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Rice Breeding for Brown Planthopper Resistance (*Nilaparvata lugens* Stål.)

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Abstract**—***Plant breeding is considered one of the prospective strategies for the BPH [Nilaparvata lugens (Stål.)] resistance development under the modernized rice production system. The BPH-resistant novel rice lines were studied via molecular SSR markers, and the BPH effects on grain yield and the agronomic performance of the rice were also reported. The seven rice lines were obtained from the parents OM6683 (donor) x OM6162 (recipient), and two TN1-susceptible and Ptb33-resistant checking cultivars were used through the standard seed box screening technique. The results showed that seven rice lines and the OM6683 variety almost contained the BPH-resistance genes Bph1, Bph3, and Bph13 by utilizing three simple sequence repeat markers, like RM1103 (200 bp), RM204 (200 bp), and RM545 (220 bp). In addition, all of these rice lines/varieties also uncovered the BPH-resistance characteristics from resistant (3, scale) to highly resistant (1, scale) for four diverse BPH populations (Can Tho, Dong Thap, Tien Giang, and Hau Giang provinces) in both Autumn-Summer and Spring-Winter seasons. Furthermore, the results of BPH-resistance characteristics, grain yield, and agronomic traits were better than in the five rice lines G1-BC_2F_5-7-1-1-5-10, G2-BC_2F_5-8-1-1-9-5, G3-BC_2F_5-11-1-1-8-7, G4-BC_2F_5-54-1-1-5-2, and G5-BC_3F_4-8-1-1-1-5 as compared to other rice lines and the parents OM6162 and OM6683. In conclusion, the studies suggested that these potential rice lines can be harnessed as donor pivotal genetic sources to develop BPH-resistant novel rice varieties combined with valid agronomic and quality traits, and high crop yield.*

Keywords**—Rice (Oryza sativa L.), Brown planthopper, BPH resistance genes, MAS (Molecular Marker-Assisted Selection), Plant breeding.**

I. INTRODUCTION

Backcrossing-based plant breeding is mostly through phenotyping selection on the segregation of progeny generations for an excellent rice plant individual. There were advanced endeavors in plant breeding through the phenotyping selection for the key characteristics; however, they generally face constraints during the selection procedure due to the interaction between genotypes and environments. These challenges shall be addressed using molecular markers in plant breeding programs to enhance selection efficiency [1-3]. The MAS (marker-assisted breeding) approaches have diverse benefits because the plant traits cannot be assessed under field conditions [4, 5]. The MAS breeding research has revealed the important roles of genetic diversity determination, the interaction of various crop species, and the sustainability of genetic materials of crop plants [6, 3].

Out of different DNA markers, microsatellites are codominant shall increase the effectiveness and accuracy of the genetic algorithm of population-aided these makers as compared with the other makers, such as AFLP and RAPD; these markers show high allelic diversity; are easily and economically experimented by PCR analysis, and the procedures can be automated and the heterogeneous can be detected clearly during the experiments [7, 8, 4]. Many potential SSR markers have been identified in rice species, and more than 25,000 genes have been known as molecular markers in plants [9-11]. Many molecular marker-based research studies involving different varieties and cultivars have been proposed related to genome mapping, genetic diversity assessment, and marker-based breeding [12, 13, 4]. Plant breeding for BPH-resistance genes in rice varieties with multiple resistant mechanisms helps to give a reliable and broad spectrum of resistance against BPH, and protection and improvement of natural enemies and predators (*Anagrus nilaparvatae*) in the activities' efficiency [14-20, 3, 21] and reducing the application of agrochemicals [22-27]. In contrast, the overuse and misuse of chemicals and fertilizers are associated with unfavorable weather conditions, leading to a changing insect pest quantity and density, damage incidence, and shifting of the BPH biotypes. After that, BPH became more tolerant; further, BPH's damage also transfers an agent as a virus on rice plants [28-33, 27, 34, 35].

Till now, in rice, scientists have categorized the BPH populations into four distinct biotypes [22, 24, 31-33]. In the case of biotype 1, this biotype is a population determined in East and Southeast Asia. The *Bph1* gene was first discovered in the IR26 rice variety from the parents IR24 and TKM6 and displayed resistance against biotype 1 [13]. While biotype 2 is reported as the dominant biotype of the BPH population and originates in Indonesia and Vietnam [36-38, 22, 39, 4, 18, 40, 41] and the *bph2* gene was known in IR36 rice variety and identified resistance to biotype 1 and 2, but this gene is not against biotype 3. The resistance of rice during the impacts of BPH populations and biotype 3 has been known by IRRI [42] and Japan. In the remaining population, biotype 4 appeared only in South Asia countries. Three specific R-genes, *bph5*, *Bph6*, and *bph7*, show resistance against biotype 4, but could not be for biotypes 1, 2, and 3 [23, 30, 32, 39, 41]. In addition, in the Mekong Delta regions of Vietnam, there were several wild rice [43], traditional rice cultivars [39, 44, 45], and many rice lines/varieties (including OM9582, OM9577, OM6976, OM7364, OM6683, etc.) [46-52, 41, 53, 54, 55] showed high resistance to reducing vary BPH populations's damage in various rice cultivation regions of Vietnam. These results also indicated that these resistance rice varieties can have the presence of strong BPH-resistance genes or a BPH-resistance gene pool in rice, therefore, future investigation needs to be executed for the identification of the presence of BPH-resistance genes in these rice varieties *via* MAS [11, 56-66]. To date, 38 resistance genes have been identified on different rice chromosomes *via* BPH resistance analysis on the cultivated *Oryza sativa* and Wild rice species of rice germplasm resources gathered from diverse regions worldwide [67, 68]. Generally, all of the BPH R-genes have extensively been studied in plant breeding programs across Worldwide, especially Asian country's rice tropical cultivation with various BPH's biotypes and virulence in the field, however not three genes *bph5, bph7,* and *bph8*, some other BPH-resistance genes, and QTLs were determined and mapped on different chromosomes and associated with the resistance characteristics to BPH on 7 chromosomes (2, 3, 4, 6, 10, 11, and 12), these chromosomes almost present in *Indica* and wild rice species [69, 6, 70-74, 67, 75, 68, 76]. The results demonstrated that not all known genes show significant functions for resistance to various BPH populations prevalent throughout many Asian countries; many of those genes are effective with tagged molecular markers [77]. Among the determined BPH-resistance genes, *Bph14* (on chr. 3), *Bph3/Bph17* (on chr. 4), *Bph26* (on chr. 12), *bph29* (on chr. 6), *Bph18* (on chr. 12), *Bph6* (on chr. 11), *Bph32* (on chr. 6), and *Bph9* (on chr. 12) have been used for heritable studies *via* the cloning approach [78-81, 67, 82-84]. Out of the BPH-resistance genes that were published, 10 genes were identified at the stage of fine-map comprising *Bph1*, *bph2*, *Bph3*, *Bph9*, *Bph14*, *Bph15*, *Bph18*, *Bph19*, *Bph20*, and *Bph21* [70, 54, 55].

The markers-based selection and breeding are considered prominent approaches for the identification of present BPH-resistance genes in the host plant because of the merits of the molecular markers comprising economics, ease to manipulate, and ubiquitous presence and uniform distribution in the genome due to genetically sources under high-throughput materials data [85-88, 41, 53, 89, 84]. The present study was carried out with the main goal is to identify the BPH-resistance genes that introgression into the back crossing-based generated rice lines from the OM6683 rice variety donor and OM6162 rice variety recipient using the controlled screening methods in the greenhouse and the field with artificial pressure screening by direct inoculation of BPH individuals on seedling rice plants, along with evaluation agronomic traits, yield components, and grain yields; and using the molecular genetic techniques for the reported gene-linked SSR markers-based genotypic assessment for the BPH resistant in rice species. The findings showed that out of the seven potential rice lines, we screened three rice lines

from the OM6162/OM6683//OM6162 hybridization combination, which exhibited high resistance against BPH and received better agronomic performances in comparison to other rice lines and the parent rice varieties. In this report, we named these rice lines G1-BC_2F_5-7-1-1-5-10, G2-BC_2F_5-8-1-1-9-5, G3-BC_2F_5-11-1-1-8-7, G4-BC_2F_5-54-1-1-5-2, and G5-BC_3F_4-8-1-1-1-5, which were used for further evaluation in farmers' fields at different regions and serve for the rice production program of new BPH-resistant rice varieties of the countries.

II. MATERIALS AND METHODS

1. Plant materials

Plants source were from seven rice lines (containing four lines BC_2F_5 and three lines BC_3F_4) of hybridization combination OM6162/OM6683//OM6162, in which OM6162 (recurrent parent) and OM6683 (donor parent) were performed as the primary parents' rice varieties (high-quality rice varieties), and susceptible check variety TN1 (non-carrying resistance gene), and resistance check variety Ptb33 (carrying resistance gene: *bph2*, *Bph3*, and *Zlh3*) [50, 77]. The BPH population sources were collected at four distinctive rice-cultivating regions of the Mekong River Delta of Vietnam, *viz.* Can Tho, Dong Thap, Tien Giang, and Hau Giang provinces.

2. Phenotypic assessment

The plant phenotype of seven rice lines was checked through the BPH damage levels recording to plants based on a standard seed-box screening test (SSST) under greenhouse conditions at a temperature of 28-30^{o}C and RH of 70-80%, the technique was offered by the International Rice Research Institute [90]. The rice seeds were presoaked in clean water for 36 hours and then kept at room temperature for 24 hours in dark conditions for sowing in a plastic/steel tray. After that, the tiny seedlings of seven rice lines were sown in rows of 35 x 25 x 10 cm, along with the donor and recurrent parents, OM6683 and OM6162 varieties, susceptible and resistant checks, TN1 and Ptb33, respectively. A total of 20 seedlings per row were maintained per line. There were three replications for each line, and these seedlings were infested at 7 days old with the 2nd to 3rd instar hopper, 5-8 nymphs per seedling (BPH population samples were collected in a test tube, given in a tray, and counted for the number of BPH per seedling). Seeds of susceptible check TN1 were sown in two border rows and half of the middle row.

After one week to ten days, old seedlings were infested with BPH; after this infestation, the hopper burn 'symptom' was observed and recorded on the rice seedlings. When 100% of the susceptible check TN1 shows wilting and drying symptoms, the plants were scaled individually at growth stage level 2 (for the greenhouse), the evaluation is based on the damage scaling system, and each seedling was scaled as 0 = no injury/damage, 1 = Very slight injury/damage, 3 = first and second leaves of most plants partially yellowing or incompletely all leaf, 5 = Pronounced yellowing and stunting or about 10 to 25% of the plants wilting or dead and remaining plants severely stunted or dying, 7 = More than half of the plant dead, 9 = All plants dead.

3. Genotypic assessment

DNA extraction was done using the mini DNA method 91, 2] described. Leaf samples were collected after phenotypic assessment, *i.e.*, about two weeks after sowing. These leaf samples were taken in the morning as salt concentration inside the leaf is low and the enzyme activity is less; this is the best condition to extract DNA. The quantity and quality of DNA samples were checked using a spectrophotometer and electrophoresis analysis, respectively. These DNA samples were run on an agarose gel (0.9%) in a solution of TAE 1X. The high-purity quality of DNA samples was stored at -20^{o}C.

4. The analysis of SSR markers

The PCR products were amplified using SSR markers following the methods by a Bio-Rad machine. The PCR amplification procedure for SSR markers is suggested and illustrated by [91, 92, 2], Tables 1 and 2.

The SSR products were checked on an agarose gel 3% in a solution of TBE 1X combined with ethidium bromide. Using 7µl of PCR products and 4µl of ethidium bromide solution in each well. Horizontal electrophoresis was used to run PCR products for 1-2 hours, depending on the characteristics of the primer pair. After that, the running of PCR samples was observed on the gel, and the gel was gently taken out to take a photo. The bands appeared on film to find the resistance and susceptible genes based on standard bands of the DNA ladder (50bp and 100bp).

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

Markers	Primers	Chr.	Linked gene	Ref.
RM1103	Forward 5' CAGCTGCTGCTACTACACCG 3' Reverse 5' CTACTCCACGTCCATGCATG 3'	12	*Bph1*	Park et al. [47]
RM204	Forward 5' GTGACTGACTTGGTCATAGGG 3' Reverse 5' GCTAGCCATGCTCTCGTACC 3'	6	*Bph3*	Jairin et al. [48]
RM545	Forward 5' CAATGGCAGAGACCCAAAAG 3' Reverse 5' CTGGCATGTAACGACAGTGG 3'	3	*Bph13*	Chen et al. [50]

Table 2: PCR solution preparation for each reaction.

Components	Stock solution	Final solution	Volume for 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

5. The field trial of the rice lines was conducted through a BPH-resistance gene pool

The rice lines from prior studies were used for this study through various elaborate analyses. The field's experiments were conducted at Cuu Long Delta Rice Research Institute (CLRRI), Can Tho city, Vietnam. The trials were designed in Randomized Complete Block Design (RCBD) with 3 replications [93] and MARD, 2011 (Vietnamese Standards, QCVN 01-55:2011/BNNPTNT) [94] - the area of a plot at 30m^2/treatment/rep. The distance between plots is 30cm, and between the replications is 40cm.

The specialized technical procedure: The rice seeds were sown on the tray, and the seedlings at 10-12 days old were transplanted into the field as seedlings after sowing. The transplanting density was one plant/hole. The transplanting distance was 15cm x 20cm spacing, around 33 plants/m^2. Fertilizer application 100N-40P_2O_5-30K_2O kg/ha in the Spring-Winter season.

The insect pests were investigated according to Vietnamese Standards (MARD, 2011) (QCVN 01-38: 2010/BNNPTNT, 2010) [94]. The evaluation of insect pest resistance in the field on the potential rice lines was performed following the SES (Standard Evaluation System) for rice [90]. The BPH (*Nilapavata lugens* Stål.) was evaluated by the different levels at the growth stage (3-9): 0-scale: No injury; 1-scale: Slight yellowing of a few plants; 3-scale: Leaves partially yellow but with no hopper burn; 5-scale: Leaves with pronounced yellowing and stunting or wilting and 10-25% of plants with hopper burn, remaining plants harshly stunted; 7-scale: More than half the plants wilting or with hopper burn, remaining plants harshly stunted; 9-scale: All plants dead. Culm strength and plant type were evaluated at the different levels: Culm strength: 1-scale: Strong (no bending); 3-scale: Moderate strong (most plants bending); 5-scale: Intermediate (moderately bending of most plants); 7-scale: Weak (nearly flat of most plants); 9-scale: Very weak (all

plants flat). Plant type: 1-scale: Simply; 2-scale: Intermediate; 3-scale: Open.

Evaluation and recording of the parameters' data of growth, development, and grain yield according to National Technical Regulation on Testing for Value of Cultivation and Use of Rice varieties - VCU (Vietnamese Standards, QCVN 01-55: 2011/BNNPTNT) (MARD, 2011) [94]. The potential rice lines were evaluated for agronomic traits, yield parameters, and grain yield. The growth period (85% of grain per panicle): the trait was recorded from the sowing date to the ripening panicle stage, > 95%. Plant height (cm): measure from the surface of the soil near the stalk to the top of the tallest panicle (awns excluded). Number of filled grain/spike (grain): Count all filled grain/spike of 10 tillers and calculate the average of 3 replications. 1,000 grains weight (gram): Weigh 1,000 grains at 14% moisture content, with 3 replications. Grain yield (ha^{-1}): Weigh the harvested grains' weight with 5 square meters at 14% moisture content, with 3 replications.

III. RESULTS AND DISCUSSION

1. Development of rice line populations based on phenotypic evaluation combined with molecular markers

The presence of BPH-resistant genes in rice lines was identified as follows. The rice lines populations in the F_1, BC_1F_1, BC_2F_1, BC_2F_2, BC_3F_1, and BC_3F_2 were evaluated on both genotypes and phenotypes. Rice lines were selected in the F_1, BC_1, BC_2, and BC_3 generations with heterozygous resistance genotypes in all 3 markers to continue backcrossing in the next generation. Rice lines were selected in the BC_2F_2 and BC_3F_2 generations with homozygous resistance genes in 3 resistance genes and self-pollinated to select pure lines in the next generation.

The backcrossing results of the combination OM6162/OM6683//OM6162 were summarized in Fig. 1 and Table 3. The number of individuals carrying the BPH resistance gene was selected through molecular markers combined with phenotypic evaluation in the F_1 (117), BC_1 (14), BC_2 (6), and BC_3 (9) generations of the combination OM6162/OM6683//OM6162 (Table 3). As the previous study has been published by [54, 55], the similarity results on the rice lines have high resistance to diverse BPH populations, but not on the donor variety OM6683.

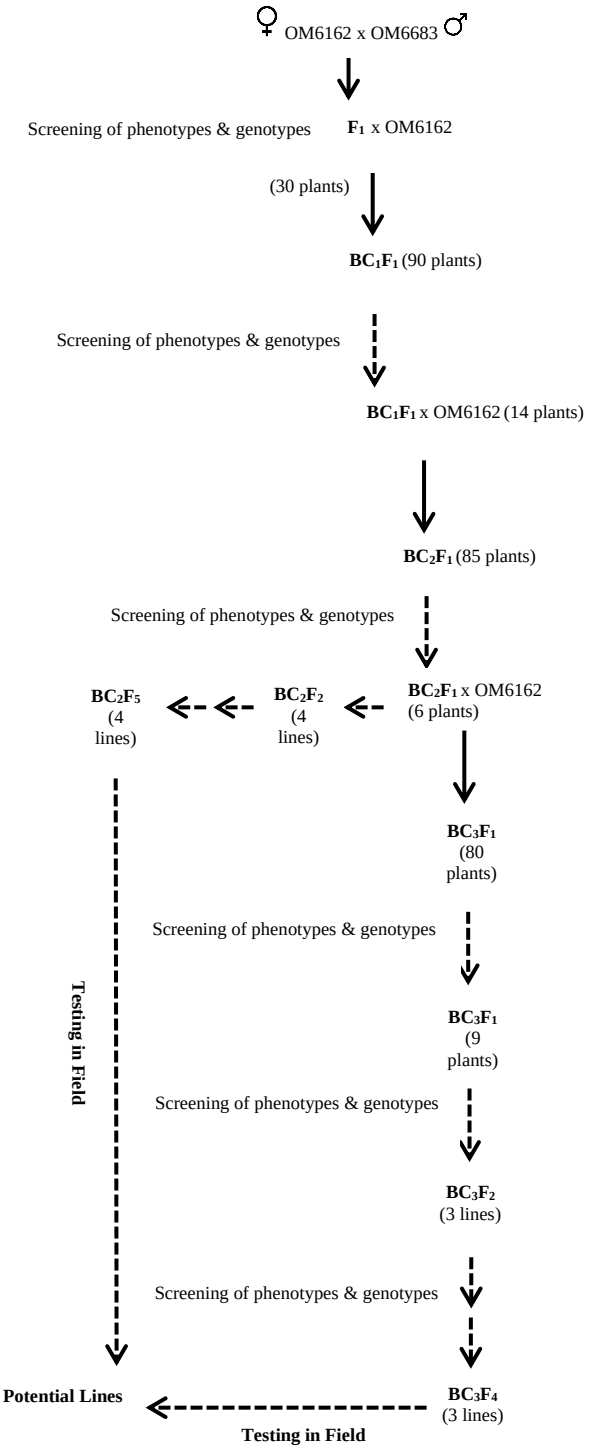

Fig.0. Scheme of hybridization and breeding of the OM6162/OM6683//OM6162 combination for BPH resistance rice lines

Table 3. The number of BC rice lines from the two combinations OM6162/OM6683//OM6162.

Generations	Total of rice lines	Number of selected rice lines *via* genotypes and phenotypes screening
F_1	180	117
BC_1F_1	90	14
BC_2F_1	85	6
BC_3F_1	80	9

+ F_1 generation: 30 rice lines were bred included of I-1; I-2; I-4; I-6; I-7; I-8; I-9; I-10; I-11; I-13; I-14; I-18; I-19; I-20; I-25; I-28; I-30; I-32; I-33; I-35; I-40; I-41; I-42; I-45; I-46; I-47; I-53; I-54; I-52; and I-56.

+ BC_1F_1 generation: 14 rice lines were bred included of I-6-1; I-7-1; I-8-1; I-9-2; I-10-2; I-11-1; I-14-2; I-19-1; I-47-1; I-53-3; I-53-4; I-54-1; I-52-1; and I-56-2.

+ BC_2F_1 generation: 6 rice lines were bred included of I-7-1-1; I-8-1-1; I-11-1-1; I-17-1-2; I-53-4-1; and I-54-1-1.

+ BC_3F_1 generation: 9 rice lines were bred included of I-7-1-1-1; I-7-1-1-2; I-7-1-1-3; I-8-1-1-1; I-11-1-1-1; I-17-1-2-1; I-53-4-1-1; I-54-1-1-1; and I-54-1-1-2.

1.1. Breeding of BPH-resistant rice lines in the field

Rice lines of the BC_2F_2, BC_2F_3, BC_2F_4, BC_3F_2, and BC_3F_3 populations were planted in experimental fields to breed pure lines resistant to brown planthoppers.

Table 4. The BPH resistance rice lines in the field were bred from the OM6162/OM6683//OM6162 combination.

Season	Generation	Total rice lines	Rice lines bred	Individuals bred
Autumn-Summer 2016	BC_2F_2	63	4	30
Spring-Winter 2016-2017	BC_2F_3	30	17	25
	BC_3F_2	45	3	20
Autumn-Summer 2017	BC_2F_4	25	10	4 (promising lines)
	BC_3F_3	20	7	3 (promising lines)

Rice lines carrying brown planthopper resistance genes were bred, as well as these lines posing good agronomic characteristics that were evaluated according to standards released by IRRI (2013), of plant hardness, shape, growth period, resistance to major pests and diseases, out of which most importantly are the yield of promising lines. The results of breeding BPH-resistant rice lines in the field of two combinations, OM6162/OM6683//OM6162 illustrated in Table 4. In other studies, the authors also reported the good agronomic components and high yield on the potential rice lines carrying the BPH resistance genes when those rice lines were trialed in the field [54, 55].

1.2. Genotype's assessment of the rice line population

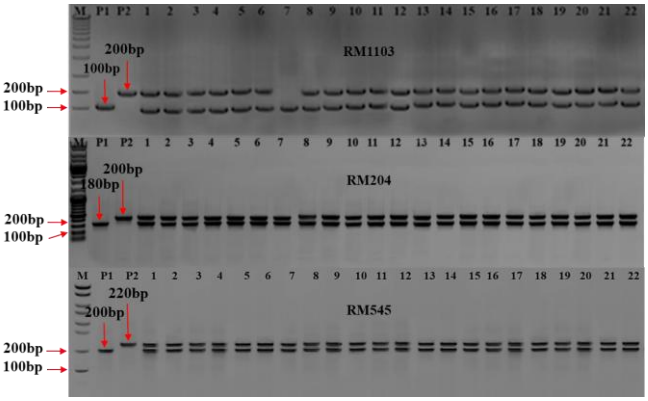

Fig.1: F_1 generation's genotypes of the OM6162/OM6683 combination were evaluated using markers *RM1103, RM204, and RM545. P1: OM6162, P2 OM6683; 1-22: F_1 hybrid lines population, M: Ladder 100bp*

In these results, based on phenotyping combined with genotyping, the authors bred 04 and 03 elite and potential rice lines at BC_2F_4 and BC_3F_3, respectively, which carry 03 codominant resistant genes (*Bph1*, *Bph3*, and *Bph13*). After that, those rice lines were self-pollinated to breed pure rice lines in the future generation (assessment results were illustrated in Fig. 1 to Fig. 8). Many researches have been used three markers RM1103, RM204, RM545 to determine the presence of three genes *Bph1*, *Bph3*, *Bph13* in rice, *Bph1* gene. For the *Bph1* gene idemtifed in some rice varieties such as Mudgo [95, 96, 66], IR747B-6 and IR28 (TKM6) [97-99], Vietnamese's rice varieties: OM6683, OM5954, OM7364, TLR493, and Tau Huong [54], *Japonica* rice varieties [100], Norin-PL3 rice variety [101]. For the *Bph3* gene known in Ptb33, Rathu Heenati [102, 103, 77]. For the *Bph13* gene explored in several rice varieties: OM6683, OM5954, and OM7364, Tau Huong, *O. officinalis* [104, 54], IR54745-2-21-12-17-6 rice variety, and *O. officinalis* wild rice [105], wild rice *Oryza eichingeri* [106].

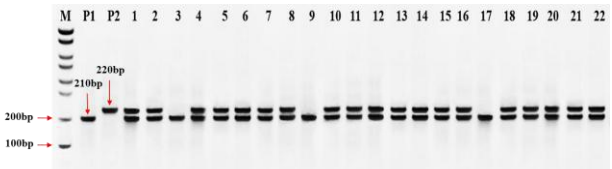

Fig.2: BC_1 generation's genotypes of OM6162*2/OM6683 combination were evaluated using marker *RM545. P1: OM6162, P2 OM6683; 1-22: BC_1 hybrid lines population, M: Ladder 100bp*

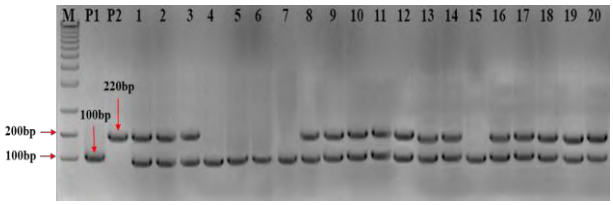

Fig.3: BC_2 generation's genotypes of OM6162*3/OM6683 combination were evaluated using marker *RM1103. P1: OM6162, P2 OM6683; 1-22: BC_2 hybrid lines population, M: Ladder 100bp*

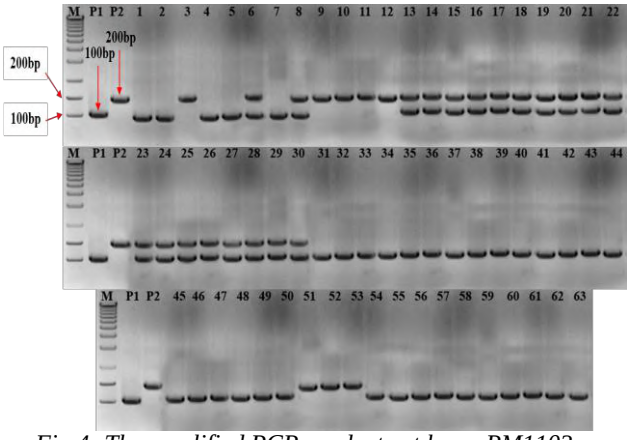

Fig.4: The amplified PCR products at locus RM1103 on chromosome 12 of BC_2F_2 rice lines of OM6162*2/OM6683 combination. *P1: OM6162, P2 OM6683; 1-63: BC_2F_2 hybrid lines population, M: Ladder 100bp*

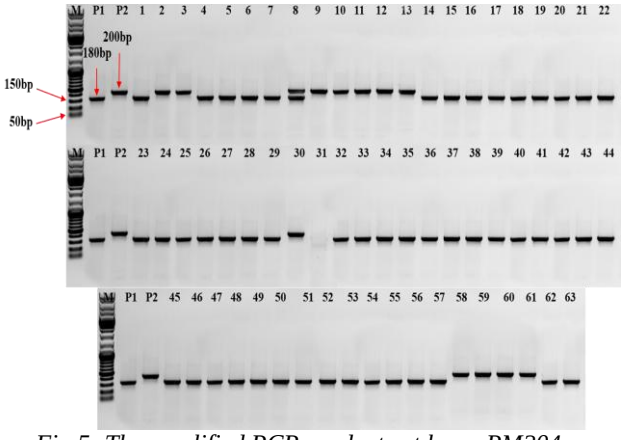

Fig.5: The amplified PCR products at locus RM204 on chromosome 6 of BC_2F_2 rice lines of OM6162*3/OM6683 combination. *P1: OM6162, P2 OM6683; 1-63: BC_2F_2 hybrid lines population, M: Ladder 50bp*

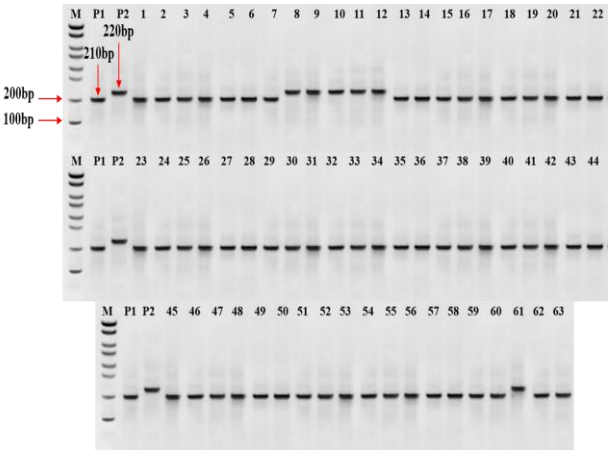

Fig.6: The amplified PCR products at locus RM545 on chromosome 3 of BC_2F_2 rice lines of OM6162*3/OM6683 combination. *P1: OM6162, P2 OM6683; 1-63: BC_2F_2 hybrid lines population, M: Ladder 100bp*

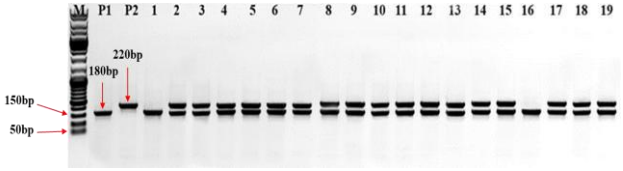

Fig.7: BC_3 generation's genotypes of OM6162*4/OM6683 combination were evaluated using marker *RM1204. P1: OM6162, P2 OM6683; 1-19: BC_3 hybrid lines population, M: Ladder 50bp*

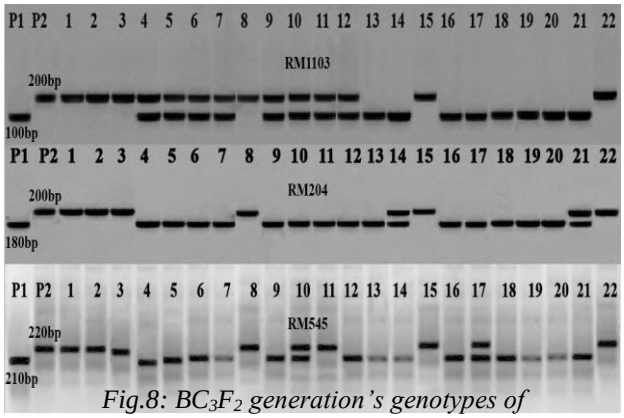

Fig.8: BC_3F_2 generation's genotypes of OM6162*4/OM6683 combination were evaluated using markers *RM1103, RM204, and RM545. P1: OM6162, P2 OM6683; 1-22: BC_3F_2 hybrid lines population, M: Ladder 100b*

2. The elite and potential rice lines for BPH-resistance

2.1. The presence of *Bph1, Bph3, and Bph13* genes for BPH-resistance in seven rice lines

The genes were for resistance to BPH from the genetic background of the backcrossing-based bred rice lines of the donor sources, the OM6683 rice variety, using the marker-assisted selection as shown in Table 5 and Fig. 9. The genotype screening assessment was performed using resistance gene-linked markers to evaluate resistance to BPH in each rice line (Fig. 9). The results of the current study showed that all seven potential rice lines had the presence of all three BPH-resistance genes *Bph1*, *Bph3*, and *Bph13 via* various resistance gene-linked SSR markers, including RM1103 (Fig. 9a), RM204 (Fig. 9b), and RM545 (Fig. 9c), respectively. For the primer RM1103, the *Bph1* gene was detected at 200bp on seven rice lines and cultivar OM6683 (P2) as compared to cultivar OM6162 (P1) (susceptible variety, at 100bp) (Fig. 9a). In the case of the primer RM204, the expression of the *Bph3* gene was determined in all the varieties and located at 200bp under this investigation, except the OM6162 cultivar at 180bp (Fig. 9b). The primer RM545 was as the results in "Fig. 9b" revealed that the *Bph13* gene was identified at a position of 220bp in cultivar OM6683 (P2), as well as seven rice lines were coded into 1-7, however this location did not detect in OM6162 cultivar, instead of that is at 210bp position. As part of this result (part 1) described above, the crucial role of three genes during the exposure of rice plants to the damage of BPH populations through stimulating the response and/or resistance characteristics. Our understanding was investigated based on the studies on phenotyping and genotyping assessment in the greenhouse and the field for BPH resistance of rice *via* the activation of the BPH resistance genes group, *viz. Bph1*, *Bph3*, and *Bph13* [54, 55, 107].

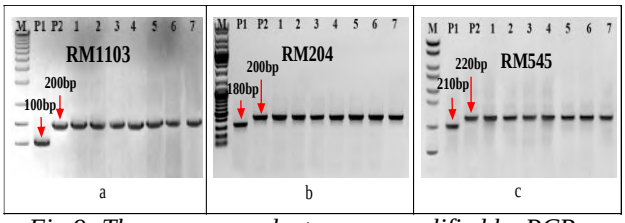

Fig.9: Three gene products were amplified by PCR on agarose gel (3%) using 03 MM: RM1103 (Bph1), RM204 (Bph3), RM545 (Bph13) for 07 rice lines of the OM6162/OM6683 combination. *P1: OM6162, P2 OM6683; 1-63: BC_2F_2 hybrid lines population, M: Ladder 50bp and 100bp*

2.2. The BPH-resistance characteristics of seven rice lines in a greenhouse through seed-box methods

The rice plant's resistance/susceptible characteristics to BPH of the potential rice lines (these lines were the products of backcrossing-based hybridization of the recipient (OM6162) and donor (OM6683) parents were evaluated on 4 brown plant hoppers populations, which have been collected in 4 provinces such as Can Tho, Dong Thap, Tien Giang, and Hau Giang, as well as the controlling varieties were TN1 (susceptible check) and Ptb33 (resistant check) were also harnessed to assess the responses of rice varieties to the BPH's damages, as the results were shown in Table 5. Those potential rice lines were found to be the resistance (R) reactions with 4 BPH populations at a damage scale range from 1-3 (resistant - R), including OM6683 rice variety (3, scale - resistant), these results were comparable to TN1 (susceptible control) variety at a scale of 9 (highly susceptible) and Ptb33 was at a scale of 3 (resistant control) (Table 5). Among 4 BPH populations, only Hau Giang's population exhibited low damage levels on seven rice lines and the Ptb33 rice variety; these results compared to the other three populations in both seasons (Autumn-Summer and Spring-Winter) (Table 5). Many studies show that the resistance characteristics of multiple genes to damage from insect pests in plants are the most effective and sustainable approaches. Especially, in brown planthopper populations, many pyramided genes were in rice plants showing higher resistance levels compared to a single gene in a particular rice variety/cultivar during the effects of diverse BPH biotypes [108, 109, 54, 55]. Multiple resistance genes present in different rice varieties/cultivars such as three genes *Bph3*, *Bph17*, and *Zlh1* in Rathu Heenati, four genes *bph2*, *Bph3*, *Bph32*, and *Zlh3* in Ptb33 [78, 103, 77]; two genes *Bph6* and *Bph12* in progeny rice lines [110]; two genes *Bph14* and *Bph15* in backcross progeny rice lines; four genes in *Bph1*, *bph4*, *Bph13*, and *Bph17* in Vietnamese's rice varieties/cultivars [111, 54, 55], family R gene - *Bph33(t)* in RIL rice lines - RP2068-18-3-5 (RP2068) (TN1 x RP2068) [112]; three *BPH4*, *BPH9*, and *BPH32* in NIL rice lines [76]; two genes *Qbph6* and *Qbph12* in Khao Dak Mali 105 [113].

Table 5. The BPH-resistance characteristics of the potential rice lines in the Summer-Autumn 2017 and Spring-Winter 2017-2018 seasons (the greenhouse).

S /N	Lines/ varieties	The damage scale of BPH populations on the rice lines (for the greenhouse test)							
		Can Tho		Dong Thap		Tien Giang		Hau Giang	
		AS 2017	SW 2018	AS 2017	SW 2018	AS 2017	SW 2018	AS 2017	SW 2018
1	G1-BC_2F_5-7-1-1-5-10	1	1	3	3	3	3	1	1
2	G2-BC_2F_5-8-1-1-9-5	3	3	3	3	3	3	3	3
3	G3-BC_2F_5-11-1-1-8-7	3	3	3	3	3	3	3	3
4	G4-BC_2F_5-54-1-1-5-2	3	3	3	3	3	3	3	3
5	G5-BC_3F_4-8-1-1-1-5	3	3	3	3	3	3	3	3
6	G6-BC_3F_4-53-4-1-1-1	3	3	3	3	3	3	3	3
7	G7-BC_3F_4-54-1-1-1-2	3	3	3	3	3	3	3	3
8	OM6162	7	7	7	7	7	7	5	5
9	OM6683	3	3	3	3	3	3	3	3
10	TN1	9	9	9	9	9	9	9	9
11	Ptb33	3	3	3	3	3	3	1	1

Annotated: AS: Autumn-Summer; SW: Spring-Winter 2018 (2017-2018). Damage scale and the responses of the rice lines/varieties: 1-3: Resistant (R); 5: Moderate Susceptible (MS); 7: Susceptible (S); 9: Highly Susceptible (HS).

3. The BPH resistance gene pool of rice lines in the field trial

To evaluate the host-plant resistance against other pests and diseases, field trial research was implemented in the Spring-Winter 2017-2018 season to validate the effectiveness of resistance genes in rice. The results of this present exploration exhibited that the set of seven rice lines and the parents' cultivar OM6683 were highly resistant (scale, 1) or resistant (scale, 3) for 5 insect pests and blast blight diseases, remaining the line G5-BC_3F_4-8-1-1-1-5 unveiled a moderate response (scale, 5) for leaf folder, similarly, the parent line OM6162 showed a medium response level (scale, 5) to planthopper and leaf folder (as illustrated in Table 5). The medium damage level of Blast blight, Thrips, and Planthopper was lower than Leaf folder and Gall midge on seven rice lines and OM6683, except for the OM6162 rice variety. Hence, the results of the present study indicated that seven rice lines are capable of carrying distinct R-genes for different resistance against insect pests such as Thrips, Leaf folder, Gall midge, and pathogen of Blast blight, rather than BPH resistance genes only for brown planthoppers (*Nilaparvata lugens* Stål.). Compare to the previous reports, the results of the present study showed moderate resistance to high resistance for mainly five insect pests (BPH, Thrips, Leaf folder, Gall midge, Blast blight) in rice plants, these results were also similar to former studies have been published in rice, like BPH [114, 115], BPH, Thrips, Leaf folder, and Gall midge [114, 116-119], BPH [120], Gall midge resistance [121], Blast disease [122-125].

Table 6. The resistance characteristics of the potential rice lines against some insect pests and diseases, Spring-Winter 2017-2018 season (the field).

S/N	Lines/ varieties	BPH (scale 0-9)	Thrips (scale 1-9)	Leaf folder (scale 0-9)	Gall midge (scale 0-9)	Blast blight (scale 0-9)
1	G1-BC_2F_5-7-1-1-5-10	1	1	3	3	3
2	G2-BC_2F_5-8-1-1-9-5	1	1	3	3	1
3	G3-BC_2F_5-11-1-1-8-7	1	1	3	3	1
4	G4-BC_2F_5-54-1-1-5-2	1	1	3	3	1

5	G5-BC_3F_4-8-1-1-1-5	1	1	5	3	1
6	G6-BC_3F_4-53-4-1-1-1	1	1	3	3	1
	G7-BC_3F_4-54-1-1-1-2	1	1	3	3	1
7	OM6162	5	3	5	3	3
	OM6683	1	1	3	3	1

Annotated: Damage scale and the responses of the rice lines/varieties: 1-3: Resistant (R); 5: Moderately Susceptible (MS); 7: Susceptible (S); 9: Highly Susceptible (HS).

3.1. The growth characteristics, yield components, and yield of the potential rice lines

The outcomes of the present study showed off that the seven rice lines and parents rice varieties OM6162 and OM6162 possessed a growth period distinguishing from 95-102 days, of which three rice lines < 100 days of the growth period containing lines like G2-BC_2F_5-8-1-1-9-5, G3-BC_2F_5-11-1-1-8-7, and G4-BC_2F_5-54-1-1-5-2, the rest lines were ≥ 100 days of the growth period. The plant height average of seven rice lines was low from 93.7-110.3 cm, among these three lines G4-BC_2F_5-54-1-1-5-2 (98.3 cm), G5-BC_3F_4-8-1-1-1-5 (93.7 cm), and G7-BC_3F_4-54-1-1-1-2 (96.7 cm) and one rice variety OM6683 (99.0 cm) retained plant height < 100 cm, whereas the rest of other lines and OM6162 rice variety had taller plant height > 100 cm. In case the number of panicles per hill was from 8.0-10.7 panicles, and the panicle length was from 22.2-24.2 cm, and virtually no non-significant difference between those lines and the parent's rice varieties, especially these three rice lines. In addition, the culm strength of rice lines uncovered at 3, scale *i.e.*, moderately strong (most plants bending) to scale 1, *i.e,*. strong level [90], and the plant type was from the moderate compact type (Table 6). Furthermore, all seven rice lines showed higher rice yield and significant differences at the P = 0.05 level compared to the OM6162 cultivar, because this cultivar was damaged by the BPH effects, inducing lower grain yield and other performance traits, as illustrated in Table 7. Among seven rice lines, five rice lines revealed higher grain yield with significant differences at the P = 0.05 level as compared to the parents' rice varieties as well as other rice lines, these lines were G1-BC_2F_5-7-1-1-5-10, G2-BC_2F_5-8-1-1-9-5, G3-BC_2F_5-11-1-1-8-7, G4-BC_2F_5-54-1-1-5-2, and G5-BC_3F_4-8-1-1-1-5; among of which, the line G4-BC_2F_5-54-1-1-5-2 impressively showed on the highest grain yield at 7.7 tons/hectare. Similarly, in the case of other traits' performance, *viz.*, a quantity of filled grain/panicle, flat grain percentage, and weight of 1.000 grains, were shown almost higher in these five rice lines as compared to other rice lines and the parents' rice varieties (Table 7, Fig. 10, 11). Overall, the increasing agronomic performance in seven rice lines in comparison to the donor OM6683 and recipient OM6162 rice varieties proved that the BPH-R genes had no yield penalty in the BPH resistance genes-carrying rice lines, these obtained findings might caused by the stimulating of genes expression inherited from the donor genomes, especially, five rice lines mentioned above part. These views again stated the effectiveness of the MAB and MAS programs in transferring R-genes into elite rice varieties and simultaneously obtaining the identical target genes for insect pest resistance. As compared to previous studies that reported, we may desmonstrate that the potential molecular events and resistance mechanisms based on the interaction between host-plant-insect pests and the central role of R-genes appear inside plant cells, in which the R-genes are activated under the impacts of BPH, helping plants survive, maintain the growth and development, promote and enhance yield components and high yield of rice plants. These BPH-resistant mechanisms resulted from the phenotyping and genotyping assessment by the participation of different reactions and mechanisms comprising the specific resistance mechanisms from the host-plant resistance [126], high-regulation of the BPH-resistance genes (including *Bph* and/or *bph* gene type) [127, 112, 54, 55, 107].

Table 7. The growth characteristics of the potential rice lines in the Spring-Winter season 2017-2018.

S /N	Lines/ varieties	Growth period (days)	Plant height (cm)	Culm strength (scale)	Plant type (scale)
1	G1-BC_2F_5-7-1-1-5-10	102	110,3a	1	1
2	G2-BC_2F_5-8-1-1-9-5	98	101,7cd	3	1
3	G3-BC_2F_5-11-1-1-8-7	95	106,7ab	3	1
4	G4-BC_2F_5-54-1-1-5-2	98	98,3de	1	1

5	G5-BC_3F_4-8-1-1-1-5	100	93,7f	1	1
6	G6-BC_3F_4-53-4-1-1-1	102	103,3bc	1	1
7	G7-BC_3F_4-54-1-1-1-2	100	96,7ef	3	1
8	OM6162	102	101,7cd	3	2
9	OM6683	100	99,0cde	1	1
	CV%		2,5		

Annotated: The values of the same column followed by the same letters indicate the statistically non-significant difference under the Duncan test at a 5% level.

Table 8. The agronomical traits and grain yield of the potential rice lines in the Spring-Winter season 2017-2018.

S/N	Lines/ varieties	No. of panicles/ hill (grains)	Panicle length (cm)	No. of filled grain/ panicle (grains)	Flat grains ratio (%)	1.000 grains weight (gr)	Yield (tons/ hectare)
1	G1-BC_2F_5-7-1-1-5-10	8,3bc	22,5b	114,0bc	20,9bcd	26,9ab	7,0b
2	G2-BC_2F_5-8-1-1-9-5	9,7ab	22,3b	124,3ab	17,9cd	27,3ab	7,4ab
3	G3-BC_2F_5-11-1-1-8-7	9,7ab	22,7b	110,0c	22,6bcd	27,1ab	6,8b
4	G4-BC_2F_5-54-1-1-5-2	10,7a	23,3ab	134,7a	15,7d	27,8a	7,7a
5	G5-BC_3F_4-8-1-1-1-5	10,7a	24,2ab	127,7a	17,8cd	27,2ab	7,3ab
6	G6-BC_3F_4-53-4-1-1-1	9,0bc	22,2b	108,3c	24,8ab	26,3b	6,2c
7	G7-BC_3F_4-54-1-1-1-2	8,0c	23,2b	105,3c	27,2ab	26,3b	6,0c
8	OM6162	6,0d	23,7ab	92,7d	30,5a	26,3b	5,2 d
9	OM6683	9,3abc	25,3a	104,7c	26,2ab	27,1ab	6,2c
	CV%	9,3	4,7	5,9	16,3	2,00	4,8

Annotated: The values of the same column followed by the same letters indicate the statistically non-significant difference under the Duncan test at a 5% level.

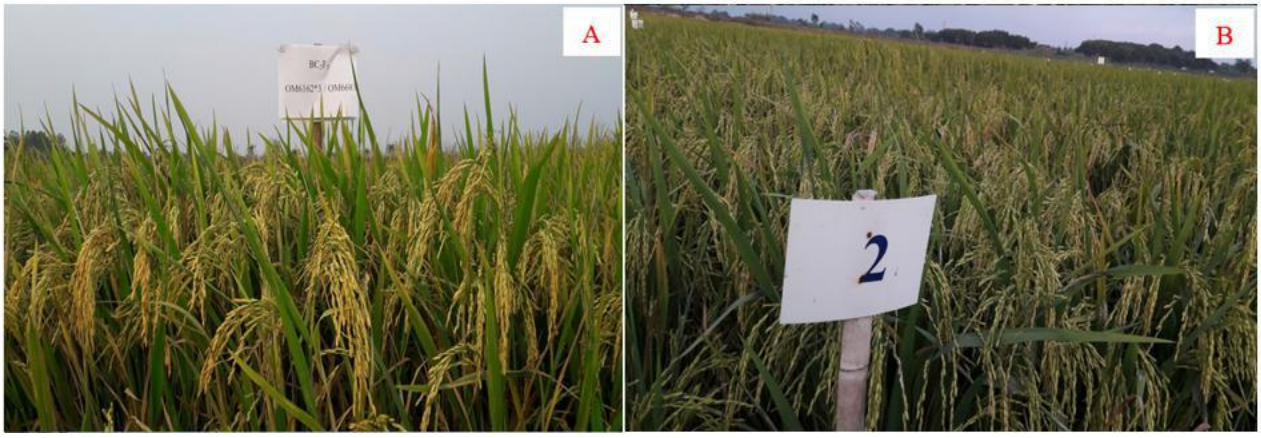

Fig.10: Field trial of promising BPH-resistance rice lines at CLRRI in Spring-Winter 2017-2018

A: Rice line G4-BC2F5-54-1-1-5-2 of OM6162/OM6683//OM6162 combination

B: Rice line G2-BC_2F_5-8-1-1-9-5 of OM6162/OM6683//OM6162 combination

Fig.11: Hybridization rice lines (surviving after resistant assessment) were planted under artificial screening in a net-house

IV. CONCLUSION

In summary, the research breeding three potential rice lines from seven introgression rice lines of the backcrossing population of OM6162/OM6683//OM6162 combination, these rice lines were G1-BC_2F_5-7-1-1-5-10, G2-BC_2F_5-8-1-1-9-5, G3-BC_2F_5-11-1-1-8-7, G4-BC_2F_5-54-1-1-5-2, and G5-BC_3F_4-8-1-1-1-5, which carry three pyramiding BPH-resistance genes *viz. Bph1* (RM1103; 200 bp), *Bph3* (RM204; 200 bp), and *Bph13* (RM545; 220 bp). Furthermore, three rice lines showed BPH resistance levels from resistant (scale 3) to highly resistant (scale 1) through the standard seedbox screening technique (SSST) for four BPH populations at four areas: Can Tho, Dong Thap, Tien Giang, and Hau Giang. Addition, three rice lines out of seven rice lines almost showing the medium damage level of Blast blight, Thrips, and Planthopper (grade 1-3), Leaf folder and Gall midge (grade 3-5), this result indicate that three genes carrying the intergration of resistance to various insect pests under the controlling conditions. Moreover, the yield components and yield of the potential rice lines also show higher levels than before the sources of varieties. In conclusion, the potential rice lines obtained from this study can be used as significant genetic materials for future studies of the breeding programme. These lines need to be studied in a deepening knowledge to understand the molecular biology of the differentially expressed BPH resistance genes and strategies for plants' response and control based on the genomic sequencing integration and the OMICs approaches (genomics, transcriptomics, proteomics, metabolomics, phenomics, ionmics). The key connection of these results is that these potential rice lines must be developed in the main rice production regions to provide novel insect-pest-resistant rice variety sources for the farmers' rice cultivation and sustainable development in the Mekong Delta of Vietnam.

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