MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT
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EXPLOITATION OF THE INITIAL MATERIALS FOR RESEARCH AND BREEDING OF BROWN PLANTHOPPER RESISTANT RICE VARIETIES

Specialization: Biotechnology
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SUMMARY OF PHILOSOPHY DOCTORAL THESES IN AGRICULTURAL BIOTECHNOLOGY

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INTRODUCTION

1. The necessity of theses

Among the insect pest of rice, the brown planthopper (BPH), *Nilaparata lugens* Stål (Hemiptera: Delphacidae), is the most serious planthopper species which destruct and widespread throughout the rice areas in the world, especially in Asian rice-growing countries crops productivity seriously reduced by BPH (Bharathi and Chelliah, 1991; Ikeda and Vaughan, 2006). In Vietnam, brown planthopper causes the annual production losses approx. ≈ 20% in the total productivity of crops (Ha Huy Nien and Nguyen Thi Cat, 2004). The outbreak cycle of BPH is from 12-13 years and the most peak is 14 years (Le Huu Hai, 2016). Therefore, the management must be positively performed during rice production to control the damage of BPH. The traditional means like insecticides/pesticides has been used to prevent BPH. However, the widespread and excessive use of insecticides/pesticides as well as nitrogenous fertilizer (urea) has been caused outbreaks as a result of selective adaptation of the insects by increasing the fecundity of BPH, and by reducing populations of natural enemies. Currently, among strategies to prevent and control BPH, breeding for developing and using resistant cultivars has become a priority in rice improvement (Ho Van Chien *et al.*, 2015). The use of resistant cultivars is the most effective, economical methods and long-term solution for BPH control as well as is a safe method for the ecological environment (Alam and Cohen, 1998; Renganayaki *et al.*, 2002). Therefore, keeping the important points in mind, the present investigation on the topic: "Exploitation of the initial materials for research and breeding of brown planthopper resistant rice varieties" was done to create valuable material sources for the sustained resistant to BPH that can contribute to the future and the current production objectives in the Mekong Delta.

2. The objectives of theses

The current investigation was performed with two major objectives: (i) Assessment of BPH resistance of the batch of converged rice cultivars and local rice varieties; (ii) Phylogenetic analysis and using marker molecular-based breeding methods to develop the excellent 2-3 cultivars with sustained resistance to BPH for providing in the Mekong River Delta.

3. The scientific and practical significance of the investigation

The achieved results of the current investigation *via* the molecular marker-assisted methods will be served for the exploring of the stacking-based BPH resistance genes in rice.

These results will be open the great opportunities for widely using of those useful information in the breeding programs. The BPH resistance genes-carried rice lines were selected in the current investigation will be served as the initial material resources and can valuably contribute to the breeding program of the sustained BPH resistance genes-contained rice cultivars in towards several years in Vietnam.

4. Subjects and investigation fields

4.1. Subjects

The subjects were used in the current study included a set of high-yield rice varieties (15 vars.); a set of local rice varieties (199 Acc.); a set of BPH-indicator rice varieties (15 vars.); and a set of popular rice varieties in Mekong Delta (14 vars.); Four known biotypes of BPH populations (Can Tho, Dong Thap, Tien Giang, and Hau Giang provinces), and together with several of BPH resistance genes-linked appropriate molecular markers.

4.2. Investigation fields

Investigation fields of the current study: (1) Phenotypic evaluation of BPH resistance of a set of high-yield rice varieties and set of local rice varieties (which cultivated in many areas of the Mekong River Delta) were performed based on the use of four different BPH populations. In addition, the investigation also used the appropriate molecular-markers to identify BPH resistance genes in some evaluated-rice varieties; (2) The presence of BPH resistance genes in the inbred-hybrid rice lines and back-crossed rice lines were identified through molecular SSR markers; (3) BPH resistance of the obtained hybrid rice lines were also evaluated.

Investigation locations: Collect sets of local rice varieties in local rice varieties growing regions of 10 Mekong Delta provinces. Collect BPH in four provinces such as Can tho, Dong Thap, Tien Giang, and

Hau Giang. The current study was conducted in the laboratory of PCR and Biotechnology Company; the laboratory of molecular genetics, greenhouse/net-house system, and the field trials of Plant Breeding and Genetics and Plant Protection Department in CLRRI. The investigation was performed in 44 months from June/2014 to February/2018.

5. The new contributions of theses

The current study provided the important genetic information on the initial material sources serve as the parent materials in the breeding strategy of new BPH resistance rice varieties.

The evaluation of efficiency of the BPH resistance genes showed maintained in Mekong River Delta regions

Out of the breeding objective for BPH resistance genes-carried rice varieties, the current investigation also studied on the high-yield rice varieties and appropriate duration rice varieties. This is a decisive requirement for the bred rice varieties products to be widely used and developed after the study is completed.

Suggestion of traditional breeding and hybridization method combined with using of molecular markers-based methods for reducing of timing research in the breeding program of BPH resistance rice varieties and the converging of BPH resistance genes.

6. The general structure of theses:

The main contents of the current investigation comprised of 127 pages, 37 tables, and 40 plates and figures. In which, Introduction, section comprised 4 pages, chapter I: Review of Literature comprised 36 pages, chapter II: Materials, Contents, and Methods comprised 21 pages, chapter III: Results and Discussion comprised 64 pages, Conclusions and Suggests comprised 2 pages. In addition, the theses also comprised appendices and bibliography section. The study used 279 references, in which used 49 references in Vietnamese and 230 references in English language.

Chapter 1. REVIEW OF LITERATURE

1.2.2.2. The achieved studies of BPH resistance genes in rice

Currently, a total of 38 BPH resistance genes has been identified and characterized in rice ((Balachiranjeevi *et al.*, 2019). In which, the important BPH resistance genes have been mapped to the different chromosomes, such as: *Bph33(t)* (Naik *et al.*, 2018), *Bph35* (Yang *et al.*, 2019) *Bph37* (IR64 variety QTLs) (Yang *et al.*, 2019) và *Bph38(t) genes* (Balachiranjeevi *et al.*, 2019) are located on chromosome 1; *Bph13 (t)* gene is located on chromosome 2 (Liu *et al.*, 2001); *bph11* (Hirabayashi *et al.*, 1998), *Bph13* (Renganayaki *et al.*, 2002; Chen *et al.*, 2006), *Bph14* (Du *et al.*, 2009), and *bph19* genes (Chen *et al.*, 2006) are located on chromosome 3; *Bph12* (Hirabayashi *et al.*, 1998; Yang *et al.*, 2002), *Bph15* (Yang *et al.*, 2004), *Bph17* (Sun *et al.*, 2005), *Bph20* (Rahman *et al.*, 2009), *Bph27* (Huang *et al.*, 2013), *Bph34* and *Bph36* genes (Kumar *et al.*, 2018) are located on chromosome 4; *Bph3*, *bph4*, *Bph25*, and *Bph29*, and *Bph32* genes are located on chromosome 6 (Kawaguchi *et al.*, 2001; Yara *et al.*, 2010; Wang *et al.*, 2015; Ren *et al.*, 2016); *Bph30* is located on chromosome 10 (Wang *et al.*, 2015); *Bph6* (Jena *et al.*, 2002), *Bph28* genes (Han *et al.*, 2014) are located on chromosome 11; *Bph1, bph2, Bph9, Bph10, Bph18, Bph21,* and *Bph26* genes are located on chromosome 12 (Sharma *et al.*, 2004; Jena *et al.*, 2006; Rahman *et al.*, 2009; Yara *et al.*, 2010) (Table 2.1 in Appendix 2).

1.2.2.3. The identified major BPH resistance genes in the present

Among the major BPH resistance genes, the genes like *Bph3*, *Bph17*, *Bph*20, *Bph*21, and *Bph32I* genes were remained effective resistance to BPH in Mekong Delta, further, some rice varieties carried multiple resistance genes in which the combining of *Bph3* gene and other gene possessed sustainable resistance. There are several BPH resistance genes have been known from the wild rice such as: *bph11*, *Bph12*, *Bph13*, *Bph14*, *Bph15*, *Bph20*, *Bph21*, *Bph29*, and *Bph30* genes were remaining effective resistance to BPH in Mekong Delta (Sun *et al.*, 2005; Jairin *et al.*, 2007a; Yasui *et al.*, 2007; Horgan *et al.*, 2015).

1.4. Recently resulted in studies on the exploitation of initial materials source, and use of MAS for the breeding of BPH resistance gene rice varieties

In the recent years, the molecular markers-based plant breeding strategies have been advanced and the obtained results in the pyramiding of several BPH resistance genes into elite varieties to breed the broad-spectrum, stable, and sustainable-BPH resistance rice lines. Sharma *et al.* (2004) used molecular-MAS marker-assisted selection-based breeding method to stack *Bph1* and *Bph2* genes into *Japonica* rice variety. Li *et al.* (2006) pyramided *Bph14* and *Bph15* genes by MAS into both the maternal and paternal parents of Chinese rice varieties. Myint *et al.* (2012) developed BPH-resistance hybrid rice lines which possessed both *Bph25* and *Bph26* genes. Zhao *et al.* (2013) pyramided all of BPH-resistance *Bph20 (t), bph21* genes, and blast-resistant Pi9 gene into BoIIIB elite variety to create new varieties with resistance to both BPH and Blast. Similarity, the obtained results in Vietnam showed that AS996 rice variety developed from the hybrids between IR64 x *Oryza rufipogon* (Acc. 106424, Tram Chim, Đong Thap Muoi), and IR65482-4-136-2-2 (IR31917-45-3-2 x *O. australiensis*), IR54742 rice variety developed from IR31917-45-3-2 x *O. Officinalis,* which have been used in the exploiting of BPH-resistance genes with broad-spectrum resistance scale to BPH populations in Mekong Delta (Bui Chi Buu *et al.*, 2013). Phung Ton Quyen, (2014) bred two *Bph3* and *BphZ(t)* resistance-carried DTR64 and KR8 hybrid rice lines by backcross hybridization combined with fertilization method. The author also showed that the important roles of combining between the BPH resistance selection method and BPH population's resistance, evaluation are based on the different resistance scores/scales, and with the traditional breeding method for developing BPH resistance rice lines (Phung Ton Quyen, 2014). Hu *et al.* (2015) performed the stacking of two QTLs *Qbph3* and *QBph4* from the progeny lines of *O. officinalis* into 9,311 rice varieties by backcross hybridization method combined with the molecular markers-based breeding method. Liu *et al.* (2016) successfully performed in pyramiding both *Bph*3 and *Bph*27 (t) BPH resistance genes into elite rice variety.

Chapter 2. MATERIALS, CONTENTS AND METHODS

2.1. The materials of the investigation

In the current investigation used a set of 15 indicator rice varieties; 14 popular rice cultivars in Mekong Delta; 115 high-yield lines/varieties; 119 local rice varieties (Acc.) Which were assembled in 10 provinces of the Mekong River Delta; four BPH populations were collected in four provinces such as Can Tho, Dong Thap, Tien Giang, and Hau Giang respectively; five molecular SSR markers and phenotypes evaluation requirements were used as the study input materials.

2.2. The main contents of the investigation

(i) Sets of rice varieties were assembled and evaluated for resistance to BPH populations; (ii) Selective populations were developed in greenhouses; (iii) Molecular markers were used to evaluate the BPH resistance genes-converged rice lines; (iv) The BPH resistance genes-contained rice lines observed and compared in the field.

2.3. The methods of the investigation

2.3.1. Assemble and assess the BPH resistance of the studied rice varieties

* Phenotypic evaluation of the studied rice varieties: Phenotypic evaluation of study rice varieties was performed according to the seedling box method by IRRI on four different BPH populations: Can Tho, Dong Thap, Tien Giang and Hau Giang. The parameters evaluation of BPH resistance and susceptible scale was followed by SES (Standard Evaluation System for Rice) (IRRI, 2013).

Based on the evaluated resistance/susceptible phenotype of sets of indicator rice varieties (the seedling box method as mentioned above) was utilized to determine the effective resistance genes to BPH populations in the Mekong Delta. The BPH resistance/susceptible phenotype of the set of the high-yielding rice varieties and set of local rice varieties were evaluated with four different BPH populations representing in the Mekong Delta to select materials source with stable resistance to many BPH populations. The data were analyzed using NTSYS-pc 2.1 software to compare multidimensional patterns of BPH resistance of study rice varieties. The genetic hierarchical cluster analysis was performed according to the SM (Simple Matching coefficient) method of Rohlf, (2002).

* Genotypic evaluation of BPH resistance of the phenotypic evaluation results based-selected rice varieties were performed. The analysis results were achieved through using of molecular SSR markers, and DNA samples studied to search the target genes against

BPH biotypes *Bph1, Bph3, bph4, Bph13,* and *Bph17* by PCR analysis to amplify DNA sections based on molecular SSR markers such as RM1103, RM204, RM217, RM545 và RM401 (Table 2.1).

2.3.2. Developing selective populations in greenhouses

Genetic parameters were evaluated in the analysis of selective efficiency of hybrid combinants in F_1 and F_2 generations to select hybrid combinants with high selective efficiency to be continuously developed. In addition, the study also performed the analysis of the resistance and susceptible dissociation by the chi-square test to determine the genetic characteristics. The backcross populations were bred and selected through phenotypic and genotypic screening technique (BC_1F_1 - BC_nF_1).

2.3.3. Using molecular markers to evaluate the converged resistance genes rice lines

Molecular marker techniques were used to identify the BPH resistance genes-carried BCn individuals. The BPH resistant was checked for the resistance genes-carried rice lines.

2.3.4. Observation and comparison of the BPH resistance rice lines in the field

The BPH resistance rice lines were selected in the field such as BC_2F_3, BC_2F_4, and BC_3F_3. The converged-BPH resistance genes-bred promising rice lines were studied under field trials. These prospected rice lines were the results of studies in greenhouses, laboratories and in the field and were also continued under field trials for the important agronomic characteristics.

2.3.5. Data analysis

Microsoft Excel 2016, IPM Statistics SPSS 20, and NTSYS-pc version 2.1 (Rholf, 2002) software were used for data analysis.

Chapter 3. RESULTS AND DISCUSSION

3.1. Converging and assessment of the BPH resistance of the studied rice varieties

3.1.1. Assessment of the virulence of four BPH populations in the Mekong Delta

The analysis results of the changed BPH resistance in the most popular rice varieties of the Mekong River Delta from 2009 to 2018 showed that there were 7 BPH resistant rice varieties in 2009, and after three years remained 2 BPH resistance rice varieties, and after nine years remained only one rice variety resistance to BPH. These results suggested that the rice varieties-BPH resistance which popularly cultivated in production conditions for a short time, so that the BPH resistance is stable and not broken. However, the rice varieties-BPH resistance would be lost as cultivated for a long term due to the adaptation of BPH. From 2009 to 2018, the BPH resistance of 11/14 rice variety decreased, in which 3 rice varieties showed stable resistance to BPH, indicating that the virulence of BPH has increased. Most of the popularly cultivated rice varieties in the Mekong Delta in 2018 possessed the response to BPH from rather susceptible up to susceptible and very susceptible.

The evaluation results of BPH resistance in the indicator rice varieties with four BPH populations showed that: When attacking two different resistance genes-carried rice varieties, the damage scale of four different BPH populations also is different. In case of the average damage scale induced by particular population on the total number of rice varieties, the damage scale of the BPH population in four regions was similar, out of which the BPH population damage scale in Dong Thap was higher as compared to the remained three BPH populations. Some rice varieties carried multiple resistance genes with high resistance level such as *O. officinalis (bph11*, *bph12, Bph13, Bph14* and *Bph15)* and *O. rufipogon (Bph29* and *Bph30)*, and some rice varieties with moderate resistance scale Ptb33 (*bph2, Bph3* and *Bph32*), Rathu Heenati (*Bph3* and *Bph17*), and Sinna Sivappu (*Wbph9 (t), wbph10 (t), wbph11 (t),* and *Wbph12 (t)*). The BPH population of Dong Thap was with higher damage scale than the other three BPH populations. This demonstrated that BPH populations in different ecological regions will have different damage scale or in other words, they included different virulence scale. These results also showed that Biotype of BPHs in the same area (Mekong River Delta) has always been the same Biotype. These results indicated that the migration of BPH populations in the same area is less likely to occur due to the availability feed source for BPH in the same region. Further, along with the pressure in the use of diverse rice varieties during the production as well as traditional means of farmers for agricultural cultivation that using of insecticides/pesticides leading to the BPH populations virulence was changed but not significantly.

The analysis results also showed that the virulence alteration of the BPH population in Mekong Delta from 2004 to 2018 in the BPH resistance single gene-carried rice varieties were from the relative susceptible up to very susceptible. For instance, the damage scale of Ptb33, the BPH resistance standard rice variety in 2004 was low (zero scale), but in 2015 the damage scale increased from 3 scale to 5 scales. This demonstrated that the BPH population virulence increased in the present. These results are comparable with other published studies has been reported the correlation between the BPH resistance genes and the Biotypes of BPH (Khush and Brar, 1991; Zhang, 2007), and the phylogenetic analysis results of the BPH resistance genes revealed the stark cluster of the BPH resistance genes. These results combined with other reports showed that the BPH Biotype in Mekong Delta was differed with the reported four Biotypes.

The phylogenetic analysis results of the BPH resistance genes-carried indicator rice varieties based on the resistance or susceptible phenotype showed the two main clusters at 0.68 correlation coefficient (r) as the following (i) the correlation coefficient of Cluster I was 74% (or 26% difference), this cluster comprised of 6 rice varieties such as *O. officinalis*, *O. rufipogon*, Swanalata, Ptb33, Sina Sivapu, and Rathu Heenati, in which Swanalata was resistance to only one BPH population in Tien Giang, the rest of three rice varieties were resistance to all of four BPH populations; (ii) the correlation coefficient of Cluster II was 78% and comprised of 9 rice varieties such as TN_1, Chin Saba, ARC10550, Pokkali, ASD7, IR54742, Babawee, T12, and Mudgo, however, all of these rice varieties possessed the susceptible phenotype to all the BPH populations.

3.1.2. Assessment of the BPH resistance on high-yielding rice varieties

The damage index of 61-70% of Can Tho BPH population was with the highest number of lines/varieties (43 lines/variety, occupied 37.39%), followed by the damage index of 51-60% was 25 lines/varieties (occupied ratio 21.74%), in case of the damage index of 30-40% was with the lowest number of lines/varieties (two lines/varieties, occupied ratio 1.74%). While in the damage index of 61-70% in the three BPH populations of Dong Thap, Tien Giang and Hau Giang were with the highest total number of lines/varieties and occupied the highest ratio, followed by the damage index of 71-80% and the damage index of 30-40% were with the lowest number of lines/varieties and the lowest ratios. Generally, the damage index of the lines/varieties for the BPH populations virulence was highest from 61% to 70%, and the lowest was 30-40%. In case of the damage index of ≤ 50% was with the low ratio at 9.57-16.52%.

The response of the lines/varieties to the BPH damage: For the response level from moderate susceptible to susceptible corresponding to the damage scale of 4.6-7.0 showed with the highest number of lines/varieties. While in the response level from resistance to moderate resistance corresponding with the damage scale of 4.6-7.0 and 3.0-4.5 respectively, and showed with 22-27 numbers of lines/varieties, occupied ratio 19.13%-23.48% (Table 3.6).

In general, among 32 rice varieties possessed phenotypic resistance and moderate resistance to 1-4 BPH populations, we were found 17 rice varieties with completely resistant to 4 BPH populations, these rice varieties were OM5954, OM6830, TLR594, OM6075, OM6683, TLR493, TLR1.030, TLR201, OM7262, TLR606, OM10040, OM6610, OM7268, OM7364, OM10041, TLR601, and OM3673. Similarly, out of 32 rice varieties, 6 rice varieties showed phenotypic resistance to 3 BPH populations, these rice varieties were OM927-1, OM28L, MNR3, OM10383, TLR444, and TLR461; while in only one OM10450 rice variety showed resistance to two BPH populations; and eight rice varieties were resistant to one Can Tho BPH population, these rice varieties were OM1015, OM10258, OM10000, OM6327, OM10396, OM10373, and TLR970.

In summary, our results suggested that among 115 high-yielding rice varieties were assessed and showed the phenotypic responses of BPH populations, only 17 rice varieties were completely resistant to all of the BPH population in Mekong Delta. These achieved results will be the basis and valuable resistance genes source to serve for the breeding strategies of new BPH resistance rice varieties in the future studies.

Table 3.6: The damage scale and response of high-yielding rice lines/varieties to the BPH damage, CLRRI Spring-Winter Season 2014-2015

The damage scale and response		Can Tho BPH population		Dong Thap BPH population		Tien Giang BPH population		Hau Giang BPH population	
Damage scale	Response	No. of lines/ varieties	Ratio (%)	No. of lines/ varieties	Ratio (%)	No. of lines/ varieties	Ratio (%)	No. of lines/ varieties	Ratio (%)
3,0	Resistance	2	1,74	2	1,74	2	1,74	0	0,00
3,1-4,5	Moderate Resistance	20	17,39	22	19,13	25	21,74	23	20,00
4,6-5,6	Moderate susceptibility	56	48,70	42	36,52	49	42,61	51	44,35
5,7-7,0	Susceptible	37	32,17	48	41,74	38	33,04	39	33,91
7,1-9,0	Very susceptible	0	0,00	1	0,87	1	0,87	2	1,74

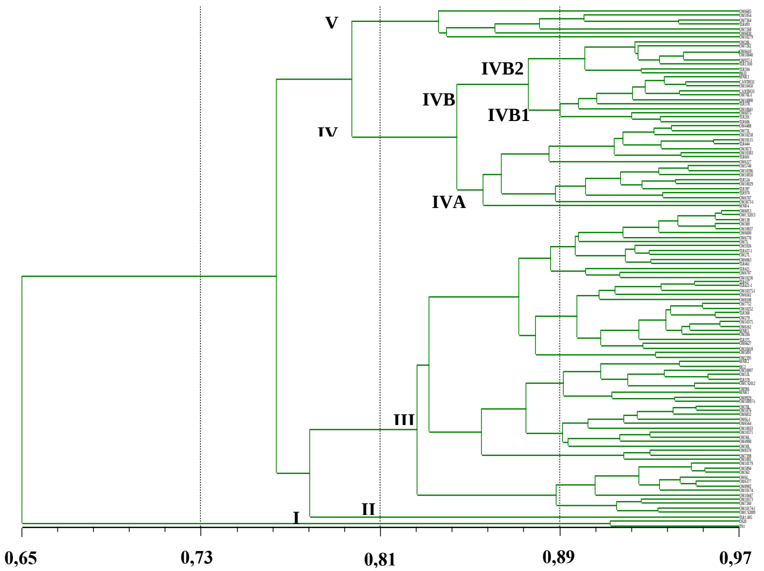

Figure 3.4: Cluster schematic of higher-yielding rice varieties based on phenotypic characteristics and damage index

The cluster analysis results of 115 high-yielding rice varieties were divided into five main clusters with a correlation coefficient (r) of 0.81 (as depicted in Figure 3.4). In which, Cluster I included two standard susceptible rice varieties TN1 and DS20, these two varieties were known as very susceptible varieties on all of BPH population. Cluster II included rice line TLR1.005 and showed completely susceptible to all of BPH populations. Cluster III included 69 lines/varieties; these varieties showed moderate susceptible response up to susceptible to many BPH populations. Cluster IV divided into sub-clusters IVA (19 varieties), and sub-clusters IVB (19 varieties), and this cluster then divided into IVB1 and IVB2. The varieties of this cluster showed the responses from moderate susceptible up to moderate resistance. Further, some varieties of this cluster also showed the wide-spectrum resistance responses *i.e.* resistance to several BPH populations, especially sub-cluster IVB2 included BPH resistance rice lines/varieties were clustered together with standard resistance rice variety PtB33 (OM28L, OM7262, OM6610, OM10040, OM927-1, TLR1.030, and TLR594). Cluster V included 7 rice varieties such as OM6683, OM5954, OM7364, TLR493, OM7268, OM6830, and OM10279. The rice varieties of this cluster showed a wide-spectrum resistance, and complete resistance to four BPH populations (except OM10279 variety).

3.1.3. The BPH resistance assessment of the local rice varieties

The number of local rice varieties revealed the damage index greater than 50% and occupied at a high ratio in BPH populations such as Can Tho, Dong Thap, Tien Giang, and Hau Giang corresponding to 79.83% (95 varieties), 77.32% (92 varieties), 69.75% (83 varieties), and 78.99% (94 varieties) respectively. In general, the BPH damage index on local rice varieties expressed at a high level. For instances, the damage index of > 80% in the Can Tho BPH population showed the highest number of varieties corresponding with 30 rice varieties, occupied 25.20%. While in the BPH populations in Dong Thap and Hau Giang, the damage index of > 71-80% showed the highest number of varieties, corresponding with 33 varieties (27.73%) and 48 rice varieties (40.34%), respectively.

The present investigation was found the majority of local rice varieties with the damage scale ≥ 4.6, in which the damage scale from 4.6-7.0 occupied with the highest ratio and assessed corresponding with the response to BPH were from the moderate susceptible scale up to susceptible scale. In addition, the number of local rice varieties from 12-18 rice varieties (Occupied 10.08-15.13%) which showed the damage scale from 3.1 to 4.5 and assessed as moderate resistance.

Table 3.9: The damage scale and response of the local rice varieties to the BPH damage, CLRRI, Autumn-Summer 2015

The damage scale and response		Can Tho BPH population		Dong Thap BPH population		Tien Giang BPH population		Hau Giang BPH population	
Damage scale	Response	No. of lines/ varieties	Ratio (%)	No. of lines/ varieties	Ratio (%)	No. of lines/ varieties	Ratio (%)	No. of lines/ varieties	Ratio (%)
3,1-4,5	Moderate Resistance	13	10,92	18	15,13	14	11,76	12	10,08
4,6-5,6	Moderate susceptibility	43	36,13	27	22,69	52	43,70	43	36,13
5,7-7,0	Susceptible	59	49,58	66	55,46	51	42,86	62	52,11
7,1-9,0	Very susceptible	4	3,37	8	6,72	2	1,68	2	1,68

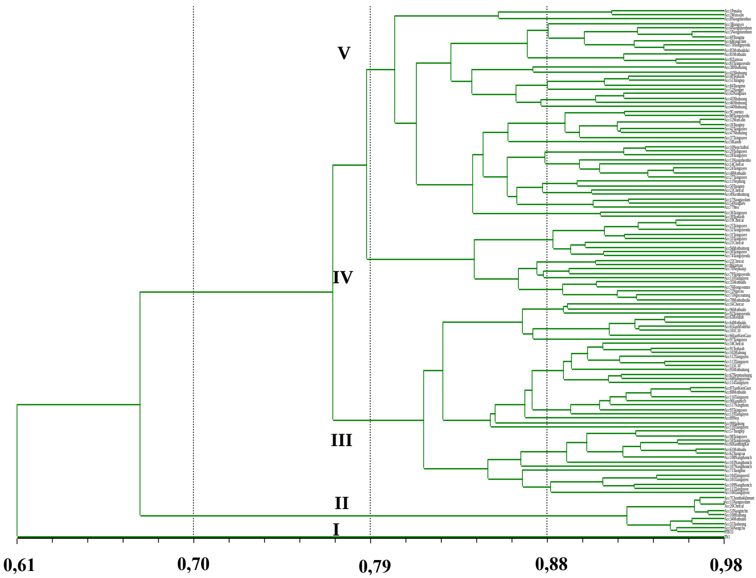

Figure 3.5: Cluster schematic of the local rice varieties based on phenotypic characteristics and damage index

The analysis results showed that among 38 local rice varieties with resistant phenotype to at least one BPH population, we were only found Chet Cut rice variety (Acc. 20) was complete resistance to four BPH populations, the rest of rice varieties (25 varieties) have only been resistance to one BPH population. In addition, another 8 rice varieties showed resistance to 2 BPH populations, these rice varieties were Nang Cha (Acc.56), Tau Huong (Acc.55), Mot Bui Do (Acc.34), Tai Nguyen (Acc.25, Acc.33, and Acc.31), Bong Sen 3 (Acc.30), and Hai Bong (Acc.99) and 4 varieties showed resistance to 3 BPH populations, these varieties were Chom Bok Khmum (Acc.7), Nang Tay Dum (Acc.3), Nang Trich Trang (Acc.53), and Hai Bong (Acc.100). Furthermore, the analysis results of resistance and susceptible response to BPH of the rice varieties which selected from the local rice varieties, these rice varieties were with same names but different Accession number (Acc.) and revealed clear differences in response to BPH populations. These achieved results can be due to the presence of several lines in the same rice varieties. Taken together, the responses to BPH of each Acc. were differed and the changes of resistance level to BPH depended on Acc. of the respective line. In another analysis, the results showed the local rice varieties divided into five major clusters with 0,79 correlation coefficients (r) (Figure 3.5).

3.1.4. Assessment of BPH resistance genotype in the studied varieties/cultivars

In the present investigation, we revealed that through the evaluation results of resistance/susceptible phenotype of rice varieties on four BPH populations as well as based on the phylogenetic scheme of two sets of high-yield rice and local rice varieties. Out of which some broad-spectrum resistant varieties were selected as gene donor lines and OM6162 rice variety was used as gene receive the line. These rice varieties were then selected for genotype analysis. For the high-yielding rice varieties, we selected seven rice varieties which showed the resistance response to BPH and arranged the same cluster, these rice varieties were OM6683, OM5954, OM7364, TLR493, OM7268, OM6830, and OM10279. For the local rice varieties, we were selected three local rice varieties from the broad-spectrum BPH resistance 8 rice varieties which they represented for three different ecological regions like upland local rice variety (Chom bok khmum (Acc.7)), lowland local rice varieties (Nang Tay Dum (Acc.15), Chet Cut (Acc.20)), coastal local rice varieties (Nang Trich Trang (Acc.53), Hai Bong (Acc.100), Mot Bui Do (Acc.34), Tau Huong (Acc. 55), Nang Cha (Acc. 56)).

Selection of *Bph1* resistance gene using RM1103 primer

The PCR analysis results showed the band position of some rice varieties such as OM6683, OM5954, OM7364, TLR493, and Tau Huong were at the size of 200bp on the gel and identified similar to the molecular size 200bp for the *Bph1* resistance gene in a Mudgo rice variety as described in Figure 3.6. In conclusion, this result demonstrated that these rice varieties contained *Bph1* resistance gene under the present study. Park *et al.* (2008) also reported that the RM1103 primer and *Bph1* resistance gene is linked together and located on chromosome 12 of rice.

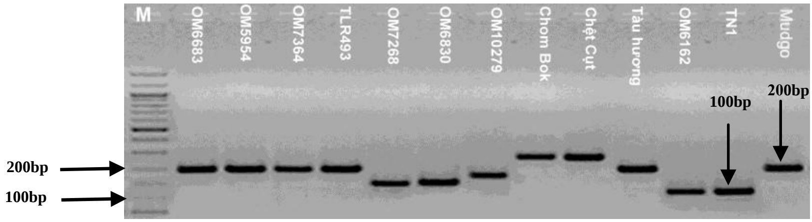

Figure 3.6: PCR amplification products of *Bph1* gene and RM1103 primer was carried on aragose gel (3%). M: Ladder 50bp

Selection of *Bph3* resistance gene using RM204 primer

The PCR analysis results showed the amplification of an allele B band for *Bph3* resistance gene were at the size of 200bp in both Ptb33 resistant rice variety control and some rice varieties like OM6683, OM7268, OM6830, OM10279, Chom Bok Khmum, and Tau Huong as described in Figure 3.7. In conclusion, this result demonstrated that these rice varieties contained *Bph3* resistance gene under the present study. In the study, Jairin *et al.* (2007) used the RM204 primer to detect the location of *Bph3* gene on chromosome 6 of rice.

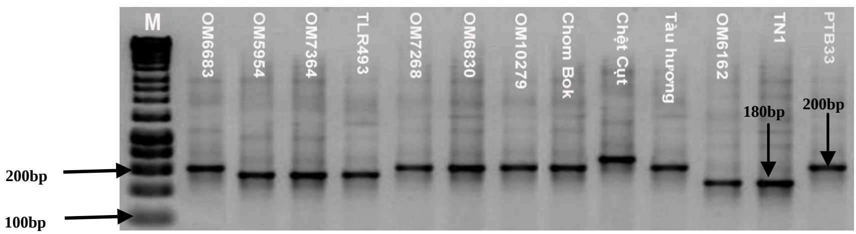

Figure 3.7: PCR amplification products of *Bph3* gene and RM1103 primer was carried on aragose gel (3%). M: Ladder 50bp

Selection of *Bph4* resistance gene using RM217 primer

The PCR amplification analysis results showed both OM7364 and Babawee rice varieties with band position on the gel were at the size of 218bp (as described in Figure 3.8). This result demonstrated that this rice variety contained resistance gene *bph4*. Kawaguchi *et al.* (2001) reported that the RM217 primer is linked with *bph4* resistance gene and located on chromosome 6 of rice.

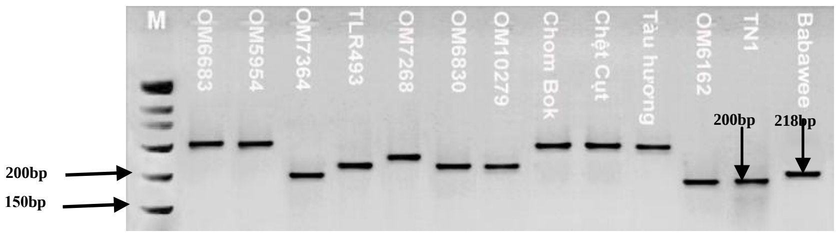

Figure 3.8: PCR amplification products of *Bph4* gene and RM217 primer was carried on aragose gel (3%). M: Ladder 50bp

Selection of *Bph13* resistance gene using RM545 primer

The PCR amplification analysis results revealed the band position at size of 220bp size for both OM6683, OM5954, OM7364, and Tau Huong rice varieties and standard resistance rice variety *O. officinalis* (as described in Figure 3.9). This result demonstrated that these rice varieties contained resistance gene *bph13*. Chen *et al.* (2006) reported that the RM545 primer is linked with the *Bph13* resistance gene and located on chromosome 3 of rice.

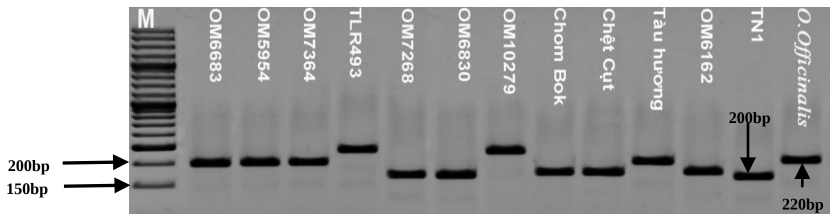

Figure 3.9: PCR amplification products of *Bph13* gene and RM545 primer was carried on aragose gel (3%). M: Ladder 50bp

Selection of *Bph*17 resistance gene using RM401 primer

Out of 11 rice varieties, only Chom Bok Khmum rice variety showed the similar molecular size of 200bp as compared to Rathu Heenati-standard resistance rice variety (as described in Figure 3.10). Sun *et al*. (2005) reported that RM401 is linked with *Bph17* resistance gene and located on chromosome 4 of rice.

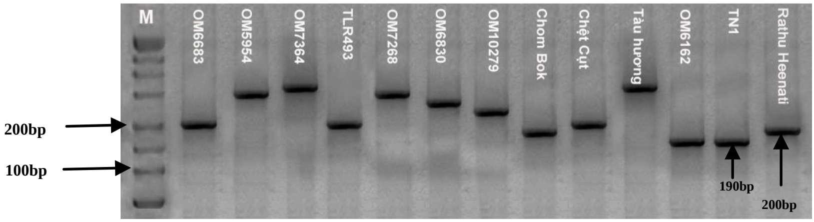

Figure 3.10: PCR amplification products of *Bph17* gene & RM401 primer was carried on aragose gel (3%). M: Ladder 50bp

Taken together, in the present investigation, we identified five rice varieties with multigenic resistance in BPH populations through the genotype assessment results of rice varieties which have been selected as the donors and recipients materials, these rice 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*). Therefore, among five rice varieties, four rice varieties such as OM6683, OM7364, Chom Bok Khmum, and Tau Huong (because rice variety OM5954 has two genes *Bph1* and *Bph13* like OM6683) were selected as the donor materials for developing hybrid populations in greenhouses.

3.2. Development of selected populations in greenhouses

Using genetic parameters in the analysis of selective efficacy of hybrid combinants in BPH resistance.

The phenotypic variance was much higher many folds as compared to the genotype variance in 2 hybrid combinants OM6162/Chom Bok Khmum, OM6162/Tau Huong, this result showed that the furious effect of the environment to the BPH resistance of these two hybrid combinants and suggested that the BPH resistance in these hybrid combinants was unstable. In contrast, the deviation between phenotypic variance and genotypic variance of two hybrid combinants

OM6162/OM6683, and OM6162/OM7364 is low, this result showed that the BPH resistance in these two hybrid combinants did not change and stable under the environment effect. These results are similar to the report recorded by Selvaraj *et al.* (2011). According to the classification result of Sivasubramanian and Menon, (1973) showed that the BPH resistance in GCV and PCV were high (occupied > 20%), and the GCV value is near equal PCV value and this revealed that a large contribution of the genotypes to the phenotypic expression of BPH resistance.

Heritability of 2 hybrid combinants OM6162/OM6683 and OM6162/OM7364 determined at a higher value (h^2_b > 0,8) for the traits against BPH. Singh, (2001) reported that the obtained high value of h^2_b for characteristics/traits against BPH and indicated that this trait is mainly controlled by the internal inheritance factors and leading to improved heritability of the traits and help for selecting of the next generation. However, the high heritability has not always produced a better selective generation for a particular trait (Falconer, 1982). The selective efficiency depends on the abundance of variation which based on the genotype's variance in a segregated population (GCV%), and when the relationship between GCV% and GA% is high and hence we can easily find the high selective efficiency on those characteristics (Nguyen Thi Lang and Bui Chi Buu, 2011). Similarly, in the previous study, Burton, (1952) suggested that the higher value of GCV and h^2_b will give high selectivity. Taken together, the heritability combines with selective efficiency are the important factors for selecting the hybrid combinant with the appropriate traits and are stably developed.

In the present investigation, we determined the heritability and selective efficiency of both hybrid combinants OM6162/OM6683 and OM6162/OM7364 were at high (h^2_b > 0,8 and GAM > 50%). These obtained results can be explained by the depression activity of dominant genes in both hybrid combinants and leading to the breeding can be implemented in the first generations. These results are similar to the achieved results by Wolie *et al.* (2013) and Ogunbayo *et al.* (2014). In the other study, Nguyen Thi Lang and Bui Chi Buu, (2011) indicated that the high value of both h^2_b and GAM, therefore the selection used according to the conventional method can also be obtained with high effective to improve this characteristic/trait. In contrast, in the case of 2 hybrid combinants of OM6162/Chom Bok Khmum, OM6162/Tau Huong showed the low value of h^2_b and GAM as well as long duration, hence the selection is so difficult to achieve the desired results. Therefore, this study only focused on 2 hybrid combinants of OM6162/OM6683 and OM6162/OM7364, while in the other two hybrid combinants will be used as the initial materials source for the future studies.

3.3. Using molecular markers to assess the BPH resistance genes-converged rice lines

In the present investigation, we have analyzed the presence of the BPH resistance genes in the hybrid progenies. The hybrid population of generations such as F_1, BC_1, BC_2F_1, BC_2F_2, BC_3F_1, and BC_3F_2 assessed phenotypes in parallel with genotypes. The hybrid lines in generations F_1, BC_1, BC_2, and BC_3 with the heterozygous resistance genotypes identified by three molecular markers were selected to continue for the backcross hybridization in the next generations.

To assess the BPH resistance genes-converged lines in the hybrid combinant OM6162/OM6683, the molecular markers were used to link the hybrid rice lines to three different SSR primers (RM1103 primer is linked with *Bph1* gene on chromosome 12 of rice, and RM204 primer is linked *Bph4* genes on chromosome 6 of rice, and RM545 primer linked to *Bph13* gene on chromosome 3 of rice.).

Similarly, in case of the BPH resistance genes-converged rice lines in the hybrid combinant OM6162/OM6683 was assessed using the specific couple primers related to the BPH resistance genes such as *Bph1, bph4,* and *Bph13*, these genes are linked with three different primers like RM1103, RM217, and RM545 in three different chromosome 12, 4, and 6 respectively.

The summary results of the backcrossed populations of two hybrid combinants OM6162/OM6683//OM6162 and OM6162/OM7364 //OM6162 as described in Table 3.19. The results showed that the number of the BPH resistance gene-carried individuals which selected through molecular markers combined with phenotypic assessment in the generations such as F1, BC_1, and BC_2, and BC_3 in the hybrid combinants like OM6162/OM6683//OM6162 is 117, 14, 6, and 9 respectively; and the hybrid combinants like OM6162/OM7364//OM6162 is 111, 10, 9, and 9 respectively.

The achieved backcross hybridization results for developing of the BPH resistance genes-carried populations as mentioned above, through those results we were selected 14 potential rice lines in two different hybrid combinants. For the hybrid combinant of OM6162/OM6683//OM6162, out of 14 rice lines, the seven rice lines carried three BPH resistance homozygous alleles (*Bph1*, *Bph3*, and *Bph13*) were selected and comprised of four rice lines in BC_2F_5 generation, three rice lines in BC_3F_4. For the hybrid combinant of OM6162/OM7364//OM6162, out of 14 rice lines, the seven rice lines carried three BPH resistances homozygous alleles (*Bph1*, *bph4,* and *Bph13*) were selected and comprised of two rice lines in BC_2F_5 generation, five rice lines in BC_3F_4.

3.4. Observation and comparison of the BPH resistance rice lines in the field

3.4.1. Selection of the BPH resistance rice lines in the field

The individuals of the populations such as BC_2F_2, BC_2F_3, BC_2F_4, BC_3F_2, and BC_3F_3 were grown in the field for the selection of the BPH resistance pure rice lines. The achieved selection results of the BPH resistance rice lines of two hybrid combinants OM6162/OM6683 //OM6162 and OM6162/OM7364//OM6162 in the field, in which progeny generations were recorded and described in Tables 3.20 and Tables 3.21.

Table 3.20: The selection results of BPH resistance rice lines in the field of hybrid combinant OM6162/OM6683//OM6162

Field Season	Generation	Total no. of the grown rice lines	Total no. of the selected rice lines	Total no. of the selected individuals
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 (*)
	BC_3F_3	20	7	3 (*)

(*) Indicates the potential rice lines

Table 3.21: The selection results of BPH resistance rice lines in the field of hybrid combinant OM6162/OM7364//OM6162

Field Season	Generation	Total no. of the grown lines	Total no. of the selected lines	Total no. of the selected individuals
Autumn-Summer 2016	BC_2F_2	50	2	20
Spring-Winter 2016-2017	BC_2F_3	20	12	20
	BC_3F_2	45	5	33
Autumn-Summer 2017	BC_2F_4	20	5	2 (*)
	BC_3F_3	33	12	5 (*)

(*) Indicates the potential rice lines

3.4.2 Assessment results of the agronomic characteristics of the potential rice lines

Assessment results of the BPH resistance of 14 potential rice lines, in which the BPH resistance of the potential rice lines assessed in the artificial conditions with four converged BPH populations in Can Tho, Dong Thap, Tien Giang, and Hau Giang. In addition, TN1 and Ptb33 were used as standard susceptible and resistant rice varieties, and OM6162, OM6683, and OM7364 were used as parents rice varieties material source. These results showed that all of the potential rice lines revealed resistance to four BPH populations with damage level 1-3, except line G5-BC3F4 of hybrid combinant OM6162/OM7364// OM6162 showed rather susceptible to Dong Thap BPH population. On the other hand, the analysis results of yield constituents and the yield of the potential rice lines were recorded in Table 3.27 and Table 3.29.

Table 3.27: Agronomic characteristics, rice yield, and yield components of the potential rice lines of hybrid combinant OM6162/OM6683//OM6162 in Spring-Winter 2017-2018

Sl No.	**Lines/varieties**	**No. of tillers/ plant**	**Panicle length (cm)**	**No. of grains /panicle**	**Flat grain ratio (%)**	**1000 grains weight (g)**	**Yield (ton/ ha)**
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	5,2d
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

Notes: The values of the same column followed the same letters indicate the statistical non-significant difference under Duncan test at the 5 % level.

Table 3.29: Agronomic characteristics, rice yield, and the yield components of the potential rice lines of hybrid combinant OM6162/OM7364//OM6162 in Spring-Winter 2017-2018

Sl No.	**Lines/varieties**	**No. of tillers/ plant**	**Panicle length (cm)**	**No. of grains /panicle**	**Flat grain ratio (%)**	**1000 grains weight (g)**	**Yield (ton/ ha)**
1	G1-BC_2F_5-3-1-1-6-9	10,3a	24,3a	141,0a	15,4e	27,9a	7,9a
2	G2-BC_2F_5-5-1-1-4-8	9,7a	22,3c	131,7ab	18,3de	27,6ab	7,5ab
3	G3-BC_3F_4-3-1-1-1-4	8,7a	23,8ab	113,7de	24,9abc	26,9abc	6,1ef
4	G4-BC_3F_4-5-1-1-2-5	8,3a	23,0bc	105,0e	26,1ab	26,9abc	5,7f
5	G5-BC_3F_4-25-2-1-3-7	9,0a	23,8ab	118,3cd	22,3bcd	26,7abc	6,8cd
6	G6-BC_3F_4-30-1-1-2-6	9,0a	22,8bc	119,3cd	19,8cde	26,6bc	6,9cd
7	G7-BC_3F_4-44-3-2-1-7	10,0a	23,7ab	127,33bc	16,0e	27,5abc	7,3bc
8	OM6162	6,0b	23,7ab	92,7f	30,5a	26,3c	5,2g
9	OM7364	9,3a	22,7bc	111,0	24,1bc	27,3abc	6,5de
	CV %	11,6	2,8	4,7	14,1	2,3	4,6

Notes: The values of the same column followed the same letters indicate the statistical non-significant difference under Duncan test at the 5 % level.

The field trials, the results of the potential rice lines showed that five rice lines in the hybrid combinant OM6162/OM6683//OM6162 were revealed the resistance to BPH and high-yield, these rice lines were G1-BC_2F_5, G2-BC_2F_5, G3-BC_2F_5, G4-BC_2F_5, and G5-BC_3F_4. While in the hybrid combinant OM6162/OM7364//OM6162, 3 rice lines revealed the resistance to BPH and high-yield, these rice lines were G1-BC_2F_5, G2-BC_2F_5, and G7-BC_3F_4.

CONCLUSIONS AND SUGGESTIONS

1. Conclusions

- Assessment results of the BPH resistance indicators rice varieties in four provinces of Mekong River Delta found the BPH resistance genes-carried rice varieties such as *O. officinalis*, *O. rufipogon*, Ptb33, Rathu Heennati, and Sinna Sivapu, and further these rice varieties showed the large-scale BPH resistance (100% resistance) to the four BPH populations that represented in four ecological regions of Mekong River Delta. The virulence of four BPH populations was similar as compared to each other, but the virulence increased in all four BPH populations.

- The phenotypic assessment results of 115 high-yielding lines/varieties with the four BPH populations found 17 completely BPH resistance rice varieties to four BPH populations, and these rice varieties were OM5954, OM6830, TLR594, OM6075, OM6683, TLR493, TLR1.030, TLR201, OM7262, TLR606, OM10040, OM6610, OM7268, OM7364, OM10041, TLR601, and OM3673.

- The phenotypic assessment results of 119 accessions local varieties with the four BPH populations found only one Chet Cut (Acc.20) rice variety was completely resistance to four BPH populations. Four rice varieties showed resistance to three BPH populations, these rice varieties were Chom Bok Khmum (Acc.7), Nang Tay Dum (Acc.3), Nang Trich Trang (Acc.53), Hai Bong (Acc.100). Eight rice varieties showed resistance to two BPH populations.

- The genotypic analysis selected multigenic resistance-contained four rice varieties like OM6683 (*Bph1*, *Bph3*, and *Bph13*), OM7364 (*Bph1*, *bph4*, and *Bph13*), Chom Bok Khmum (*Bph3* and *Bph17*) and Tau Huong (*Bph1*, *Bph3*, and *Bph13*). These four rice varieties and

OM6162 were selected as the donor and recipient material sources in the present breeding program respectively.

- The four hybrid combinants were developed, in which our study only focused for developing two backcrossed populations which carried resistance genes such as OM6162/OM6683 and OM6162/OM7364, while in another two hybrid combinants OM6162/Chom Bok Khmum and M6162/Tau Huong will be used as the initial material sources for the future studies.

- The BPH resistance genes-converged hybrid progenies rice lines were bred *via* the phenotypic assessment combined with using of molecular markers, out of which 14 potential rice lines were selected under the present investigation. In which, from the hybrid combinant OM6162/OM6683//OM6162 selected seven rice lines which carried three homozygous resistance alleles (*Bph1*, *Bph3* và *Bph13*), these 7 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, G5-BC_3F_4-8-1-1-1-5, G6-BC_3F_4-53-4-1-1-1, and G7-BC_3F_4-54-1-1-1-2.

- The eight potential rice lines with high-yield were selected. Among of eight, five rice lines like 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 were the progeny generations of the hybrid combinant OM6162/OM6683//OM6162, and three rice lines of the hybrid combinant OM6162/OM7364//OM6162 (G1-BC_2F_5-3-1-1-6-9, G2-BC_2F_5-5-1-1-4-8, G7-BC_3F_4-44-3-2-1-7).

2. Suggestions

- In the future line of the study, the progeny generations populations of two hybrid combinants OM6162/Chom Bok Khmum and OM6162/Tau Huong will be continuously studied for developing the new BPH resistance rice varieties in the breeding strategy.

- Continuing to implement the field trials of the potential rice lines and then can provide for the wider production regions.

- Continuing to hybridization of the BPH resistance genes-carried rice lines with the parent generations for studying of the sustainable BPH resistance of these rice lines.

NOTABLE PUBLICATIONS
RELATED TO THE DISSERTATION

1. Phuc N.V., Chau L.M., Huu N.T. and Vang P.T.K. (2014), Arthropods biodiversity of rice ecosystems in Can Tho. *Agriculture & Rural Development*, 1: 31-37.

2. Vang P.T.K., Chau L.M. and Lang N.T. (2016), Evaluation of rice varieties and breeding lines for brown plant hopper resistance in the Mekong River Delta. *Vietnam Agricultural Technology & Science*, 6(67): 30-34.

3. Vang P.T.K., Chau L.M. and Lang N.T. (2016), Evaluation of local rice varieties for brown plant hopper resistance in the Mekong River Delta. *Vietnam Agricultural Technology & Science*, 9(70): 43-46.

4. Ha P.T.T., Lang N.T., Tam D.M., Vang P.T.K. and Ramin R. (2017). Phenotypic Screening of Drought-Tolerant Lines for Brown Planthopper, Blast and Phytic Acid Content Assay of Rice (*Oryza sativa* L.). *International Journal of Environment, Agriculture, and Biotechnology (IJEAB)*. 2(6): 3160-3165.

5. Vang P.T.K., Phuoc N.T., Ha P.T.T. and Lang N.T. (2018). Using molecular markers to detect resistance genes to brown plant hopper from rice backcross OM6162*3 / OM6683 population. *Vietnam Agricultural Technology & Science*, 3 (88): 8-12.

6. Vang P.T.K., Huu N.T., Cat H.D., Lan N.T.P. and Thach T.N. (2018). Identification of rice varieties for resistance to brown plant hopper in Mekong Delta. *Vietnam Agricultural Technology & Science*, 3 (88): 13-17.

7. Vang P.T.K., Lang N.T. and Chau L.M. (2018). Virulence of four brown planthopper populations from Mekong Delta against resistant rice varieties. Technology and Scientific Conference Book specialized in Cultivation and Plant Protection in the period 2013-2018. Ministry of Agriculture and Rural Development, 485-490.

8. Vang P.T.K., Phuoc N.T. and Lang N.T. (2019). Using molecular markers to detect resistance genes to brown plant hopper from rice backcross OM6162*3/OM7364 population. National Scientific Conference Book: The Universities, Scientific Researches in the period of Industrial Revolution 4.0, Kien Giang University, 166-174.