNA markers such as *Bph17* need to be evaluated for identifying BPH biotypes. The use of MAS to suspect and avail the selection of simply inherited traits is increasingly important in breeding programs, allowing an acceleration of breeding process, and is not affected by the environment or growing conditions (Guimarães et al. 2007; Bahagiawati 2012). Many of MAS are used as a marker assisted breeding (MAB) for selection of segregated population, but sometimes cannot be used for selection of non-breeding populations as well as landraces or local rice (depending on the marker trait). Carsono et al. (2016) found 63 selected lines from F2 progenies of resistant parent based on the linked marker of *Bph17* allele of Rathu Heenati as the check variety.

Landrace is a germplasm containing resistance genes to pests and diseases (Sitaresmi et al. 2013). Some landraces and new-improved varieties of rice have been identified for resistance to BPH (Yunani et al. 2014; Jamil et al. 2015). However, the presence of resistance genes, especially *Bph17* in Indonesian landraces and some new-improved varieties has not been intensively studied. To support breeding program based on landrace populations, it is necessary to study the presence of these BPH resistance genes in Indonesian landraces and several new-improved varieties of rice and find out the association of *Bph17* allele position on Rathu Heenati.

The study aimed to determine the association between *Bph17* allele in landraces and improved varieties of rice and resistance to BPH.

MATERIALS AND METHODS

The study was conducted in 2015 in DNA Laboratory of Plant Breeding Division, Indonesian Center for Rice Research (ICCR) at Subang, West Java.

Plant Materials

The study used 21 rice genotypes, consisted of 13 landraces from various provinces in Indonesia, one positive check variety (Rathu Heenati), five new improved varieties and three popular varieties (Table 1). The rice genotypes belonged to ICRR. Ten to twenty seeds of each accession were germinated in the planting medium then put into the germinator cabinet. The 21 day-old rice seedings were transplanted into polybags containing a mixture of soil and sand growth medium (50:50). The plants were kept in the greenhouse of Plant Breeding Division, ICRR, and maintained according to protocol of The Crop Manager version 1.0 by IRRI (http://webapps.irri.org).

Molecular Analysis

Molecular analysis was done using simple sequence repeat (SSR). The analysis consisted of five major activities, namely DNA isolation, DNA quantity and quality test, polymerase chain reaction (PCR) amplification, electrophoresis of PCR products, and visualization of electrophoresis products.

DNA Isolation and DNA Quantity and Quality Test

Five young leaves of ten-day old rice seedlings of each accession were taken and used for DNA isolation. The DNA was extracted following the method of Murray and Thompson (1980) by small modification on leaf crushing. The leaves were crushed in a mortar without liquid nitrogen and homogenized with 800 µℓ CTAB buffer. DNA quality and quantity were measured using NanoDrop 2000/UV-Vis Spectrophotometer at 260 and 280 nm.

PCR Amplification

DNA of *Bph17* allele in the leaf samples was amplified using SSR markers, i.e. RM8213 and RM5953 (Sun et al. 2005) (Table 2). Extracted DNA was amplified using PCR machine (BIO-RAD T100™ Thermal Cycler) applying the ICRR DNA Laboratory procedure. PCR cocktail was made consisting of 50 ng DNA sample, 0.25 µM forward and reverse primers, 100 µM dNTPs, 1x PCR buffer (consisting of 20 mM Tris pH 8.3, 50 mM KCl, 1.5 mM M_gCl_2, and 0.01% gelatin), and 0.5 units Taq DNA polymerase. PCR amplification was performed under the following conditions: denaturation at 95ºC for 5 min, 35

Table 1. List of accessions and origin of rice varieties used in the study.

No. Lab	No. accession	Accession/ variety	Subspecies	Origin/pedigree	Resistance to BPH	Reference
		Landraces				
1	33	Bandang Si Gadis	*Indica*	North Sumatra	S	Yunani et al. (2014)
2	144	Padi Kuning	*Indica*	Jambi	S	Yunani et al. (2014)
3	268	Siawak	*Indica*	Bengkulu	MR	Yunani et al. (2014))
4	289	Takong	*Indica*	East Kalimantan	MS	Yunani et al. (2014)
5	673	Pare Ndele A	*Javanica*	North (?) Nusa Tenggara	S	Yunani et al. (2014)
6	1039	Mentik wangi	*Indica*	Central Java	S	Yunani et al. (2014)
7	1240	Cinta kasih	*Indica*	Bengkulu	MS	Yunani et al. (2014)
8	1546	Ratu Heenati	*Indica*	Introduction	R	Yunani et al. (2014)
9	2733	Padi serai	*Indica*	East Kalimantan	S	Yunani et al. (2014)
10	2734	Selasih	*Indica*	East Kalimantan	S	Yunani et al. (2014)
11	4771	Mayas	*Indica*	East Kalimantan	S	Yunani et al. (2014)
12	7787	Marahmay	*Indica*	Banten	S	Yunani et al. (2014)
13	7944	Jadul	*Japonica*	Central Kalimantan	R	Yunani et al. (2014)
		New Improved Varieties				
14		Inpari 34	*Indica*	BR41XIR6190-3B-22-2	MS	Jamil et al. (2015)
15		Inpari 35	*Indica*	IR10206-29-21XSUAKOKO	S	Jamil et al. (2015)
16		Inpari 36	*Indica*	IR58773-35-3-1-2/IR65475-62-3-1-3-1	S	Jamil et al. (2015)
17		Inpari 37	*Indica*	CT9162-12/SeratushariT36//Membramo/ Cibodas///IR66160-121-4-5-3/Membramo	S	Jamil et al. (2015)
18		Inpari 38	*Indica*	IR68888/BP68*10/Selegreng/Guarani/ Asahan	MS	Jamil et al. (2015)
		Popular Varieties				
19		Ciherang	*Indica*	IR18349-53-1-3-1-3/IR9661-131-3-1// IR19661-131-3-1-3///IR64///IR64	R	Jamil et al. (2015)
20		Rojolele	*Javanica*	Local Delanggu Klaten	S	Yunani et al. (2014)
21		Batanghari	*Indica*	Cisadane/IR19661-131-1-3-1-3	MR	Suprihatno et al. (2010)

BPH = brown planthopper; Resistance to BPH: S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant.

Table 2. Markers linked for the aplification of *Bph17*allele DNA.

Marker	Chr	Forward (5'-3')	Reverse (5'-3')	Tm	Size	Reference
RM8213	4	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG	55	177	Sun et al. (2005)
RM5953	12	AAACTTTCTGTGATGGTATC	ATCCTTGTCTAGAATTGACA	55	129	Sun et al. (2005), Shabanimofrad (2015)

cycles of 1 min denaturation at 94ºC, 1 min annealing at 55ºC, 1 min extension at 72ºC, and a final extension at 72ºC for 5 min. The amplification was verified by continuous polyacrylamide gel electrophoresis (8%) to ascertain the presence of amplifiable DNA under 100 volts for 60 minutes in 1 x TBE buffer.

Data Analysis

The amplification data were analyzed based on the presence (1) or absence (0) of DNA bands. DNA polymorphism of Rathu Heenati was used as a reference of the *Bph17* allele. The presence of *Bph17* allele on each accession was ascertained at a distance of 177 bp and 129 bp based on RM5953 and RM8213 markers existing on the check variety Rathu Heenati. Association of the presence of *Bph17* allele bands and plant resistance to BPH was analyzed using correlation analysis by Minitab version 13 software.

RESULTS AND DISCUSSION

BPH Resistance

Resistance levels of rice genotypes to BPH biotype 3 varied (Table 3), ranging from susceptible to resistant. Resistance trait was owned by Rathu Heenati, Jadul and Ciherang, while moderately resistance trait was owned by Si Awak and Batanghari. Takong, Cintakasih and Inpari 38 were moderately susceptible, while Bandang Si Gadis, Padi Kuning, Pare Ndele A, Mentik Wangi, Padi Serai, Selasih, Mayas, Marahmay, Inpari 35, Inpari

36, Inpari 37 and Rojolele were susceptible to BPH. Data on resistance to BPH biotype 3 from ICRR Gene Bank showed that biotype 3 is the most virulent biotype. Therefore, the biotype was selected to investigate the existence of *Bph17* allel.

Brown planthopper has a high genetic plasticity, making it easier and faster in forming a new biotype (Baehaki and Widiarta 2009). Therefore, breeding of rice varieties having durable resistance to BPH is needed to compensate the development of BPH biotypes (Baehaki 2012; Baehaki and Mejaya 2014). Resistance trait to BPH has a narrow genetic variability. Nugaliyadde et al. (2016) reported that resistance to BPH was monogenic dominant based on the damage reaction at seedling stage of F1 and F2 generations from crosses between PTB33 and susceptible variety. On the other hand, Sai Harini et al. (2013) stated that resistance trait to BPH had a wide genetic variability based on molecular analysis results.

Bph17 allele could be used for marker assisted selection (Sun et al. 2005). For rapid identification requirements associated with resistance trait to BPH in Indonesian rice landraces, the role of this allele and its presence need to be investigated clearly. Specific markers for *Bph17* were mapped at RM8213-177bp and RM-5953-129bp on Rathu Heenati.

Table 3. The presence of *Bph17* allel fragments based on two specific primers RM8213 and RM5953 on several landrace, improved and popular rice accessions.

No. accession	Accession/variety	Resistance*)	RM8213-177bp	RM5953-129bp
	Landraces			
33	Bandang Si Gadis	S	+	+
144	Padi Kuning	S	-	+
268	Si Awak	MR	+	+
289	Takong	MS	+	+
673	Pare Ndele A	S	-	-
1039	Mentik Wangi	S	+	+
1240	Cinta Kasih	MS	+	+
1546	Rathu Heenati*	R	+	+
2733	Padi Serai	S	+	+
2734	Selasih	S	-	+
4771	Mayas	S	+	+
7787	Marahmay	S	+	+
7944	Jadul	R	-	-
	Ciherang	MR	-	-
	Improved varieties			
	Inpari 34	MS	-	-
	Inpari 36	S	+	-
	Inpari 37	S	+	-
	Inpari 38	MS	+	-
	Popular varieties			
	Rojolele	S	-	-
	Inpari 35	S	-	-
	Batanghari	MR	+	-

*)Source: Yunani et al .(2014),

R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible. + = allele presence, - = allele absence.

Identification of *Bph17* Allele

Table 3 shows that RM8213-177 bp was not only present in positive-check variety (Rathu Heenati), but also in nine landraces, three new improved varieties and Batanghari. Meanwhile, based on the results of RM5953 amplification at 129 bp, specific band was present on check variety and 10 landraces, but the band was absent in new improved varieties tested (Figure 1).

RM8213 and RM5953 gave different results regarding the presence of *Bph17* allele. The genotypes of Bandang Si Gadis, Si Awak, Takong, Mentik Wangi, Cinta Kasih, Padi Serai, Mayas and Marahmay had both of the markers (RM8213-177bp and RM5953-129bp). Most of the landraces had *Bph17* allele. Eight accessions had the two allele markers, including Bandang Si Gadis, Si Awak, Takong, Mentik Wangi, Cinta Kasih, Padi Serai, Mayas and Marahmay. On the other hand, RM8213-177 bp allele only appeared on Inpari 36, Inpari 37, Inpari 38 and Batanghari, while RM5953-129 bp allele was only present on Padi Kuning and Selasih (Table 3).

One landrace (Si Awak) had *Bph17* allele and medium resistance to BPH biotype 3. The contradictive results

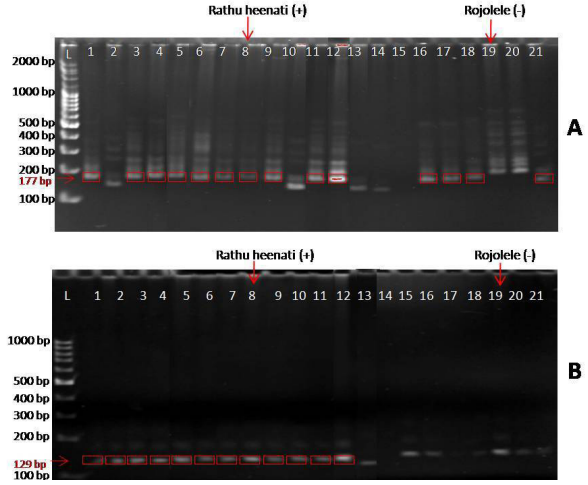

Fig. 1. Visualization of polymerase chain reaction products of RM8213 (A) and RM5953 (B).

Table 4. Association between *Bph17* allele and BPH resistance traits based on correlation analysis.

Correlation	Resistances	RM8213-177bp
RM8213-177bp	-0.119	
Prob.	0.608	
RM5953-129bp	-0.023	0.430
Prob.	0.921	0.052

Prob. > 0.05 shows no significant correlation.

were observed on Rathu Heenati vs Jadul and Ciherang. Rathu Heenati had *Bph17* allele and was resistant to BPH, while Jadul which did not have *Bph17* allele, was resistant to BPH.

Association Between Bph17 Allele and BPH Resistance

The presence of the bands that indicate *Bph17* had a very low relationship with BPH resistance. The analysis result showed that the correlation between the presence of RM8213-177bp and RM5953-129bp and BPH resistance was very low and not significant (r = -0.019 and -0.023, respectively). This means that the presence of *Bph17* allele had very low association with resistance traits to BPH in Indonesian landraces and new-improved varieties of rice.

The presence of *Bph17* allele mostly contradicted with the resistance information on landraces and new-improved varieties. The data revealed that some Indonesian landraces that had a *Bph17* gene were not necessarily resistant to the most virulent BPH and the resistant varieties did not necessarily have a *Bph17* gene. Some landraces that have a *Bph17* allele (the same allele with Rathu Heenati, positive check variety) based on SSR analysis were susceptible to BPH. It is allegedly because although the size of DNA bands are the same, the DNA sequences are completely different.

The same result was observed by Damayanti (2014) on *bph4* alelle. The allele was absent in resistant genotypes, but it was present in susceptible genotypes. The *Bph1* allele also had a low association with resistance to BPH in promising lines. Rahmini et al. (2012) reported that IR42 has a *bph2* allele, but the feeding activity of BPH of bitype 3 on this variety is very high.

Sun et al. (2006) reported the great progress in MAS development in recent years, but relatively few varieties or lines successfully developed by this method due to low association of specific marker and resistance. The presence of one kind of *Bph/bph* allele was not enough information to guest plant resistance to BPH. Satoto et al. (2008) reported that pyramiding analysis is needed as resistance to BPH is controlled by many genes. Su et al. (2006) said that not all *Bph/bph* allele markers can be used as MAS. Bogadhi et al. (2015) found more than one BPH resistance genes in each resistant genotype.

Microarray analysis showed that BPH resistance in Rathu Heenati (donor of *Bph17* gene) may be controlled by a series of resistance-related genes (Wang et al. 2012). Based on this research result, the presence of *Bph17* allele in Indonesian landraces and new-improved varieties does not merely show plant resistance to BPH. Association of *Bph17* alleles in landraces and new-improved varieties of rice does not constantly express resistance to BPH. Landraces and varieties resistant to BPH in this study had different resistance genes to those of Rathu Heenati. So that, RM5953-129bp and RM8213-177bp cannot be used for analyzing rice varieties resistant to BPH of biotype 3. The presence of these markers had no correlation with resistance trait on new-improved rice varieties, i.e. Inpari 34, Inpari 35, Inpari 36, Inpari 37 and Inpari 38. Therefore, it is necessary to search for new genes that control the resistance trait to BPH in landraces or varieties originated from Indonesia.

CONCLUSION

The association of *Bph17* alleles in landraces and new-improved varieties of rice resistant to brown planthopper was very low (r = -0.019 and -0.023, respectively). The presence of *Bph17* allele does not constantly express resistance to brown planthopper. The study suggests that *Bph17* allele cannot be used as a tool of MAS for evaluating resistance of Indonesian landraces and new-improved varieties of rice to BPH. Further research is needed to obtain a specific gene marker that can be used as MAS and applicable for differential rice varieties from Indonesia.

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