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USING OF MOLECULAR MARKERS TO STUDY OF THE IMPROVEMENT OF THE CHALKINESS RATIO IN HIGH-YIELDING RICE VARIETIES (*Oryza sativa* L.)

Specialization: Biotechnology

Code No.: 9420201

SUMMARY OF PHILOSOPHY DOCTORAL THESES IN AGRICULTURAL BIOTECHNOLOGY

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The full text of theses can be found in the following libraries:

1. The National Library of Vietnam
2. The Library of Vietnam Academy of Agricultural Sciences
3. The Library of Cuu Long Delta Rice Research Institute

Chapter I. INTRODUCTION

1.1. The necessity of theses

Rice (*Oryza sativa* L.) is one of the most staple crop plants, which provide the food and living for more than half of the world's population. The economic value of rice has been obtained no longer limited in providing of the food for people, but it is now a major export good to provide a high foreigner exchange. Among the strategies to increase the economic value of rice is to change novel rice variety, and introduce to the production procedure of high-quality rice varieties because good quality rice is consumed at a higher price than average- and poor-quality rice (Tran Duy Quy, 2002).

The amount of chalkiness is one of the important characteristics of grain quality of rice varieties directly involved to milling qualities. Chalkiness creates a translucent dot in the grain embryo. The high percentage of chalkiness in white rice will affect to the cracking ratio of grain at high in the milling quality. Moreover, the endosperm type of rice grain is one of the factors that play important role in rice export demand (Nguyen Thi Lang and Bui Chi Buu, 2011). Concomitant, together with the development of global economic, demand and favorite of consumers require the highest quality of grain in more and more increasing. In addition, the chalkiness character is controlled by multiple genes and influenced by environmental factors. Therefore, it is difficult to find the completely un-chalkiness rice varieties, but the study focuses on the improvement of rice varieties with less chalkiness amount. Hence, the topic "using of molecular markers to study of the improvement of the chalkiness ratio in high-yielding rice varieties (*Oryza sativa* L.)" was performed to generate the rice lines/varieties have high-yielding and less or un-chalkiness.

1.2. The objectives of theses

To generate the novel rice lines/varieties with high-yielding and low chalkiness ratio for the improvement of chalkiness traits of rice grains on the high-yielding rice varieties with the help of Microsatellite-molecular markers linked with the genes that control chalkiness characteristics on chromosome 7 of rice.

1.3. The scientific and practical significance of the investigation

The first successes in the assembly of genes govern chalkiness trait at low level using molecular markers in rice plant, which shall be

open the ability of wide apply to the breeding program in general, not only for chalkiness characteristic but also for several of the other important agronomy parameters.

The study selected the potential rice lines that improved chalkiness trait (*i.e.* less chalkiness level) and adapted to external environmental conditions to provide into high-quality rice varieties group with the purposes to serve for the demands of Vietnam and enhance the competition ability with other countries in the world on export rice.

The study contents in the investigation can use for the breeding program in the present. The products of the study are the materials source and the methods of the study are the document's source for the future study programs. In addition, the study also contributes to the service of the study and education.

1.4. Subjects and investigation fields

* **Subjects**: are higher-yielding rice varieties at Mekong Delta.

* **Investigation fields**:

- Locations: The experiments of the study were designed and conducted in net-house and in the fields of Cuu Long Delta Rice Research Institute (CLRRI) which represent for the different ecological areas. The parameters of the study were analyzed at a laboratory of grain quality analysis, molecular biology laboratory of Plant Breeding and Genetics Department, and Genetic Technology laboratory of the Mekong Delta Research Institute of High Technology.

- Dates: from 2013 to 2017.

- Methods: the traditional methods combined with modern methods.

1.5. The new contributions of theses

The study assessed the parent’s materials to exploit the un-chalkiness characteristic.

Beside the breeding goal of un-chalkiness genes-carried rice varieties, the study also focuses on high-yielding and suitable growing time. These are decisive requirements of the rice varieties products which can be widely used and developed after the completion of the present study.

The methods are the combination of the traditional breeding, molecular biology and Bioinformatics during the study.

1.6. The general structure of theses

The main contents of theses were shown on 99 pages, 16 tables, and 34 figures. Introduction section is 4 pages; Chapter 1: Review of Literature is 28 pages; Chapter 2: Contents and Methods are 10 pages; Chapter 3: Results and Discussion are 55 pages; Conclusions and Suggestions are 2 pages. In addition, theses also have an appendix section. The study used 133 references, in which included 22 references in Vietnamese and 111 references in English language.

Chapter 2. REVIEW OF LITERATURE

Chalkiness creates opaque spots in the endosperm of grain due to the disruption of the grain filling process that result the air spaces between the starch granule cells (starch granules in the chalkiness area are loosely arranged, and structure are poorly tighter than the translucent area) forming waxy spots in rice grains visible by light reflection (Tashiro and Wardlaw, 1991).

The transparency of the rice grain depends on the nature property of endosperm, spots can appear on the belly, back or center of the rice grain. Because of such a structure, the rice grain is easy to crack at the site of opaque when milling, and reducing the commercial value of the rice grain (Nguyen Thi Lang and Bui Chi Buu, 2011).

Chalkiness of rice grain is a complicate characteristic that governed by various genetic factors and influenced by external environmental conditions (Nakata and Jackson, 1973; Seetharaman, 1964). These genes controlled by the endosperm and maternal cytoplasm. According to Ebron, (2013), the chalkiness is manifested by the opaque areas in many parts of the rice grain, which occur when protein and starch components are loosely packed in the endosperm when the seeds are firmed. Chalkiness is the trait show phenotype of the quality and most assessed *via* the percentage of grain with chalkiness (PGWC), the area of endosperm chalkiness grain (AEC), and the degree of endosperm chalkiness (DEC). The inheritance of these traits and thus is considered to be very complex (Pooni *et al.*, 1992; Zhu and Weir, 1994; Mo, 1995). According to Del Rosario *et al.* (1968), the results showed that high PGWC values decreased the density of starch granules and the grains are easily broken during milling.

Previously, many studies on the genetics of chalkiness of rice grain in India and American showed that the rice grain had white spot in the center, which controlled by the recessive *wc* gene (USDA, 1963). Other studies suggested that the opaque granules in center are regulated by the single dominant gene (Nagai, 1958; Chalam and Venkatesvarlu, 1965), However, there were several later studies with the help of biotechnology, the chalkiness trait of rice grain was determined to be controlled by multiple genes. These genes are influenced by the environment (Le Doan Lien *et al.*, 1977) and can be affected by many combined factors, including: physical, biochemical, physiological, water quality, etc. (Chen *et al.*, 2012).

The studies result of Truong Ba Thao, Nguyen Thi Lang and Bui Chi Buu, they studied on the analysis of genetic variation of the chalkiness trait on rice grain of the hybrid combinations IR64/Jasmine and IR64/DS20 and showed that the chalkiness rate is ratio of multiple genes and is strongly influenced by environment and it is suggested that in the further research by molecular markers to find polymorphism in the dissociation ratio of the hybrid combinations.

Chapter 3. CONTENTS AND METHODS OF THE STUDY

3.1. Location and timing of the study

* Location: The experiments were conducted at Cuu Long Delta Research Institute, Mekong Delta High-tech Agricultural Research Institute and at 6 locations of 6 provinces in Mekong Delta, including: Can Tho, Hau Giang, Long An, An Giang, Bac Lieu, and Tra Vinh.

* Timing: the study was conducted from January/2013 to December/2017.

3.2. The materials of the study

* Rice varieties: 105 high-yielding rice varieties were collected from the gene bank of CRRI and were used to assess phenotype and genotype. The materials were used for hybridization were rice varieties selected from 105 high-yielding rice varieties which previously collected. Maternal parent: has high-yielding, but rice grain is at a high level of chalkiness, including OM3673. Paternal parent: has a high rate of chalkiness at 0 level, including: RVT and TLR434. In addition, control rice varieties were KDML105 and IR50404

* Chemical and equipment: were included glasswares, plasticwares, equipment, chemical, etc. were used in molecular biology laboratory, and quality analysis laboratory.

* Molecular markers:

i. Molecular markers Indel5 and RM21938 were used in the breeding study of the improved backcross rice varieties.

ii. Fifteen molecular markers were used to form GGT genetic map.

3.3. The main contents of the study

- Assessment of parent materials used in the breeding study of high-quality rice varieties with low chalkiness level.

- Breeding of backcross hybrid populations have a low chalkiness rate *via* Marker Assisted Selection (MAS).

- Selection of backcross hybrid populations BC_nF_2 *via* the formation of GGT map.

- Assessment and selection of individuals have low chalkiness level and high-yielding rice varieties on backcross hybrid populations BC_nF_3 in various environments.

3.4. The methods of the study

3.4.1. Hybridization methods: including single hybridization, and backcross hybridization methods.

3.4.2. The methods in laboratory:

- The percentage of chalkiness amount was assessed according to the standards of IRRI with four various levels: 0, 1, 5, and 9 level.

- The genotype assessment methods were conducted *via* molecular markers based on the assessment standards of Nguyen Thi Lang (2002).

3.4.3. Statistical analysis methods and data analysis:

The data were analyzed using the software of Microsoft Excel 2010, genetic hierarchical analysis used by NTSYSpc 2.1 (Adams and Rohlf, 2000), interaction analysis between genotype and environment were performed using IRRISTAT software.

Chapter 4. STUDY RESULTS AND DISCUSSION

4.1. The selection of parents for rice hybridization with low chalkiness level

4.1.1. The assessment of chalkiness level of hybrid materials, rice varieties

The successes of novel rice varieties breeding depend not only on the hybridization method and breeding, but also the contribution of the parent genetic materials. Therefore, the selection of suitable parents is an important factor for the future characteristics of hybrid progenies. In this study, 105 rice lines/varieties from Cuu Long Delta Rice Research Institute were assessed on the chalkiness level to find the most potential parent couples to serve in the hybridization program of rice varieties with low chalkiness level.

The rice varieties harvested at 25, 26, 27, 28, 29, 30, 31, and 32 days when rice was at 50% of flowering. The assessment of chalkiness level performed when rice varieties harvested and dried (moisture content at 13%). The assessment results showed that chalkiness level tended to increase when rice harvested as late as after the 25th day as 50% of flowering (Figure 4.1). These results are similar to several previous studies (Lang *et al.,* 2010; Tran Thanh Son, 2008; Le Thu Thuy *et al.*, 2005).

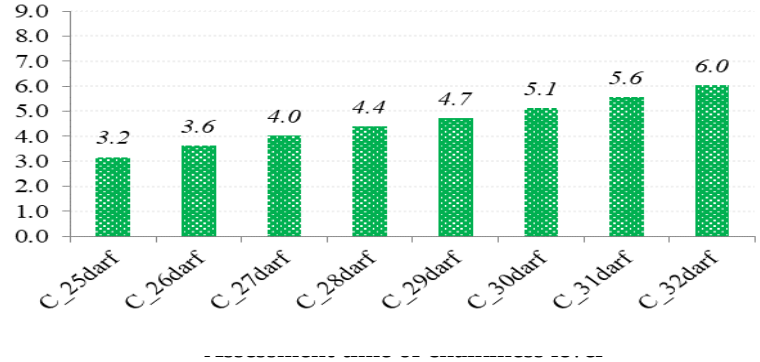

Figure 4.1: The average of chalkiness level in the different states

Notes: C: Chalkiness; DARF: Days after rice flowering

When considering on each chalkiness level, chalkiness level 1-3 (chalkiness rate is less than 10%) tends to decrease while in chalkiness level > 3 (chalkiness rate ≥ 10%) increases as the harvest time is long (Figure 4.2). When the rice was harvested at 25-26 days after 50% of flowering, the rice grains had the lowest chalkiness rate, and at 29 days onwards, the rice grain chalkiness rate increased at the highest level. These results showed that the harvesting stage at correctly time shall be leading rice translucent rate (un-opaque) higher than as compared to the late of harvesting stage.

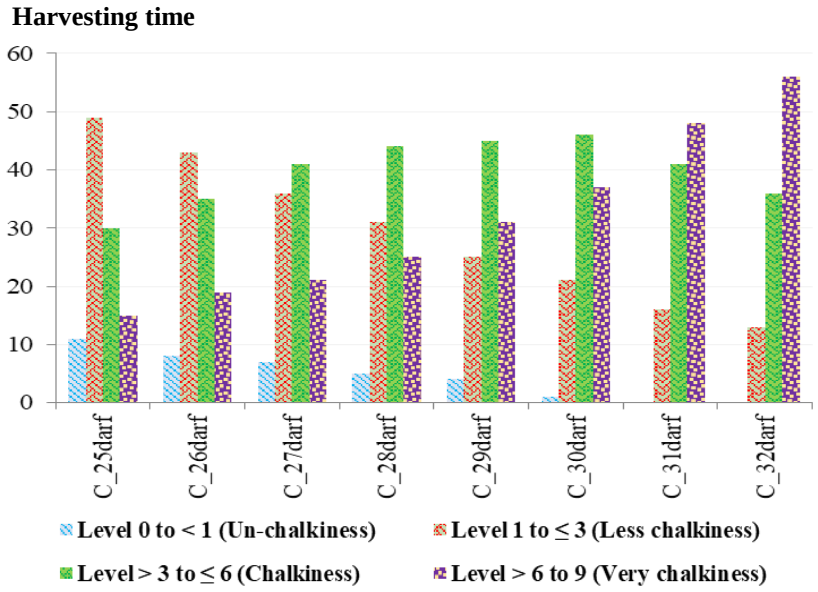

Figure 4.2: The variety of the different chalkiness levels under the harvesting time

Notes: C: Chalkiness; DARF: Days after rice flowering

4.1.2. Genetic hierarchical cluster analysis of the hybrid material rice varieties based on the assessment results of the chalkiness level

The analysis of the genetic hierarchical cluster of phenotyping shall be helped to find faster seeking of rice lines/varieties group which suited to the goals in the breeding program. However, in this study, 105 rice varieties clustered on genetic, based on different chalkiness levels. The analysis result of the genetic hierarchical cluster, the rice varieties of A_1 group are rice varieties with the chalkiness rate at low level,

hence, these individuals used for the donor materials with the characteristics of translucent rice. While in the elite rice varieties are TLR434 and RVT (chalkiness level at 0-1), un-chalkiness or low chalkiness level.

4.1.3. The genotyping analysis related to the chalkiness level of the hybrid material rice varieties

There are several characteristics of rice quality, the chalkiness trait always mentioned by scientists and customers. However, the chalkiness level is multiple gene trait and highly influenced by the environmental conditions (Sun *et al.*, 2015; Tran Thanh Son, 2008). In this study, two molecular markers Indel5 and RM21938 linked with gene for chalkiness characteristic on chromosome 7 (Lang *et al.*, 2015; Zhou *et al.*, 2009), which used to assess the genotype of different rice varieties.

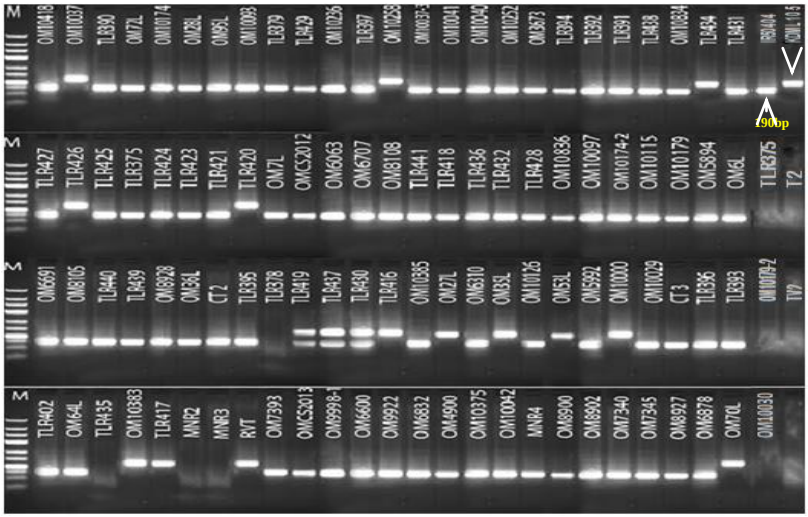

Figure 4.4: The PCR products of the hybrid rice varieties samples at Indel5, located on chromosome 7

Note: M: ladder (1Kb$^+$)

In Indel5 molecular marker, the PCR products amplified up-to 91,4% (9/105 samples are without bands). The results of PCR products with Indel5 molecular marker on 3% agarose gel showed multiple bands with the presence of 2 alleles with the molecular size are 190bp and

200bp. In the control rice varieties KDML105 with less chalkiness level (chalkiness rate < 10%) and showed the band at the size of 200bp, while in, IR50404 rice variety is very less chalkiness level (chalkiness rate > 10%) and the band is at 190bp. This result showed that at size of 200bp is respective site, which marked with gene of less chalkiness level in rice grains.

The electrophoresis results in Figure 4.4 showed that 15 rice varieties had the band size at 200bp or carried gene with less chalkiness level. The rest of rice varieties showed the bands at size of 190bp (without target gene) or without any bands.

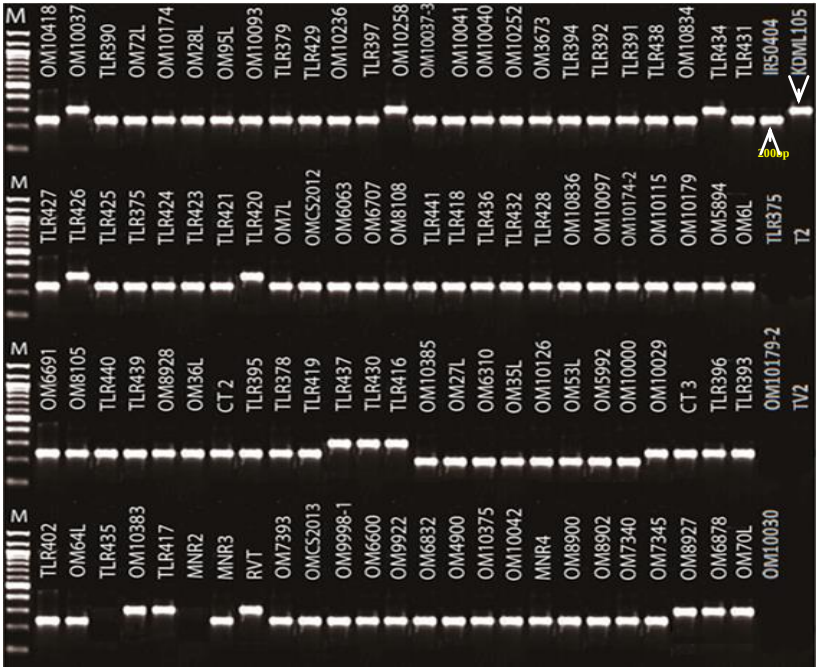

Figure 4.5: The PCR products of the hybrid rice varieties samples at RM21938, located on chromosome 7

Note: M: ladder (1Kb$^+$)

In the molecular marker RM21938, there were 98/105 rice varieties showed the bands or the DNA amplification efficiency occupied 93.3%. In the electrophoresis gel at 3% of the concentration, two band size at 200bp and 210bp were recorded (Figure 4.5). These polymorphisms separated between rice varieties with less chalkiness,

and high chalkiness level. Similarity with the band at 210bp is the positive control rice variety KDML105 (less chalkiness level), this is position represented for the target gene-non-carried rice varieties, the respective is band position of the negative rice variety IR50404 (high chalkiness level). Among 105 different rice varieties, 19 rice varieties showed the band at 210bp through the assessment result of target genes, which related to chalkiness trait in rice grains. The rest of rice varieties were at size of 200bp (un-carrying the target gene) or without the bands. There weren't heterozygous bands appeared on agarose gel.

Table 4.2: Accuracy ratio of genotype compared to phenotype based on two molecular markers Indel5 and RM21938

Molecular markers	The number of rice lines/ varieties	Genotype				Accuracy ratio of genotype and phenotype
		Un-chalkiness	Chalkiness	Hetero zygous	Un-determined	
Indel5	105	14	79	3	9	66,7% (70/105)
RM21938	105	18	80	0	7	67,6% (71/105)

The assessment results of accuracy ratio of genotype compared to phenotype showed that molecular markers Indel5 occupies 66,7%, while in molecular marker RM21938 is 67,6%. These results are similar to other study results of Nguyen Thi Lang *et al.* (2015). These also showed that the using of two Indel5 and RM21938 are to mark or identify genes, which govern less chalkiness level of rice grain and these results are useful and very significant.

Therefore, through the genotype analysis with two molecular markers Indel5 and RM21938, the expression of rice varieties carries genes with less chalkiness level (homozygous), which are same both molecular markers, including the rice varieties KDML105, OM10037, OM10258, OM10383, OM70L, TLR416, TLR417, TLR420, TLR426, TLR434, and RVT. These rice varieties are of A_2 group, also are candidates of less chalkiness level-carried rice varieties (paternal rice varieties). The rice varieties belong to the A1 and A_3 group carried the genes of less chalkiness level, but heterozygous or only expressed to

carry the target gene with this molecular marker, but un-carrying genes for other molecular markers.

4.1.4. The selection of the parents for hybridization of rice varieties with low chalkiness level

The breeding program requires the first steps and important for the selection of suitable parents. This study creates the inheritance hybrid progenies with the best characteristics of their parents, enhance hybrid superiority. In this study, rice variety selected maternal is OM3673 with the desired inheritance characteristics of high-yielding, widely-adaptation, good quality. However, the defect requires to be overcome of the maternal is the high chalkiness level, not carrying the gene with less chalkiness level (Figure 4.8). The rice varieties selected paternal are TLR434 and RVT with the desired genetic characteristics of the very low chalkiness level and carrying less chalkiness gene (Figure 4.8). The two of hybrid populations expected are (OM3673 and TLR434) and (OM3673 and RVT).

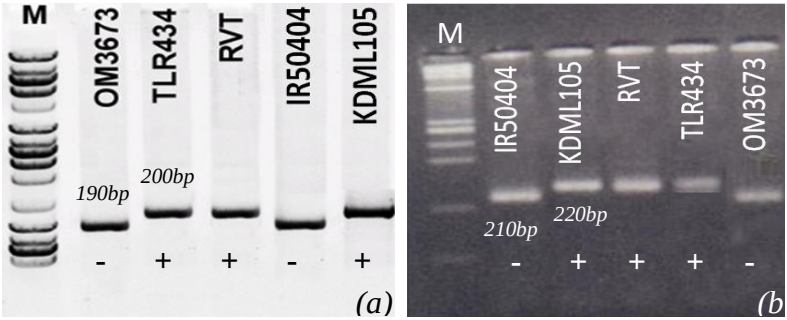

Figure 4.8: The PCR products at the locus of Indel5 (a) RM21938 (b) on chromosome 7

Notes: M: DNA Ladder (1Kb$^+$)

4.2. The breeding of backcross hybrid populations related to the characteristics of rice grains with less chalkiness level using molecular marker

4.2.1. The breeding results of backcross hybrid population OM3673/RVT//OM3673

The backcross hybrid population between OM3673 (maternal parent) and RVT (paternal parent) started for the hybridization from

2013 and the hybrid individuals were continually selected based on molecular markers up-to BC_3 generations. The individuals of each generation were selected based on the chalkiness phenotypes and genotypes with two molecular markers Indel5 and RM21938. The number of individuals was generated and selected, which recorded as described in Table 4.3.

Table 4.3. The number of selected individuals in F_1 up to BC_3

Generation	Total of generation	Number of the individuals selected for the genotype assessment	Number of the individuals selected via the genotype assessment
F_1	138	-	-
BC_1F_1	107	-	-
BC_1F_2	327	100	5
BC_2F_1	159	-	-
BC_2F_2	255	100	14
BC_3F_1	350	-	-
BC_3F_2	201	77	10

** The result of the backcross hybrid population breeding BC_3 of OM3673/RVT//OM3673*

The similarity as the populations like BC_1, BC_2, and BC_3 of hybrid combination OM3673/RVT//OM3673, which were bred in the net-house in Winter-Autumn 2014. In order to find the target gene that is expressed in the offspring, these populations are also self-pollinated up to BC_3F_2 population. The seventy-seven excellent individuals were selected from the populations including 255 plants. The two molecular markers of Indel5 and RM21938 were used to detect the target genes on the individuals of rice plants. The PCR products with two molecular markers on 3% agarose gel were shown in Figure 4.14 and Figure 4.15.

The band position of individuals carrying the target gene is at 200bp with molecular marker Indel5 and 220bp with molecular marker RM21938.

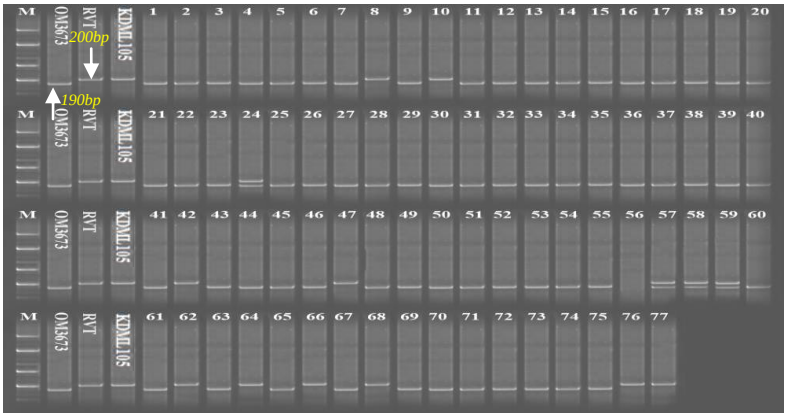

Figure 4.14: The PCR products of genes in the population OM3673/RVT//OM3673 in BC_3F_2 generation with Indel5 molecular marker on 3% gel agarose

Notes: M: Ladder (1 Kb^+); 1-77: individuals of BC_3F_2

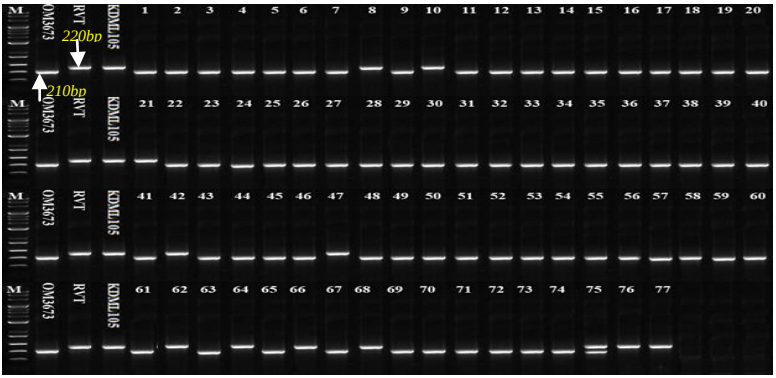

Figure 4.15: The PCR products of genes in the population OM3673/RVT//OM3673 in BC_3F_2 generation with RM21938 molecular marker on 3% gel agarose

Notes: M: Ladder (1 Kb^+); 1-77: individuals of BC_3F_2

The similarity level between the genotyping results of these two molecular markers in this assessment is almost perfect (90,9%). The number of the heterozygous individuals also decreased compared to the previous generations. These also showed that the increasing level of genetic stability of the individuals as backcross hybridization as more as. The individuals had the potential posing genes with less chalkiness level characteristic, which assessed by two molecular markers Indel5 and RM21938, these individuals were marked with numerical codes 8, 10, 42, 47, 62, 64, 66, 68, 76, and 77.

These ten individuals were marked, and harvested at 25^{th} day after the flowering and were assessed about the chalkiness level. Most of the rice lines showed chalkiness rate lower 10%, the average chalkiness level of these rice lines was 4,07, in which, maternal parent material rice variety OM3673 was at a level of 5,35 and paternal parent material rice variety of the target gene (RVT) revealed the chalkiness level at 1,31. Among the chalkiness assessed-ten rice lines, two rice lines (number 8 and number 42) were recorded with the lowest chalkiness level (rice grains with least chalkiness level) and different significant as compared to the rest of rice lines. These two lines were selected to develop on the field and the large scale.

4.2.2. The results of backcross hybrid population breeding OM3673/TLR434//OM3673

The backcross population between OM3673 (maternal parent) and TLR434 (paternal parent) were bred from last year of 2013 and the hybrid progenies continuously selected based on the molecular markers up to BC_2. The individuals of each generation were selected based on the phenotype of chalkiness characteristic and genotype with two molecular markers Indel5 and RM21938. The number of the created- and selected-individuals were recorded as described in Table 4.5.

Table 4.5: The number of individuals were selected from the F_1 generation to BC_2 generation

Generation	Total of individuals	Number of the individuals selected for the genotype assessment	Number of individuals selected via the genotype assessment
F_1	197	-	-
BC_1F_1	244	-	-
BC_1F_2	372	100	7
BC_2F_1	156	-	-
BC_2F_2	434	100	4

** The result of the backcross population breeding BC_2 of OM3673/TLR434//OM3673*

During the Winter-Autumn season in 2014, BC_2F_1 population created by 156 individuals. These individuals were self-pollinated up to BC_2F_2 population, including 434 plants. In BC_2F_2 population, the genotype assessment related to the chalkiness characteristic performed with more than 100 healthy rice plants, which were selected in the current study. Similarity in BC_1 generation, two molecular markers Indel5 and RM21938 were also used to seek the target genes. On 3% agarose gel, the band position showed the target gene-carried individuals are at a size of 200bp for Indel5 and 220 bp for RM21938.

In molecular marker Indel5, the gene products result was shown in Figure 4.20. The individuals carrying homozygous-target gene (the band position at 200bp) were assessed and occupied about 13%, including the individuals marked such as 10, 14, 22, 30, 50, 60, 62, 64, 66, 68, 80, 97, and 100. While in, the heterozygous target gene-carried individuals were assessed and occupied about 11%, the rest of other individuals didn't express the target gene.

In molecular marker RM21938, the PCR products on 2,5% agarose gel expressed polymorphism with the bands at size of 210bp

and 220bp (Figure 4.21). In which, the respective band position carrying the target gene is 220bp (position of KDML105, TLR434). The individuals carrying the homozygous target genes, including the individuals marked with numerical codes of 2, 14, 30, 50, 57, 59, 60, 61, 63, 65, 67, 79, 80, 89, 95, and 96 (occupied about 16%). The seven individuals carrying heterozygous gene and the expression of other individuals didn't carry the target gene.

The genotype analysis of BC_2F_2 populations with two molecular markers Indel5 and RM21938 showed that the result of the similarity level is 70%. The individuals carrying homozygous genes (AA) selected *via* the genotype analysis are individuals 14, 30, 50, 60, and 80. These rice lines were used in the further studies, and other rice lines carrying target genes but heterogeneous between two molecular markers/carrying heterozygous genes, and were kept for further studies.

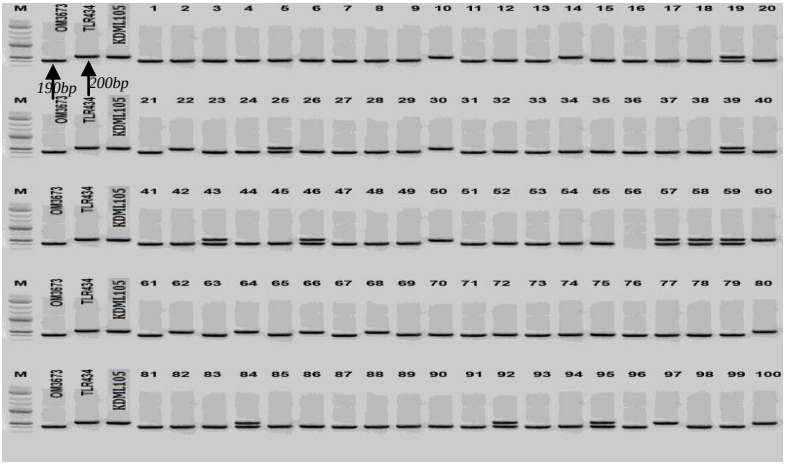

Figure 4.20: The gene products of population OM3673/TLR434// OM3673 in the generations of BC_2F_2 with molecular marker Indel5 on 3% agarose gel.

Notes: M: Ladder (1 Kb^+); 1-100: Individuals of BC_2F_2

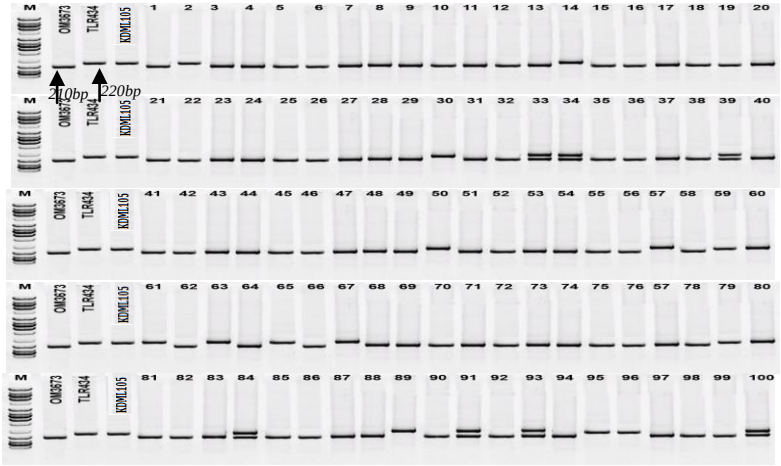

Figure 4.21: The gene products of population OM3673/TLR434// OM3673 in the generations of BC_2F_2 with molecular marker RM21938 on 3% agarose gel.

Notes: *M: Ladder (1 Kb$^+$); 1-100: Individuals of BC_2F_2*

There were five rice lines (14, 30, 50, 60, and 80) were marked, harvested and analyzed about the chalkiness level, with two controls rice varieties are OM3673 and TLR434. The assessment results showed that most of rice lines had a low chalkiness rate, in which, four rice lines had chalkiness rate from average to very low, these rice lines were BC_2F_2-30 (level of 1,81), BC_2F_2-80 (level of 1,93), BC_2F_2-50 (level of 2,39), and BC_2F_2-14 (level of 2,51). While in rice line BC_2F_2-60 had high chalkiness level at 9 (30%), this line shall not be selected for further study on rice with less chalkiness level.

4.4. The interaction analysis between genotype and environment of the promising rice lines

The potential rice lines BC_3F_3 from these studies used for the further study, which selected in the field through some of self-pollinated generations. These rice lines selected according to pure rice line methods.

The pure rice lines achieved after the selecting including 11 rice lines of combinations such as the hybrid combination OM3673/RVT// OM3673 (BC_3F_3-8-10-8; BC_3F_3-14-5-1; BC_3F_3-24-25; BC_3F_3-33-74; BC_3F_3-42-27; BC_3F_3-57-2-6; and BC_3F_3-63-7-2); and the hybrid

combination OM3673/TLR434//OM3673 (BC_2F_3-14-1; BC_2F_3-30-10; BC_2F_3-50-80; and BC_3F_3-80-20-3).

These eleven promising rice lines were tested in six different ecologies areas to assess the interaction between genotype and environment based on the yield and chalkiness rates on these rice lines.

4.4.1. The interaction assessment between genotype and environment of the promising rice lines based on the yield in Winter-Spring season in 2016-2017

The experiments were conducted at various 6 locations represented in the rice production areas in Mekong Delta such as Hau Giang, An Giang, Long An, Can Tho, Tra Vinh, and Bac Lieu.

The results of rice yield were assessed in 6 locations on the set of promising rice lines in Winter season 2016-2017 were shown in Table 4.7. When considering on rice variety, most of rice lines showed the average yield higher rice variety RVT (6,94 tons/hectare), TLR434 (6,99 tons/hectare), and some of rice lines were higher control rice variety OM3673 (7,52 tons/hectare). The rice lines of the hybrid combination OM3673/RVT//OM3673 revealed the highest yield, these rice lines were are BC3F3-33-74 (8,03 tons/hectare), BC3F3-63-7-2 (7,82 tons/hectare), BC3F3-42-27 (7,68 tons/hectare), BC3F3-24-25 (7,62 tons/hectare); and two rice lines BC2F3-30-10 (8,06 tons/hectare), and BC2F3-14-1 (7,83 tons/hectare) of the hybrid combination OM3673/TLR434//OM3673 showed the yield at higher value than control rice variety OM3673. The difference in the yield of rice lines are highly significant at the level of 95%, these results obtained from the yield-assessment standard table through multiple points.

Table 4.7: The yield (tons/hectare) of the set of the promising rice lines at various 6 locations in Winter-Spring season in 2016-2017

Sl No.	Rice lines names	Yield (tons/hectare)						
		Can Tho	**Hau Giang**	**Long An**	**An Giang**	**Bac Lieu**	**Tra Vinh**	***Average***
1	BC_3F_3-8-10-8	7,32	7,21	7,45	7,95	7,11	7,64	**7,45 ef**
2	BC_2F_3-14-1	7,71	7,59	7,84	8,34	7,49	8,02	**7,83 b**
3	BC_3F_3-63-7-2	7,69	7,58	7,82	8,32	7,48	8,01	**7,82 b**
4	BC_3F_3-33-74	7,91	7,79	8,04	8,54	7,69	8,22	**8,03 a**
5	BC_3F_3-24-25	7,50	7,38	7,62	8,12	7,28	7,81	**7,62 cd**
6	BC_3F_3-57-2-6	7,29	7,18	7,42	7,92	7,08	7,61	**7,42 ef**
7	BC_2F_3-30-10	7,94	7,81	8,06	8,56	7,71	8,24	**8,05 a**
8	BC_3F_3-14-5-1	7,20	7,08	7,32	7,82	6,97	7,51	**7,32 f**
9	BC_2F_3-80-20-3	7,19	7,06	7,31	7,81	6,96	7,49	**7,30 fg**
10	BC_3F_3-42-27	7,56	7,44	7,69	8,19	7,34	7,87	**7,68 bc**
11	BC_2F_3-50-80	7,19	7,07	7,31	7,81	6,97	7,51	**7,31 fg**
12	*RVT*	*6,82*	*6,69*	*6,94*	*7,44*	*6,59*	*7,13*	**6,94 h**
13	*TLR434*	*6,87*	*7,75*	*7,00*	*7,50*	*6,65*	*7,18*	**7,16 g**
14	*OM3673*	*7,40*	*7,27*	*7,52*	*8,02*	*7,17*	*7,71*	**7,52 de**
***AY** (tons/hectare)*		**7,40 d**	**7,35 d**	**7,52 c**	**8,02 a**	**7,18 e**	**7,71 b**	
I_j		***-0,132***	***-0,181***	***-0,007***	***0,493***	***-0,353***	***0,180***	

Notes: I_j: Environmental index; AY: Average Yield

When considering on the tested locations, the places showed the average yield at the highest level are An Giang (8,02 tons/hectare), Tra Vinh (7,71 tons/hectare), and followed by Long An (7,52 tons/hectare).

ANOVA analysis of the yield in 16 rice varieties under 6 environments conditions are the difference between rice varieties with statistically significant at a level of 99%.

In addition, based on the analysis of the interaction between genotype and environment in Winter-Spring season in 2016-2017 showed that the set of rice varieties resulted in high-yielding and good adaptation, including rice varieties such as **BC_2F_3-50-80**; **BC_3F_3-14-5-1**; **BC_3F_3-63-7-2**; **BC_2F_3-30-10**; and **BC_3F_3-42-27**. The cultivation conditions at An Giang and Can Tho are most favourable for the development of the local rice varieties in this season.

4.4.2. The interaction assessment between genotype and environment of the promising rice lines based on unchalkiness rice ratio on the Winter-Spring season in 2016-2017

In Winter-Spring season in 2016-2017, beside the yield, the interaction between genotype and environment based on the chalkiness rate were also analyzed. The set of rice lines/varieties were tested in the different six ecological areas (Hau Giang, An Giang, Long An, Can Tho, Tra Vinh, and Bac Lieu). The results of the chalkiness rate at the different locations showed that statistically significant at a level of 99% on the linear hypothesis of environment, rice variety, and interacting of rice variety and environment.

Table 4.9: The ratio of un-chalkiness rice (%) of the promising rice lines in the different ecological areas in Winter-Spring season in 2016-2017

Sl No.	Rice lines names	Ratio of un-chalkiness rice (%)						
		Can Tho	Hau Giang	Long An	An Giang	Bac Lieu	Tra Vinh	*Average*
1	BC_3F_3-8-10-8	85,21	86,40	83,60	85,90	82,89	82,46	**84,41 b**
2	BC_2F_3-14-1	79,53	80,72	77,92	80,67	77,21	76,78	**78,81 c**
3	BC_3F_3-63-7-2	77,98	79,17	76,38	78,67	75,66	75,23	**77,18 d**
4	BC_3F_3-33-74	78,85	80,04	77,24	79,02	76,53	76,10	**77,96 cd**
5	BC_3F_3-24-25	78,33	79,52	76,73	75,22	76,01	75,58	**76,90 d**

6	BC_3F_3-57-2-6	74,53	75,72	72,92	75,97	72,21	79,78	**75,19 e**
7	BC_2F_3-30-10	75,28	76,47	73,67	78,55	72,96	72,53	**74,91 e**
8	BC_3F_3-14-5-1	77,86	79,05	76,25	78,55	75,54	75,11	**77,06 d**
9	BC_2F_3-80-20-3	74,87	76,05	73,26	75,56	72,54	72,12	**74,07 e**
10	BC_3F_3-42-27	74,80	75,99	73,19	75,49	72,48	72,05	**74,00 e**
11	BC_2F_3-50-80	68,25	69,44	66,64	68,94	65,93	65,50	**67,45 f**
12	*RVT*	*94,10*	*95,28*	*92,49*	*94,78*	*91,77*	*91,35*	**93,30 a**
13	*TLR434*	*93,10*	*94,96*	*92,16*	*94,46*	*91,45*	*91,82*	**92,99 a**
14	*OM3673*	*40,26*	*41,44*	*38,65*	*40,95*	*37,93*	*37,51*	**39,46 g**
	Average (%)	**76,64ab**	**77,88a**	**75,08c**	**77,34b**	**74,37c**	**74,57c**	
	I_J	***0,662***	***1,898***	***-0,898***	***1,361***	***-1,612***	***-1,411***	

Notes: I_J: Environmental Index; Average: Average ratio of chalkiness

When considering aspects on rice varieties, most of hybrid rice lines/varieties showed the average ratio of chalkiness were higher than control rice variety OM3536 (39,46%). The difference of the chalkiness ratio of rice lines/varieties was shown very significant at a level of 95%. The rice lines with un-chalkiness rice ratio was almost more than > 70%, in which, the rice lines with highest un-chalkiness ratio are BC_3F_3-8-10-8 (84,4%), BC_2F_3-14-1 (78,8%), BC_3F_3-33-74 (78,0%),…

The locations were revealed the average ratio of un-chalkiness rice at the highest level are Hau Giang (77,88%) and An Giang (77,34%), respectively; and followed by Bac Lieu (74,37%).

Therefore, on the interaction analysis between genotype and environment based on the characteristic of un-chalkiness ratio, the promising rice lines showed that the un-chalkiness rice grain ratio at high level and same to each other, the difference are very significant as compared to the parents control rice varieties. The rice lines only showed clear differences in stability and adaptability. Hence, these suggested that through the analysis of multiple dimensions, the most

promising rice lines are **BC_3F_3-24-25**, **BC_2F_3-30-10**, **BC_3F_3-57-2-6**, and **BC_3F_3-8-10-8**.

In the Winter-Spring season in 2016-2017, the eleven promising rice lines of two backcrossing hybrid combinations OM3673/RVT// OM3673 and OM3673/TLR434//OM3673 were analyzed for interaction between genotype and environment. Depending on the analytical characteristics, the rice lines manifested the different values of stability and adaptability. Of which, some rice lines completely revealed many good characteristics, including high ratio of un-chalkiness rice grains, high-yielding, stability and adaptability, in particular is rice line **BC_2F_3-30-10**. Especially, this rice line showed adaptation to unfavorable environmental conditions with bi<1 *i.e.* under favorable or unfavorable, this rice line still shows high-yielding and rice grain with less chalkiness level.

Similarity, the analysis results of the interaction between genotype and environment through the assessment on the yield traits and the un-chalkiness rice ratio in Summer-Autumn in 2017, the rice lines pooled with many good characteristics such as high-yielding, low chalkiness ratio, and the most stability and adaptability, these rice lines were **BC_3F_3-8-10-8**, **BC_2F_3-14-1**, **BC_3F_3-63-7-2**, **BC_3F_3-33-74**, and **BC_2F_3-30-10**.

In summary, the eleven promising rice lines showed the different results in yield and the un-chalkiness rice ratio when analyzing the interaction between genotype and environment throughout two continuous cultivation seasons. In other some rice lines showed the best results in Winter-Spring season in 2016-2017, but not the best in Summer-Autumn season in 2017. Therefore, depending on the characteristics of each variety, the selection also altered under the effects of the particular environmental condition. In two cultivational seasons, rice line **BC_2F_3-30-10** considered to be the most suitable for the Winter-Spring season and rice line **BC_3F_3-8-10-8** is the most elite rice lines for the Summer-Autumn season.

Chapter 5: RESULTS AND SUGGESTIONS

5.1. Results

1. In the obtained current study results were performed on the 105 high-yielding rice varieties/samples, of which OM3673 was selected and used as recurrent maternal parent rice variety due to this is rice variety have known with the prominent characteristics like high-yielding, widely stability and adaptability, but the very high chalkiness ratio of rice grains. On the other hand, the rice varieties were used for donor target gene including RVT and TLR434, which used as paternal parent, these rice varieties showed that with a low chalkiness ratio of rice grains.

2. The promising rice lines were selected using the improved backcross hybridization method and showed that in BC_3F_2 generation of the backcross population OM3673/RVT//OM3673, the results recorded the homozygous target genes-carried ten individuals in both molecular markers Indel5 and RM21938, however, only two rice lines (BC_3F_2-8 and BC_3F_2-42) were selected for further study because of the lowest chalkiness level. Similarity, the BC_2F_2 population of OM3673/TLR434//OM3673, there were 5 individuals carrying homozygous target genes, but only 4 rice lines (BC_2F_2-14, BC_2F_2-30, BC_2F_2-50, and BC_2F_2-80) were selected based on the least chalkiness level on rice grains.

3. The analysis results of the GGT genetic map revealed that 11 individuals carrying the target gene region on chromosome 7 (0-140 cM) of rice, the homologous individuals carrying the genes set of paternal parents (RVT, TLR434) were selected for further study. The eleven rice lines including BC_3F_3-8, BC_3F_3-14, BC_3F_3-24, BC_3F_3-33, BC_3F_3-42, BC_3F_3-57, and BC_3F_3-63 are the products of the hybrid combination OM3673/RVT//OM3673); and the rice lines BC_2F_3-14, BC_2F_3-30, BC_2F_3-50, and BC_2F_3-80 are the products of the hybrid combination OM3673/TLR434//OM3673.

4. The analysis results of the interaction between genotype and environment of the eleven promising rice lines based on the yield and the un-chalkiness rice grain ratio in during the two cultivational seasons (the Winter-Spring in 2016-2017 and Summer-Autumn in 2017) showed that these eleven rice lines with high-yielding are similar with maternal parent (OM3673) and decreased rice grains ratio with high

chalkiness level, these characteristics values were the difference with high significant level as compared to the control rice variety OM3673, other these rice lines also showed that the high stability and adaptability to the different environmental conditions. Out of which, both two rice lines **BC_2F_3-30-10** and **BC_3F_3-8-10-8** manifested with the best characteristics.

The methods of hybrids selection of the improved backcross hybrid populations were used with the help of suitable molecular markers, which located on chromosome 7, these study methods and results are highly effective and shortens the breeding time.

5.2. Suggestions

Continuing to develop the promising rice lines carrying the target genes with the un-chalkiness level of chromosome 7 of rice, together with the deeper scientific study contents on the assessment of genotype based on the sequencing method (GBS: genotyping by sequencing) to increasing the accuracy of breeding results of rice lines in the future.

Besides, the chalkiness rate assessment presented under the harvesting time, the investigation needs to further studies on the effects of storage time to chalkiness level of rice lines/varieties as well as to the chalkiness level on the various positions of rice grains on the panicle.

NOTABLE PUBLICATIONS RELATED TO THESES

1. Truong Anh Phuong, Nguyen Thi Lang, Nguyen Thi Ngoc An (2016). "Exploitation of initial materials for rice varieties without chalkiness". *Journal of Vietnam Agricultural Technology & Science*, 6:3-6.

2. Truong Anh Phuong, Nguyen Thi Lang, Pham Thi Thu Ha, Bui Chi Buu (2017). "Using molecular markers to detect tolerance genes to whine from rice backcross OM3673/RVT//OM3673 population". *Journal of Agriculture & Rural Development*, 2:20-27.

3. Nguyen Thi Lang, Phan Ho Truc Giang, Pham Thi Thu Ha, Tran Bao Toan, Truong Anh Phuong, Bui Chi Buu (2017). "Identifying the grain chalkiness gene using molecular marker techniques in Rice (*Oryza sativa* L.)". *International Letters of Natural Sciences*, 63:18-26.

4. Truong Anh Phuong, Nguyen Thi Lang, Nguyen Thi Ngoc An, Bui Chi Buu (2018). "Study of the interaction genotype and environment of chalkiness in rice at Mekong Delta". *Conference Book on Technology & Science with Specialization of Horticulture, and Plant Protection, during 2013-2018*, 18-28.

5. Truong Anh Phuong, Nguyen Thi Lang, Nguyen Thi Ngoc An, Bui Chi Buu (2019). "Study of the interaction genotype and environment of yield in rice at Mekong Delta". *Journal of Agriculture & Rural Development*, 1:3-10.

6. Truong Anh Phuong, Nguyen Thi Lang, Nguyen Thi Ngoc An, Bui Chi Buu (2019). "Study of the interaction genotype and environment of yield in rice at Mekong Delta". *Conference Book on National Science with Scientific research in universities Industrial Revolution period 4.0*, 186-199.