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Determination of The Presence of Brown Planthopper Resistance Genes (*Nilaparvata lugens* Stål.) in Rice (*Oryza sativa* L.)

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Abstract*—The main goal of this current study is to determine the presence of brown planthopper (BPH)-resistance genes in some rice varieties to provide initial materials for the breeding program of BPH resistant rice varieties. According to the investigation, several molecular markers were used, such as RM1103, RM204, RM217, RM545, and RM401; and associated with BPH resistance genes Bph1, Bph3, bph4, Bph13, and Bph17. Our study was conducted in the laboratory of molecular genetics, the greenhouse, the field trials in CLRRI, and the laboratory of PCR and Biotechnology Company. The outcome indicated that there were 10 varieties showed with sustained resistance to some BPH populations in the Mekong Delta; a few indicator-resistance and indicator-susceptible varieties were assessed the genotyping through Simple Sequence Repeat (SSR) markers. Furthermore, they showed the presence of genes in the varieties such as TLR493 (Bph1), OM7268, OM6830, OM10279 (Bph3), OM6683, and Tau Huong (Bph1, Bph3, and Bph13), OM7364 (Bph1, bph4, and Bph13), OM5954 (Bph1 and Bph13), Chom bok Khmum (Bph3 and Bph17), but the Chet Cut variety was not showing the presence of five genes.*

Keywords*—*Molecular markers, Brown planthopper (BPH), BPH resistance genes, BPH resistance varieties, rice (Oryza sativa L.).

I. INTRODUCTION

Brown planthopper 's outbreak is growing of toxicity and has always been obsessed for farmers as well as scientists, managers in the world, especially in Asia's tropical regions, BPH is known as a most threat pest to rice crop production in Asia for many year decades and until now [1, 2, 3]. In annually, Asia's rice yield loss has been estimated at about > 300 million US$ dollars caused by the serious damage of BPH to rice production [4]. In China, the loss reported around 2.7 million tons of rice due to direct damage by BPH during 2005-2007 [5, 6, 7, 8]. In Vietnam, the rice yield loss due to the combined of BPH direct effect and two virus diseases has been estimated from 700.000 and 1 million tons in 2006 and 2007, respectively [9, 7, 10]. Brown planthopper is not only directly damage to rice growth, development and production about 10-75%, known as "hopper-burn" by feeding in the field conditions [11, 12], but also is mediatory for the transmitting of dangerous viral diseases such as rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV) lead serious losses of rice yield and productivity [13, 14, 15, 16]. In susceptible cultivars, the rice yield loss has been recorded up to 60% caused by the outbreaks of BPH [17].

The BPH is sophisticated for insecticides resistance development led to more and more serious damage and significantly affect to the yield of rice crops based on natural co-evolution rules and BPH's biological and behavioral characteristics such as short cycle's life, high fecundity, and long-distance migratory behavior which has been evolved to adapt, overcome, and survive on the new rice host plant, which expressed under selective pressure as well as for developing its resistance against several insecticides through various mechanisms [18, 19, 20, 21, 22]. While in the rice plant parallelly developed complicated and manifested specific defense mechanisms against the effect of BPH [23]. Addition, together with intensive rice, increasing of the rice crop season, increasing of planting area of aromatic rice varieties to serve for the exporting purpose, and resistant rice cultivars have been used against the ecological power of BPH and to stabilize the insect pest under economic threshold levels [24]. Among BPH control management, BPH-resistance genes-

carrying breeding method is always a priority approach to reduce the damage of BPH with the advantages of low-cost input and highly effective as well as this is a friendly ecological method as compared to chemical insecticides approach has been used to control BPH, however, misuse and overuse easily induced the issues of society and ecology during the cultivation as well as leading to the resistance evolution, reducing of effectiveness and led to decreasing of enemies and predators of BPH in the field [25, 26, 16]. Furthermore, firstly in 1967, the BPH-resistance gene has been known through the expression of resistance in the host plant to BPH [27]. At the present, the number of BPH-resistance genes have been genetically identified and sequenced in the cultivated landrace and in wild species and have been reported involved in the resistance of rice to BPH as well as it served as a study tool for interaction between the brown planthopper and rice host plant *i.e.* gene and gene interaction for the resistance and co-revolution of those [28, 29, 30, 31, 32, 33]. There were many BPH-resistance genes that have been introduced into popular rice varieties or elite cultivars or BPH susceptible cultivars for developing BPH-resistance new varieties and have been used as an environmental approach to control the damage of BPH at a low economic cost. Among of which, the *Bph1* gene was firstly identified in IR62 variety [34, 35], in Mudgo, CO22, and MTU15 cultivars [34], in MGL2 variety [36], and in the line IR747B2-6 of the crosses of susceptible parents [37]; the *Bph1* gene also reported that it's segregated independently for dwarf virus resistance in Kanto PL3 and stripe disease resistance in Kanto PL2 [38]. Zhao et al. [39] has been reported that the resistance of the *Bph1* gene to BPH in rice species through a map-based cloning approach. The *Bph3* gene was firstly identified in Rathu Heenati and in its introgression lines IR56 and IR60 [40], and in another seven resistant varieties [41]. In recent years, *Bph3* gene has been determined and assessed under map-based cloning technique and exhibited more resistance levels to BPH [42, 43]. The *bph4* gene identified in another ten resistant varieties [41], and in two varieties, Babawee, IR66 [44]. The *Bph13* gene identified in introgression line IR54741-3-21-22 of *O. officinalis* and *O. eichingeri* [45]. The *Bph17* gene identified in Rathu Heenati [46]. Therefore, the study of the determination of the presence of BPH-resistance genes in various local and popular rice varieties at Mekong Delta (MD) provinces of Vietnam is necessary and was conducted to find out multi-gene resistance varieties under the impact of BPH in MD. This result can be used to serve the breeding strategies to generate primary material resources for the development of BPH-resistant new rice varieties to fight the annual BPH outbreaks in MD. In this current study, MAS methods along with several molecular markers (SSR – Simple Sequence Repeat) were used to detect the presence of the BPH-resistance genes on the chromosome of various rice varieties.

II. MATERIALS AND METHODS

1. Plant materials

Ten rice varieties (OM6683, OM5954, OM7364, TLR493, OM7268, OM6830, OM10279, Chom bok Khmum, Chet Cut, and Tau Huong) have been shown the phenotype with stable resistance to four BPH populations in Mekong Delta. OM6162 rice variety has many good characteristics, but susceptible to BPH and selected as gene recipient variety; other rice varieties carrying resistance gene need to study such as Mudgo (*Bph1*), Ptb33 (*Bph3*), Babawee (*bph4*), *O. officinalis* (*Bph13*), and Rathu heenati (*Bph17*); and susceptible check variety TN1 (un-carrying resistance gene). Molecular SSR markers were used, including RM1103, RM204, RM 217, RM545, and RM401 (Table 1).

Table 1: The list of primers was used in PCR reactions.

Markers	Primers	Chr.	Linked gene	References
RM1103	Forward 5' CAGCTGCTGCTACTACACCG 3'	12	*Bph1*	Park et al. [47]
	Reverse 5' CTACTCCACGTCCATGCATG 3'			
RM204	Forward 5' GTGACTGACTTGGTCATAGGG 3'	6	*Bph3*	Jairin et al. [48]
	Reverse 5' GCTAGCCATGCTCTCGTACC 3'			
RM217	Forward 5' ATCGCAGCAATGCCTCGT 3'	6	*bph4*	Kawaguchi et al. [49]
	Reverse 5' GGGTGTGAACAAAGA			

	CAC 3’			
RM545	Forward 5’ CAATGGCAGAGACCCAAAAG 3’	3	*Bph13*	Chen et al. [50]
	Reverse 5’ CTGGCATGTAACGACAGTGG 3’			
RM401	Forward 5’ TGGAACAGATAGGGTGTAAGGG 3’	4	*Bph17*	Sun et al. [46] Liu et al. [51]
	Reverse 5’ CCGTTCACAACACTATACAAGC 3’			

2. DNA extraction

DNA samples extracted from rice plants using the mini method as described by IRRI [52, 53] and Nguyen Thi Lang [54]. The quantity of DNA samples was determined by spectrophotometer and the quality of DNA samples was checked by electrophoresis analysis under agarose gel (0.9%) in a solution of TAE 1X. DNA samples with high purity were stored at -20^{o}C.

3. SSR analysis

The PCR products were amplified through microsatellite markers (SSR) following by the method of IRRI [52, 53] and Nguyen Thi Lang [54].

Table 2: PCR solution preparation for each reaction.

Components	Stock solution	Final solution	Volume per each reaction
Duplicated Distilled H_2O	-	-	8,5µl
PCR buffer (10X)	10X	1X	1,5µl
dNTPs	1mM	0,1mM	1,0µl
Forward primer	5µM	0,25µM	0,5µl
Reverse primer	5µM	0,25µM	0,5µl
Taq polymerase	0,75U/µl	0,75U/10µl	1,0µl
DNA sample	30ng/µl	60ng/15µl/reaction	2,0µl
Total volume			15µl

Reference: IRRI

4. Duration and location of the study

The study was conducted from 2014 to 2015, and all of the experiments were implemented in the molecular genetic analytical laboratory, greenhouse, and field trials of CLRRI and the laboratory of PCR and biotechnology company in Can Tho, Vietnam.

III. RESULTS AND DISCUSSION

1. Determination of the presence of *Bph1* gene

The amplified products of multiple bands RM1103 showed 5 alleles along with the molecular size are 100bp (TN1 and OM6162), 150bp (OM7268 and OM6830), 190bp (OM10279), 200bp (OM6683, OM5954, OM7364, TLR493, Tau Huong, and Mudgo), 210bp (Chom bok Khmum, and Chet Cut) (Figure 1). The similarities to the band position of Mudgo variety at the molecular size at 200bp are including OM6683, OM5954, OM7364, TLR493, and Tau huong. In previous studies, Park et al. [47] also reported that the RM1103 marker is linked with the *Bph1*-resistance gene located on chromosome 12. In another study, Shabanimofrad et al. [55] also used RM1103 to determine the presence of BPH resistance gene located on chromosome 12. As mentioned in the upper text, *Bph1*-resistance gene not only mapped on rice chromosome 12 by RM1103 (SSR), but also detected using various molecular markers like G148 (RFLP) on chromosome 12 [56, 57]; em5814N (AFLP) on chromosome 12L [58, 59]; BpE18-3 (STS) on chromosome 12 [60] (Kim and Sohn 2005); XNpb248, XNpb336 (RFLP) on chromosome 12L [56]; OPD-7 RD7 (RAPD), RG869, RG457 (RFLP), RM247 (SSR) on chromosome 12 [61].

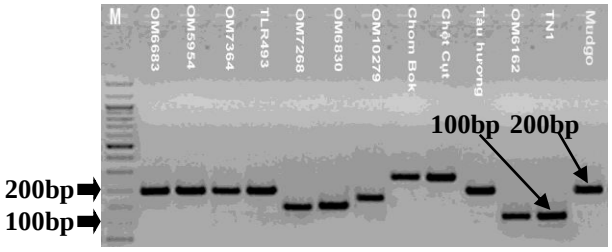

Fig.1: The amplified PCR products of the Bph1 gene at locus RM1103 on chromosome 12 of rice. M: Ladder 50bp

2. Determination of the presence of *Bph3* gene

In the case of using RM204 gene, the PCR products were completely amplified at the level of 100% and four alleles A, B, C, D were determined by molecular size 180bp, 190bp, 200bp, and 210bp, respectively. In which, based on the allele frequency of the bands on gel resistance control variety, Ptb33 showed a band of allele B at 200bp, and other varieties also revealed a band at the same size of 200bp, these varieties were OM6683, OM7268, OM6830, OM10279, Chom bok Khmum, and Tau Huong, this result indicated that these rice varieties carrying *Bph3* resistance gene. Susceptible indicator TN1 and OM6162 varieties showed the band of allele D with the size of a small 180bp. These suggested that OM6162 variety does not carry the *Bph3* resistance gene. In addition, the bands of alleles C and A also revealed at molecular size 190bp (OM5954, OM7364, and TLR493) and 210bp (Chet Cut) (Figure 2). Similarly, in a previous study Jairin et al. [48] also used molecular marker RM204 to detect the *Bph3* resistance gene on chromosome 6 of rice. In the study, the author also used RM589 (SSR) to know whether the presence of this gene on chromosome 6S of rice [48]. Further, the RM204 marker has been used to detect the presence of the *Bph5*-resistance gene on chromosome 6 and showed that this gene located together in chromosome 6 of ADR52 rice variety and progeny rice line in ADR52 [62].

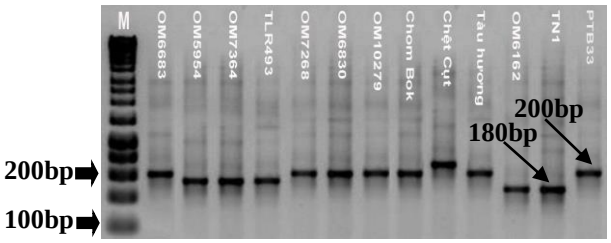

Fig.2: The amplified PCR products of the Bph3 gene at locus RM1204 on chromosome 6 of rice. M: Ladder 50bp

3. Determination of the presence of *bph4* gene

The multiple-bands were determined when using molecular marker RM217 and detected genes in various rice varieties with five alleles A, B, C, D, and E together with molecular size 200bp, 218bp, 240bp, 250bp, and 260bp, respectively. This result suggested that the genetic variation in different rice varieties is rather distinct. Kawaguchi et al. [48] have been reported that the RM217 molecular marker linked with the *bph4*-resistance gene on chromosome 6S. The author has also used other markers such as RM190 (SSR), C76A (RFLP) for the determination of the map of this gene on rice chromosome 6S [48]. In another study Sai et al. 2013 [63] also used the RM217 marker to detect *bph4* and resulted in multiple-bands with four alleles. However, in the previous study *bph4* gene has been identified on chromosome 10 through the trisomic analysis [64]. In the present study, OM7364 rice variety revealed the band position is the same as the position band of Babawee rice variety at the size of 218bp (Figure 3). In another report also demonstrated this variety is carrying *bph4* gene, and this result is similar to the report of Tran Nhan Dung [65]. Further, OM6162 variety showed the band position is the same with susceptible variety check TN1-control at 200bp, this indicated OM6162 is susceptible variety. In addition, the rest of the rice varieties showed other alleles with different band positions, these varieties were TLR493, OM6830, OM10279 (240bp), OM7268 (250bp), OM6683, OM5954, Chom bok Khmum, Chet Cut, Tau Huong (260bp). In other study, the *bph4* gene has been identified for semidwarf characteristics based on its combination with the *sd1* gene, but *bph4* and *Xa4* genes inherited independently for the resistance to bacterial blight [66]. In recent years, the *bph4* gene has been identified in the improved cultivars for the resistance to BPH during the breeding works in several countries of Southeast Asia region [48].

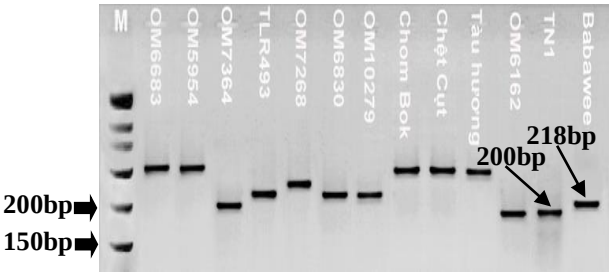

Fig.3: The amplified PCR products of the bph4 gene at locus RM217 on chromosome 6 of rice. M: Ladder 50bp

4. Determination of the presence of *Bph13* gene

In a previous study, Renganayaki et al. [67] have been reported that the *Bph13*-resistance gene determined to close the *bph19* gene at 5.18-5.70 cM on chromosome 3S of rice and located in the RG100 and RG19 (RFLP) flanked region. The amplified PCR products showed a multiple-bands with four alleles A, B, C, D when using molecular marker RM545 at four different sizes of 200bp, 210bp, 220bp, and 230bp (Figure 4). The susceptible check variety, TN1 showed a band of an allele at 200bp. While in OM6683, OM5954, and OM7364, and Tau Huong showed band similar to resistance check variety, *O. officinalis* at 220bp which is a resistance band. This result is comparable with the report of Shabanimofrad et al. [51], the authors also used molecular marker RM545 to detect this BPH resistance gene that locates on chromosome 3S of rice. In addition, other rice varieties revealed the bands with a

distinct size, including OM6162, OM7268, OM6830, Chom bok Khmum, and Chet Cut is at the size of 210bp, and TLR493 and OM10279 is at the size of 230bp. In the two progenies lines of *O. eichingeri* and *O. officinalis* detected the location of the *Bph13*-resistance gene at 6.1 cM and 5.5 cM on rice chromosome 2L through using of RM240 and RM250 markers, respectively [68]. An inherited *Bph13(t)* gene also identified on chromosome 3 of *O. officinalis* line, IR54741-3-21-22 from $AJ09b_{230}$ (RAPD), and its closely linked marker, AJ09c (STS) at 1.3cM on chromosome 3S [64]. Hence, these results exhibited that the duplicated genes, *Bph13* and *Bph13(t)* mapped on distinct two chromosomes of different parents following the law of independent segregation of the Mendel hypothesis.

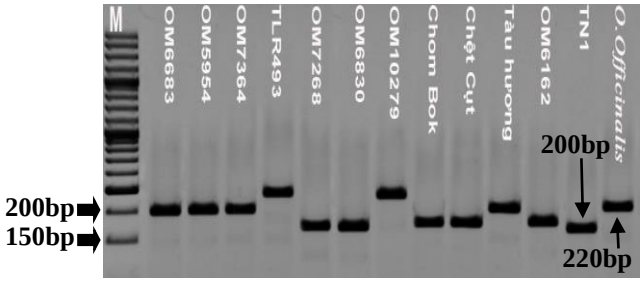

Fig.4: The amplified PCR products of the Bph13 gene at locus RM1545 on chromosome 6 of rice. M: Ladder 50bp

5. Determination of the presence of *Bph17* gene

The amplified PCR products showed a multiple-bands of six alleles when using RM401, with different molecular size are of 190bp, 200bp, 210bp, 230bp, 240bp, 250bp, and 260bp, respectively (Figure 5). Chom bok Khmum revealed the band similar to resistance check variety Rathu Heenati (200bp), which carries the resistance gene. This result is suitable with the reported study of Liu et al. [51], the authors also used RM401 molecular marker to determine the location of the BPH resistance gene, *Bph17* on rice chromosome 4S. In other studies, Rahman et al. [69] and Sun et al. [46] have been used other SSR markers, RM8213 and RM5853 to identify the location of this gene and detected *Bph17* resistance gene mapped at 4.40-9.60 cM on the short arm of chromosome 4S of rice, addition, the *Bph17*-resistance gene was tentatively designated with *Bph15* gene also detected located on chromosome 4 between two RM8213 and RM5953 markers (SSR) [70]. Part of *Bph17* gene stacked with the *Bph20* gene on chromosome 4 of varieties Rathu Heenati and *O. minuta*, respectively [69]. The OM6162 variety showed the band position similar to the band position of susceptible check variety TN1 with molecular size at 190bp, this indicated that OM6162 variety does not carry the resistance gene. The rest of the varieties showed the bands of alleles with the molecular size including 210bp (OM6683, TLR493, and Chet Cut), 230bp (OM10279), 240bp (OM6830) 250bp (OM5954, OM7268), and 260bp (OM7364, Tau Huong). In addition, the *Bph17* resistance gene has been mapped linked with *Qbph4* loci of chromosome 4S of rice, which identified as resistance genes in two different sources of IR64 and Rathu Heenati [46].

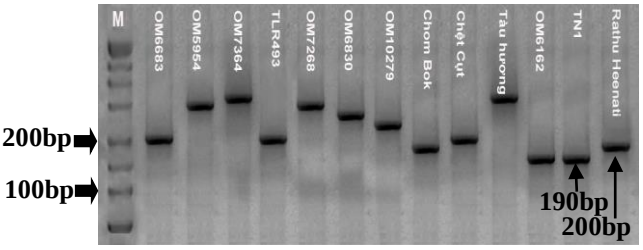

Fig.5: The amplified PCR products of the Bph17 gene at locus RM1401 on chromosome 6 of rice. M: Ladder 50bp

The result of genotype assessment determined five varieties expressed with multi-gene resistant to BPH, these varieties were OM6683 (*Bph1*, *Bph3*, and *Bph13*), OM7364 (*Bph1*, *bph4*, and *Bph13*), OM5954 (*Bph1* and *Bph13*), Chom bok Khmum (*Bph3* and *Bph17*), Tau Huong (*Bph1*, *Bph3*, and *Bph13*). Four varieties expressed by single-gene resistant; Chet Cut variety un-carrying the resistance gene. Both two genes *Bph1* and *bph2* were introduced into japonica variety for pyramiding between these genes based on molecular marker technique and showed that the pyramided lines possess the resistant degree at a higher level as compared to the line with single *bph2*, but the resistance is un-significantly different with *Bph1* [71]. Similarly, in another study, Li et al. [72] reported that both two genes *Bph4* and *Bph5* inserted into the genome of several hybrid rice-parental lines using MAS method and suggested that the pyramided lines of *Bph4* and *Bph5* exhibited the resistance to BPH with more power resistance than the introgression lines with a single gene with low resistance degree to BPH in the total of 92.3% introgression lines of single gene, *Bph4* [72]. In summary, the results of the present study combined with the previous studies obviously indicated that the rice plant possesses the polygenes leading to stronger BPH resistance degrees than the rice plant with single gene and vice versa.

Table 3: Correlation analysis between phenotype and genotype

No.	Variety name	*Bph 1* gene	*Bph 3 gene*	*bph 4 gene*	*Bph1 3 gene*	*Bph1 7 gene*
1	OM6683	+	+		+	
2	OM5954	+			+	
3	OM7364	+		+	+	
4	TLR493	+				
5	OM7268		+			
6	OM6830		+			
7	OM10279		+			
8	Chom bok Khmum		+			+
9	Chet Cut					
10	Tau Huong	+	+		+	
11	OM6162					
12	TN1 (susceptible check)					
13	Mudgo (*Bph1*)	+				
14	Ptb33 (*Bph3*)		+			
15	Babawee (*bph4*)			+		
16	*O.officinalis (Bph13)*				+	
17	Rathu heenati *(Bph17)*					+

+: positive resistance gene

IV. CONCLUSION

The presence of BPH-resistance genes investigated in various varieties, including TLR493 (*Bph1*); OM7268, OM6830, OM10279 (*Bph3*); OM6683 and Tau Huong (*Bph1*, *Bph3*, and *Bph13*), OM7364 (*Bph1*, *bph4*, and *Bph13*), OM5954 (*Bph1* and *Bph13*), Chom bok Khmum (*Bph3* and *Bph17*); Chet Cut variety was not carrying any five resistance genes, which reported in the current study. However, in the future study, the progenies population of the introgression lines shall be continuously developed in the net-house and in the field trials, these rice varieties comprised of OM6683, OM7364, Chom bok Khmum, and Tau Huong, which shall be served as the donor varieties of resistance gene to BPH in the breeding program for developing of new BPH resistance rice varieties. These new BPH-resistance varieties can contribute and provide new rice cultivars for the increasing of rice production and productivity, and improving on the quality and quantity of rice in Mekong River Delta regions of Vietnam. This result can also help for the sustainable rice production strategy in the present and future of Vietnam.

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