

## 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 seedlings 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  $\mu$ l 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  $\mu$ M forward and reverse primers, 100  $\mu$ M dNTPs, 1x PCR buffer (consisting of 20 mM Tris pH 8.3, 50 mM KCl, 1.5 mM  $MgCl_2$ , and 0.01% gelatin), and 0.5 units Taq DNA polymerase. PCR amplification was performed under the following conditions: denaturation at 95°C 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   | <i>Indica</i>   | North Sumatra  | S                 | Yunani et al. (2014)     |
| 2                      | 144           | Padi Kuning        | <i>Indica</i>   | Jambi  | S                 | Yunani et al. (2014)     |
| 3                      | 268           | Siawak             | <i>Indica</i>   | Bengkulu   | MR                | Yunani et al. (2014)     |
| 4                      | 289           | Takong             | <i>Indica</i>   | East Kalimantan  | MS                | Yunani et al. (2014)     |
| 5                      | 673           | Pare Ndele A       | <i>Javanica</i> | North (?) Nusa Tenggara  | S                 | Yunani et al. (2014)     |
| 6                      | 1039          | Mentik wangi       | <i>Indica</i>   | Central Java   | S                 | Yunani et al. (2014)     |
| 7                      | 1240          | Cinta kasih        | <i>Indica</i>   | Bengkulu   | MS                | Yunani et al. (2014)     |
| 8                      | 1546          | Ratu Heenati       | <i>Indica</i>   | Introduction   | R                 | Yunani et al. (2014)     |
| 9                      | 2733          | Padi serai         | <i>Indica</i>   | East Kalimantan  | S                 | Yunani et al. (2014)     |
| 10                     | 2734          | Selasih            | <i>Indica</i>   | East Kalimantan  | S                 | Yunani et al. (2014)     |
| 11                     | 4771          | Mayas              | <i>Indica</i>   | East Kalimantan  | S                 | Yunani et al. (2014)     |
| 12                     | 7787          | Marahmay           | <i>Indica</i>   | Banten   | S                 | Yunani et al. (2014)     |
| 13                     | 7944          | Jadul              | <i>Japonica</i> | Central Kalimantan   | R                 | Yunani et al. (2014)     |
| New Improved Varieties |               |                    |                 |  |                   |                          |
| 14                     | Inpari 34     |                    | <i>Indica</i>   | BR41XIR6190-3B-22-2  | MS                | Jamil et al. (2015)      |
| 15                     | Inpari 35     |                    | <i>Indica</i>   | IR10206-29-21XSUAKOKO  | S                 | Jamil et al. (2015)      |
| 16                     | Inpari 36     |                    | <i>Indica</i>   | IR58773-35-3-1-2/IR65475-62-3-1-3-1                                    | S                 | Jamil et al. (2015)      |
| 17                     | Inpari 37     |                    | <i>Indica</i>   | CT9162-12/SeratushariT36//Membramo/Cibodas//IR66160-121-4-5-3/Membramo | S                 | Jamil et al. (2015)      |
| 18                     | Inpari 38     |                    | <i>Indica</i>   | IR68888/BP68*10/Selegreng/Guarani/Asahan                               | MS                | Jamil et al. (2015)      |
| Popular Varieties      |               |                    |                 |  |                   |                          |
| 19                     | Ciherang      |                    | <i>Indica</i>   | IR18349-53-1-3-1-3/IR9661-131-3-1//IR19661-131-3-1-3//IR64//IR64       | R                 | Jamil et al. (2015)      |
| 20                     | Rojolele      |                    | <i>Javanica</i> | Local Delanggu Klaten  | S                 | Yunani et al. (2014)     |
| 21                     | Batanghari    |                    | <i>Indica</i>   | 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 amplification of *Bph17* allele DNA.**

| Marker | Chr | Forward (5'-3')     | Reverse (5'-3')      | Tm | Size | Reference                                  |
|--------|-----|---------------------|----------------------|----|------|--|
| RM8213 | 4   | AGCCAGTGATACAAAGATG | GCGAGGAGATACCAAGAAAG | 55 | 177  | Sun et al. (2005)                          |
| RM5953 | 12  | AAACTTCTGTGATGGTATC | ATCCTTGTCTAGAATTGACA | 55 | 129  | Sun et al. (2005),<br>Shabanimofrad (2015) |

cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C, and a final extension at 72°C 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* allele.

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* allele fragments based on two specific primers RM8213 and RM5953 on several landrace, improved and popular rice accessions.**

| No. accession      | Accession/variety | Resistance <sup>a)</sup> | 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                       | +            | -            |

<sup>a)</sup>Source: Yunani et al. (2014),

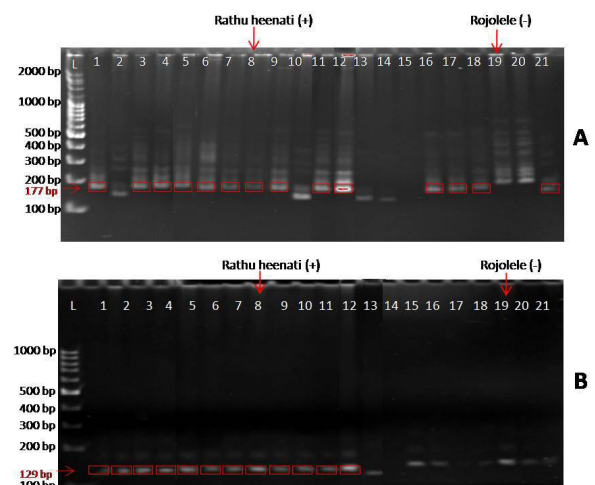
R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible. + = allele presence, - = allele absence.

## 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* allele. 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 biotype 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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### REFERENCES

- Baehaki, S.E. (2012) Perkembangan biotipe hama wereng coklat pada tanaman padi. *Iptek tanaman Pangan*. 7 (1), 8–17.
- Baehaki, S.E. & Mejaya, I, M.J. (2014) Wereng coklat sebagai hama global bernilai ekonomi tinggi dan strategi pengendaliannya. *Iptek Tanaman Pangan*. [Online] 9 (1), 1–12. Available from: <http://pangan.litbang.pertanian.go.id/files/01-Iptek012014-Baehaki.pdf>.
- Baehaki & Widiarta, I.N. (2009) Hama wereng dan cara pengendaliannya pada tanaman padi. Aan Darajat et al. (eds.) *Padi Inovasi Teknologi Produksi*. Buku 2. Jakarta, LIPI Press, pp.347–383.
- Bahagiawati (2012) Kontribusi teknologi marka molekuler dalam pengendalian wereng coklat. *Pengembangan Inovasi Pertanian*. 5 (1), 1–18.
- Bhogadhi, S.C. & Bentur, J.S. (2015) Screening of rice genotypes for resistance to brown plant hopper biotype 4 and detection of BPH resistance genes. *International Journal of Live Science Biotechnology and Pharma Research*. 4 (2), 90–95.
- Brar, D.S., Virk, P.S., Jena, K.K. & Khush, G.S. (2009) Breeding for resistance to planthoppers in rice. In: Heong KL, H.B. (ed.) *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. Los Banos, International Rice Research Institute, pp.401–428.

- Carsono, N., Prayoga, G.I., Rostini, N. & Dono, D. (2016) Seleksi berbasis marka molekuler pada padi generasi F2 guna merakit galur padi harapan tahan wereng coklat. *Jurnal Agrikultura*. 27 (1), 9–15.
- Direktorat Perlindungan Tanaman Pangan (2016) *LAKIN 2015: Laporan Kinerja Perlindungan Tanaman Pangan 2015*. Jakarta.
- Francia, E., Tacconi, G., Crosatti, C., Barabaschi, D., Bulgarelli, D., Dall'Aglio, E. & Valè, G. (2005) Marker assisted selection in crop plants. *Plant Cell, Tissue and Organ Culture*. [Online] 82 (3), 317–342. Available from: doi:10.1007/s11240-005-2387-z.
- Iswanto, E.H., Susanto, U. & Jamil, A. (2015) Perkembangan dan tantangan perakitan varietas tahan dalam pengendalian wereng coklat di Indonesia. *Journal Penelitian dan Pengembangan Pertanian* 34 (4), 187–193.
- Jamil, A., Satoto, Sasmita, P., Guswara, A. & Suharna (2016) *Deskripsi varietas unggul baru padi*. Jakarta, Badan Penelitian dan Pengembangan Pertanian.
- Jena, K.K., Jeung, J.U., Lee, J.H., Choi, H.C. & Brar, D.S. (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene, Bph18(t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*. [Online] 112 (2), 288–297. Available from: doi:10.1007/s00122-005-0127-8.
- Liu, G., Yan, H., Fu, Q., Qian, Q., Zhang, Z., Zhai, W. & Zhu, L. (2001) Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. *Chinese Science Bulletin*. [Online] 46 (17), 1459–1462. Available from: doi:10.1007/BF03187031.
- Liu, Y., Su, C., Jiang, L., He, J.U.N., Wu, H.A.N. & Peng, C. (2009) The distribution and identification of brown planthopper resistance genes in rice. *Hereditas*. [Online] 73, 67–73. Available from: doi:10.1111/j.1601-5223.2009.02088.x.
- Pertiwi, W., Carsono, N. & Amien, S. (2014) Seleksi berbasis marka SSR untuk karakter ketahanan terhadap wereng coklat dan pengamatan fenotipik untuk daya hasil tinggi pada padi F 2. *Agricultural Science Journal I* (4), 275–285.
- Prashant, Shivshankar, T., Chandrashekharaiyah, Naveena, N.L. & Mallikarjun (2012) Incidence of brown planthopper (BPH) *Nilaparvata lugens* Stal. (Delphacidae : Hemiptera) in relation to age of the rice crop. *International Journal of Agricultural Science*. 3 (3), 197–200.
- Rahman, M.L., Jiang, W., Chu, S.H., Qiao, Y., Ham, T.H., Woo, M.O., Lee, J., Khanam, M.S., Chin, J.H., Jeung, J.U., Brar, D.S., Jena, K.K. & Koh, H.J. (2009) High-resolution mapping of two rice brown planthopper resistance genes, Bph20(t) and Bph21(t), originating from *Oryza minuta*. *Theoretical and Applied Genetics*. [Online] 119 (7), 1237–1246. Available from: doi:10.1007/s00122-009-1125-z.
- Sai, H., Sai, K., Padma, B., Richa, S., Ayyapa, D. & Vinay, S. (2013) Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. *African Journal of Biotechnology*. [Online] 12 (19), 2515–2525. Available from: doi:10.5897/AJB2013.11980.
- Satoto, Sulistyowati, Y., Hartana, A. & Slamet-Loedin, I.H. (2008) The segregation pattern of insect resistance genes in the progenies and crosses of transgenic Rojolele rice. *Indonesian Journal of Agricultural Science*. 9 (2), 35–43.
- Sitairesmi, T., Wening, R.H., Rakhmi, A.T., Yunani, N. & Susanto, U. (2013) Pemanfaatan plasma nutfah padi varietas lokal dalam perakitan varietas unggul. *Iptek Tanaman Pangan*. 8 (1), 22–30.
- Sonnino, A., Carena, M.J., Guimarães, E.P., Baumung, R., Pilling, D. & Rischkowsky, B. (2007) An assessment of the use of molecular markers in developing countries. In: Guimarães, et.al. (ed.) *Marker-assisted selection: Current status and future perspectives in crops, livestock, forestry and fis*. Rome, Food and Agriculture Organization of the United Nations, pp.15–26.
- Su, C. C., Zhai, H. Q., Wang, C. M., Sun, L. H. & Wan, J. M. (2006) SSR mapping of brown planthopper resistance gene Bph9 in Kaharamana, an indica rice (*Oryza sativa* L.). *Acta Genetica Sinica*. [Online] 33 (8), 717–723. Available from: doi:10.1016/S0379-4172(06)60104-2.
- Sun, L. H., Wang, C. M., Su, C. C., Liu, Y. Q., Zhai, H. Q. & Wan, J. M. (2006) Mapping and marker-assisted selection of a brown planthopper resistance gene bph2 in rice (*Oryza sativa* L.). *Acta Genetica Sinica*. [Online] 33 (8), 717–723. Available from: doi:10.1016/S0379-4172(06)60104-2.
- Sun, L.H., Su, C.C., Wang, C.M., Zhai, H.Q. & Wan, J.M. (2005) Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. *Breeding Science*. 55 (4), 391–396.
- Wang, Y., Li, H., Si, Y., Zhang, H., Guo, H. & Miao, X. (2012) Microarray analysis of broad-spectrum resistance derived from an indica cultivar Rathu Heenati. *Planta*. [Online] 235 (4), 829–840. Available from: doi:10.1007/s00425-011-1546-1.
- Wang, Y., Wang, X., Yuan, H., Chen, R., Zhu, L., He, R. & He, G. (2008) Responses of two contrasting genotypes of rice to brown planthopper. *Molecular Plant-Microbe Interactions: MPMI*. [Online] 21 (1), 122–132. Available from: doi:10.1094/MPMI-21-1-0122.
- Watanabe, T., Matsumura, M. & Otuka, A. (2009) Recent occurrences of long-distance migratory planthoppers and factors causing outbreaks in Japan. In: Heong KL, H.B. (ed.) *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. Los Banos, International Rice Research Institute, pp.179–190.
- Yunani, N., Wening, R.H., Pramudika, E. & Maryati, E. (2014) *Katalog Plasma Nutfah Padi*. Sukamandi, Balai Besar Penelitian Tanaman Padi.