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IMPROVEMENT OF RD 15 RICE CULTIVAR BY MOLECULAR MARKERS ASSISTED BACKCROSSING FOR PHOTOPERIOD INSENSITIVE, SEMI-DWARF AND GLUTINOUS RICE

VO VAN TRUNG

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By

VO VAN TRUNG

APPROVED BY

Advisory Committee Chairperson ..... Varaporn Sangtong

(Assistant Professor Dr. Varaporn Sangtong)

25 / July / 2011

Advisory Committee Member ..... Adisak Joomwong

(Assistant Professor Dr. Adisak Joomwong)

25 / July / 2011

Advisory Committee Member ..... Saengtong Pongjaroenkit

(Assistant Professor Dr. Saengtong Pongjaroenkit)

25 / July / 2011

Chairperson, Committee on Master of Science Program in Biotechnology ..... Mayura Srikanlayanukul

(Dr. Mayura Srikanlayanukul)

25 / July / 2011

CERTIFIED BY

GRADUATE SCHOOL .....

(Assistant Professor Dr. Chamnian Yosraj)

2 / August / 2011

Title Improvement of RD 15 Rice Cultivar by Molecular Markers Assisted Backcrossing for Photoperiod Insensitive, Semi-dwarf and Glutinous Rice

Author Mr. Vo Van Trung

Degree of Master of Science in Biotechnology

Advisory Committee Chairperson Assistant Professor Dr. Varaporn Sangtong

ABSTRACT

This thesis was conducted to study the improvement of RD 15 rice cultivar for non-photoperiod sensitive, semi-dwarf and glutinous rice. RD 15 is a non-glutinous rice variety in Thailand with good cooking qualities. However, the plant is tall and photoperiod sensitive which carries homozygous dominant Hd1Hd1Sd1Sd1WxWx genotype. With the aid of functional markers, RD 15 variety was successfully improved by introgressions of the recessive *hd1, sd1* and *wx* genes from improved RD 6 line, which is non-photoperiod sensitive, semi-dwarf and glutinous, containing homozygous recessive hd1hd1sd1sd1wxwx genotype. Molecular markers assisted backcrossing (MAB) was applied in two backcrosses and one self-cross to select the four best lines of RD 15 containing genotype with corresponding phenotypes, namely: RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype), RD 15 NTG line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype), RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype). These lines were then tested for photoperiod response under long-day condition of light exposure for 14 hours per day and were later checked for yield and other traits in field trials at Maejo University, Chiang Mai province, Thailand during the rainy season of 2010. Results showed that the selected four best lines of RD 15 showed flowering when the original RD 15 was not under

long-day condition. Nevertheless, grain yields and other most important traits such as number of seeds per panicle, fertility, weight of 1,000 seeds and characteristics of paddy and brown rice grain were not significantly different with the original RD 15 rice variety.

On the other hand, the study inheritance of photoperiod response, plant height and endosperm rice traits as controlled by *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes using three markers (*hd1, sd1* and *wx*, respectively) in BC_3F_2 population. A total of 150 BC_3F_2 plants were planted in the greenhouse from August 2010 to March 2011 and exposed to light for 14 hours per day. Chi-square test (χ^2) was later applied for each individual gene. Results showed that genotypic and phenotypic ratios were found to follow the First Law of Mendel as shown by 107 non-flowering : 43 flowering plants, 110 tall : 40 short plants and 117 non-glutinous : 33 glutinous seeds ratios. Genetic analysis of these plants indicated that the rice plants were non-photosensitive with homozygous recessive hd1hd1 genotype only; rice plants which were short, had homozygous recessive sd1sd1 genotype only and rice seeds which were glutinous even when their plants had a homozygous recessive wxwx genotype only. Moreover, genetic inheritance in combination of two genes together was studied to analyze their genotypic and phenotypic ratios by chi-square test (χ^2), such as *Hd1/hd1* and *Sd1/sd1*; *Hd1/hd1* and *Wx/wx*; *Sd1/sd1* and *Wx/wx*. Results showed that both genotypic and phenotypic ratios followed the Second Law of Mendel. Furthermore, when combining three genes together such as *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes, results also indicated that genotypic and phenotypic ratios followed the Tri-hybrid Cross.

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Vo Van Trung

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CHAPTER 1

Introduction

Rice *(Oryza sativa* L.*)* is the most important staple food crop for more than half of the world's population. Asia accounts for more than 90% of the world's total rice production; the balance is divided almost equally between Africa and Latin America, where the demand for rice is increasing. It provides over 21% of the calorific needs of the world's population and up to 76% of the calorific intake of the population of South East Asia (Juliano, 2003). In the developing countries, rice accounts for 715 kcal/caput/day, 27 percent of dietary energy supply, 20 percent of dietary protein and 3 percent of dietary fat. Countries in Southeast Asia are heavily reliant upon rice such as Bangladesh, the Lao People's Democratic Republic, Thailand, Vietnam, Myanmar and Cambodia. Rice was grown on approximately 2.52 million hectares with a total production of 5.02 million tons, out of which 3.69 million tons was exported and earned a foreign exchange worth 69,325.1 million rupees (FAOSTAT, 2001).

Rice is short-day plant because it progresses most rapidly toward flowering and reproduction when the day length is shortened. Long-day period can prevent or considerably delay its flowering time. Photoperiod sensitive rice can grow only in the rainy season. When grown in the dry season (time light upper 12 hours per day), its flowering time is delayed but non-photoperiod sensitive riee can be grown in all seasons of the year (Swaminathan, 1985). Studies on quantitative trait loci (QLTs) showed that photoperiod sensitive rice was found to be controlled by six loci: *Hd1, Hd2, Hd3a, Hd3b, Hd5* and *Hd6*; with *Hd1* as the major gene. The *Hd1* allele works in long-day condition by delaying inflorescence in rice but in short-day condition, *Hd1* allele promotes inflorescence. *Hd1* allele in a photosensitive variety of Nipponbare is a functional allele while *hd1* allele in non-photosensitive varieties of HS66, HS110 and Kasalath is considered a non-functional allele because gene mutation occurs (Yano *et al.,* 2000).

Besides, plant height is one of the most important traits of rice cultivar in relation to collapsing or resistance effect of environment condition such as wind and rain. Tall plants are easy to collapse or break when they are exposed to wind and rain thus reducing 25% of grain yield (Monna *et al.,* 2002), while short plants can resist the wind and rain. Moreover, rice plant

height would allow the simulation of processes with a significant impact on yielding, lodging, floodwater effect on leaves temperature and crop-weeds eompetition for radiation interception (Roberto *et al.*, 2010). Plant height in riee is found to be controlled by the gene *Sd1/sd1* with *Sd1* gene for tallness and *sd1* gene for semi-dwarfness (Khush, 2001).

On the other hand, presence of endosperm amylose is one of the most important traits of rice cultivar to determine qualities of riee grain. Amylose content is related to rice being either non-glutinous or glutinous. Rice endosperm is non-glutinous if its amylose content is higher than 10%, while rice endosperm is glutinous if its amylose content is lower than 10% (Fitzgerald, 2004). Rice endosperm was found to be controlled by *Wx/wx* gene with non-glutinous phenotypes controlled by dominant *Wx* gene and glutinous phenotypes controlled by single recessive *wx* gene (Mikami *et al.*, 1999).

One of the most exciting developments in rice biotechnology was the advent of molecular markers. In rice breeding, molecular markers assisted backcrossing (MAB) was applied widely much recently (Collard and Mackill, 2007). These reviews mainly addressed the application of DNA markers in the improvement of crop plants in general and emphasized the marker application in heterosis breeding. They also described the limitation of marker use in crop improvement. The use of molecular markers will help the rice breeder to save time, have consistency, bio-safety, efficiency and accuracy in selecting complex traits. A series of molecular markers, such as Random Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Microsatellites or Simple Sequence Repeat (SSR) have become available. These markers offer new opportunities for various studies in genetic and breeding research, particularly in the construction of saturated molecular maps, gene tagging, Quantitative Trait Loci (QTLs) mapping, gene pyramiding, physical mapping of genome, map-based gene cloning, alien introgression and DNA finger-printing of pathogen populations (Tanksley *et al.*, 1989).

RD 15 rice is a non-glutinous variety in Thailand with good cooking qualities. However, the plant is tall and photoperiod sensitive which carries homozygous dominant Hd1Hd1Sd1Sd1WxWx genotype. That is why the aim of this research is to use molecular markers assisted backcrossing to improve RD 15 for photoperiod insensitive, semi-dwarf and glutinous rice.

1.1 Objective of this study

This study aims to:

1.1.1 Produce BC_3F_1 seeds to BC_5F_1 seeds by using molecular markers assisted backcrossing.

1.1.2 Select four best lines of RD 15 by using markers that specific for *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes and test for photoperiod response under long-day condition of light exposure for 14 hours per day of selected four best lines of RD 15:

1.1.2.1 RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype).

1.1.2.2 RD 15 NTG line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype).

1.1.2.3 RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype).

1.1.2.4 RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype).

1.1.3 Study yield, yield components and seed physically characteristics of selected four best lines of RD 15.

1.1.4 Study inheritance of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes using markers in BC_3F_2 populations.

1.2 Expected results

1.2.1 Successful use of molecular markers assisted backcrossing to produce BC_3F_1 seeds to BC_5F_1 seeds.

1.2.2 Successful use of markers in selecting genotypes corresponding to phenotypes to get four best lines of RD 15 and knowing yield, yield components and seed physically characterized of selected four best lines of RD 15.

1.2.3 To know inheritance of photoperiod response, plant height and endosperm traits of BC_3F_2 populations by using markers that specific for *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes, respectively.

1.3 Scope and Limitation

This experiment aims to improve RD 15 rice cultivar from photoperiod sensitive, tall and non-glutinous to non-photoperiod sensitive, short and glutinous rice by using molecular markers assisted backcrossing; to test photoperiod response of the selected four best lines of RD 15 under long-day condition of light exposure for 14 hours per day; to test yield abilities and some agronomic characteristics of the selected four best lines of RD 15; and to study the inheritance of photoperiod response, plant height and endosperm traits of BC_3F_2 populations by using markers that are specific for *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes, respectively.

CHAPTER 2

Literature Reviews

2.1 Rice breeding

Rice, *Oryza sativa* L. (*O. sativa* L.) with 2n = 24 belonging to the family *Graminae* and subfamily Oryzoidea is a cultivated, inbreeding species that was domesticated in Asia from a complex ancestral species, *O. rufipogon*. Evidence from fossilized remains of rice grains found in tombs in China, India, and Vietnam suggested that rice cultivation began approximately 10,000 years ago, though archaeological and genetic evidence suggested that rice domestication may be a more recent phenomenon. Two major varietal groups within *O. sativa* are *indica* and *japonica*, have been recognized since ancient times by many different ethnic groups. Estimates of sequence divergence and transposing insertion polymorphism between *O. sativa indica* and *O. sativa japonica* suggested that these groups last shared a common ancestor more than 100,000 years ago (Cheng *et al.*, 2003).

2.2 Rice production

Rice is the second largest produced cereal crop in the world after maize. At the beginning of the 1990s, annual production was around 350 million tons and by the end of the century it had reached 410 million tons. World production totaled 395 million tons of milled rice in 2003, compared with 387 million tons in 2002 (Khoso, 2008). The FAO forecast of world rice stocks at the close of the marketing years ending in 2010 has been raised by 6 million tons to 123 million tons, representing a 1% drop from opening levels. Much of the contraction is expected to be in the five major exporting countries, which, as a group, are predicted to close the year with a 24% draw down to 24.5 million tons (FAO, 2009).

FAOSTAT (2009) shows in table 1 that the general cultivated area of rice in the world has increased over the years from 2003 to 2008 but not significantly. In 2003 world rice area was 148.6 million hectares and in 2008, the world rice area was 158.96 million hectares. From 2003 to 2008, rice yield did not increase significantly from 3.94 tons per hectare (2003) increased to 4.31 tons per hectare (2008). Through the years, rice production increased slowly

from 2003 to 2004. However, from 2004 to 2008, rice production increased significantly. In 2008 world rice production reached 685.10 million tones.

Table 1 Areas, yield and production of rice in the world from 2003 to 2008

Year	Areas (million hectare)	Yield (tons/hectare)	Production (million tons)
2003	148.60	3.94	585.48
2004	150.65	4.04	608.63
2005	155.13	4.09	634.49
2006	155.78	4.12	641.81
2007	156.00	4.21	656.75
2008	158.96	4.31	685.10

(Source: FAOSTAT, 2009)

In table 2, FAOSTAT (2009) shows that in 2008, rice area of India was the largest with 44.00 million hectares, next was China with 29.49 million hectares, then Indonesia with 12.31 million hectares, Bangladesh 11.74 with million hectares and Vietnam with 7.41 million hectares. U.S. and China were the two countries with the highest rice yield in the world with 7.67 and 6.29 tons per hectare, respectively. The countries with the most rice production in 2008 were India with 215.37 million tons, followed by China with 185.65 million tons, Indonesia with 68.38 million tons, Bangladesh with 40.81 million tons, Vietnam with 38.72 million tons, Myanmar with 34.44 million tons and Thailand with 23.19 million tons.

Table 2 Areas, yield and rice production of some countries in the world in 2008

Countries	Area (million hectare)	Yield (tons/hectare)	Production (million tons)
India	44.00	4.89	215.37
China	29.49	6.29	185.65
Indonesia	12.31	5.56	68.38
Bangladesh	11.74	3.48	40.81
Vietnam	7.41	5.22	38.72
Myanmar	8.20	4.20	34.44
Thailand	10.25	2.26	23.19
U.S.	1.20	7.67	9.24
Philippines	4.46	2.00	8.92
Pakistan	2.96	2.00	5.93
Nigeria	2.38	2.42	5.76
Japan	1.70	3.37	5.74
Cambodia	2.61	1.30	3.40
Brazil	2.86	1.20	3.43

(Source: FAOSTAT, 2009)

Workman (2008), the global rice market in 2007 is estimated at 30 million tons in which Asia had 22.1 million tons, occupying 76.3% of its rice exports, followed by North and Central America with 3.1 million tons (10.6%), and Europe with 1.6 million tons (5.4%). South America had 1.2 million tons (4.2%), Africa was 952 thousand tons (3.3%). Six leading rice exporters in 2007 such as Thailand (10 million tons) occupied 34.5% of total rice export, India with 4.8 million tons (16.5%), Vietnam with 4.1 million tons (14.1%), America with 3.1 million tons (10.6%), Pakistan with 1.8 million tons (6.3%) and China (including Taiwan) had 901 thousand tons (3.1 %).

2.3 DNA marker

DNA marker (or genetic marker) is a gene or DNA sequence with a known physical location on a chromosome that can be used to identify cells, individuals or species. Genetic markers can help link an inherited disease with the responsible gene. DNA segments close to each other on a chromosome tend to be inherited together. Genetic markers are used to track the inheritance of a nearby gene that has not yet been identified, but whose approximate location is known. The genetic marker itself may be a part of a gene or may have not known function (Hurle, 2011).

Molecular markers are widely used to track loci and genome regions in several crop species as molecular markers tightly linked to a large number of agronomic and disease resistance traits are available in major crop species (Varshney *et al.*, 2005). For plant breeding applications, Microsatellite or Simple Sequence Repeat (SSR) markers have been recommended as markers of choice because of their co-dominant inheritance and often differ considerably in length due to variations in the number of tandem repeats within the microsatellite region (Gupta and Varshney, 2000). Moreover, molecular markers have proven to be a powerful tool in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Several molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSRs), and Single Nucleotide Polymorphism (SNPs) are presently available to assess the variability and diversity at the molecular level (Joshi *et al.*, 2000). Information regarding genetic variability at the molecular level could be used to help, identify and dcvclop genetically unique germplasm that compliments existing cultivars (Kalyan and Rambabu, 2006).

However, genetic markers are specific DNA sequence differences that can be identified through biochemical assays. There are two main types, such as SSRs and SNPs.

2.3.1 Simple Sequence Repeats (SSRs)

One of the markers widely applied in breeding programs is the SSRs marker, also called microsatellite. SSR markers are DNA sequences that consist of two to five nucleotides core units which are tenderly repeated. These small repetitive DNA sequences provide the basic of a Polymerase Chain Reaction (PCR) base, multi-allelic, eo-dominant genetic marker system.

The region flanking the microsatellite is generally conserved among genotypes of the same species. PCR primers to the flanking regions are used to amplify the SSRs containing DNA fragment length polymorphism which is created when PCR product from different individuals vary in length as a result of a variation in the number of repeat units in the SSRs. Moreover, SSR markers have been reported to be highly polymorphic in plants and hence highly informative in plants and can be analyzed by a rapid, technically simple and inexpensive PCR-based assay. SSR markers can be used to analyze and filter the plant genome. Now, it had been applied to different plants genome researches. A large number of SSR loci have been genetically mapped in several agronomical important plant species (Lu *et al.*, 2010).

Moreover, SSRs, the second generation of molecular marker, exists extensively in plant genome. In addition, application of SSRs in plant genetics and breeding including construction of plant molecular genetics linkage map; identification of gene or QTLs; evolution and classification of plant species, genetic diversity, protection and purity identification of varieties, determination of parentage and prediction of heterosis were discussed (Guo *et al.*, 2005).

On the other hand, Gaafar (2010) used SSR markers linked to the *Hd1* heading date time QTLs in rice revealed that these markers showed low level of allelic diversity. As seen in the results, most SSR markers showed one to three different amplicons (alleles). This indicates that this region is conserved and there is a low level of recombination which is true in the gene regions.

2.3.2 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are the most abundant variations in the genome. They can contribute directly to a phenotype or can be associated with a phenotype as a result of linkage disequilibrium. Most conventional trait markers and molecular markers, such as RFLP and cleaved amplified polymorphic sequence (CAPS) markers, are based on SNPs, *etc.*, nucleotide substitutions or insertions/deletions. Because of their abundance and co-dominance, the use of SNPs as a marker system has the potential for providing the highest map resolution. Typing of SNPs has progressed remarkably over the last several years, making genome-wide linkage analysis and molecular breeding rapid and efficient (Risch and Merikangas, 1996). SNPs distribute numerously and high-density throughout rice (*O. sativa* L.) genome. A total of 80,127 SNP sites were identified in rice genome, and one SNP every 154 bp was found between two rice

subspecies *indica* and *japonica*. The SNP rate is 0.65%. SNPs are also very considerable among within-subspecies cultivars, it can even be found between closely related cultivars, in which it has been difficult to find polymorphic sites by conventional methods. The frequency of SNPs in the rice genome varied between chromosomes; moreover it showed uneven distribution of polymorphism-rich and polymorphism-poor regions along each chromosome. Several routes have been used for the identification of SNP in rice, such as sequencing PCR products of DNA samples, screening of SNPs in SSR fragments, and searching for SNPs through the rice genome sequences and EST databases. A number of genotyping systems have been developed to identify SNPs in the rice genome. High automation in SNPs identification has become a very convenient operation by using automatized systems. SNPs can be converted to CAPS or dCAPS (derived-CAPS), and allele-specific PCR markers. SNP has shown huge potential in establishing rice genetic maps, gene cloning and functional genomics, MAS (marker assisted selection) in rice breeding, and studies on classification and evolution of germplasm (Yi, 2006).

On the other hand, using SNP markers for linkage analysis has three advantages: 1) analysis can be performed in the early growth stage of plants, requiring only a small quantity of DNA; 2) a large number of samples can be processed systematically in 96-well plates from sowing to SNP-typing; and 3) time and labor can be saved, as no electrophoresis is needed (Nasu *et al.*, 2002).

Nasu *et al.* (2002) searched for SNPs in 417 regions distributed throughout the genome of three *O. sativa* ssp., *japonica* cultivars, two *indica* cultivars, and a wild rice (*O. rufipogon*). They found 2,800 SNPs in approximately 250,000 aligned bases for an average of one SNP every 89 bp, or one SNP every 232 bp between two randomly selected strains. Graphic representation of the frequency of SNPs along each chromosome showed uneven distribution of polymorphism-rich and polymorphism-poor regions, but little obvious association with the centromere or telomere. The 94 SNPs that they found between the closely related cultivars 'Nipponbare' and 'Koshihikari' can be converted into molecular markers.

2.4 Molecular marker assisted backcrossing (MAB)

Molecular markers are now increasingly being employed to trace the presence of target genes (foreground selection) as well as for accelerating the recovery of the recurrent parent

genome (background selection) in backcross programmes. Marker Assisted Backcrossing (MAB) improves the efficiency of backcross breeding in three ways: (1) if the phenotype of the desired gene cannot be easily assayed, backcross (BC) progeny possessing a marker allele from the donor parent at a locus near/within the target gene can be selected with a good probability of carrying the gene; (2) markers can be used to select BC progeny with least amounts of donor parent germplasm in the genome outside the target region; and (3) markers can be used to select rare progeny that are the result of recombination near the target gene, thus minimizing the effects of linkage drag (Babu *et al.*, 2004).

Moreover, there are three levels of selection in which markers may be applied in backcross breeding. In the first level, markers were used to screen for the target trait, which are useful for traits that have laborious phenotypic screening procedures or recessive alleles. The second level of selection involves selecting backcross progeny with the target gene and tightly-linked flanking markers in order to minimize linkage drag (recombinant selection). The third level of MAB involved selecting backcross progeny (that have already been selected for the target trait) with 'background' markers. In other words, markers can be used to select against the donor genome, which may accelerate the recovery of the recurrent parent genome. With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent (Collard and Mackill, 2007).

In addition, the main advantages of MAB are: (1) it is an efficient foreground selection for the target locus, (2) it is an efficient background selection for the recurrent parent genome, (3) it minimizes linkage drag surrounding the locus being introgressed, and (4) it rapidly breeds new genotypes with favorable traits. The effectiveness of MAB depends on the availability of closely linked markers or flanking markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch and Melchinger, 2005).

The basis of a MAB strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. The use of molecular markers, which permit the genetic dissection of the progeny at each generation, increases the speed of the selection process, thus increasing genetic gain per unit time (Hospital, 2003).

One research showed that molecular markers that were tightly linked with *Sub1*, flanking *Sub1*, and unlinked to *Sub1* were used to apply foreground, recombinant, and background seleetion, respectively, in backcrosses between a submergence tolcrant donor and the widely grown recurrent parent Swarna. By the BC_2F_2 generation a submergence tolerant plant was identified that possessed Swarna type SSR alleles on all fragments analyzed exeept the tip segment of rice chromosome 9 that possessed thc *Sub1* locus. A BC_3F_2 population double recombinant plant was identified to have homozygous for all Swarna type alleles except for an approximately 2.3 - 3.4 Mb region surrounding the *Sub1* locus. The results showed that the mega variety Swarna could be efficiently converted to a submergence tolerant variety in threc backcross generations, involving a time of two to three years (Neeraja *et al.*, 2007).

On the other hand, MAB has previously been used in rice breeding to incorporate the bacterial blight rcsistance gene *Xa21* (Chen *et al.*, 2001) and *waxy* gene (Zhou *et al.*, 2003) into elite varieties also.

2.5 Mendel's Laws

Mendel discovcrcd that by erossing white flower and purple flower plants, the result was not a blend. Rather than being a mix of the two, the offspring was purple flowered. He then conceived the idea of heredity units, which he called "factors", one of which is a recessive characteristic and the other dominant. Mendel said that factors, later called genes, normally occur in pairs in ordinary body cells, yet segregate during the formation of sex cells. Each mcmber of the pair becomes part of the separate sex cell. The dominant gcne, such as the purple flower in Mendel's plants, will hide the recessive gene, the white flower. Aftcr Mendel self-fertilized the F_1 generation and obtained the 3:1 ratio, he correctly theorized that genes can be paired in three different ways for each trait: AA, aa, and Aa. Thc capital "A" represents the dominant factor and lowercase "a" represents the recessive. Mendel stated that each individual has two factors for eaeh trait, one from each parent. The two factors may or may not contain the same information. If the two factors are identical, the individual is callcd homozygous for the trait. lf the two factors have different information, the individual is called heterozygous. The alternative forms of a factor are called alleles. The genotype of an individual is made up of the many alleles it possesses. An individual's physical appearance, or phenotype, is detcrmined by its alleles as well as by its

environment. An individual possesses two alleles for each trait; one allele is given by the female parent and the other by the male parent. They are passed on when an individual matures and produces gametes: egg and sperm. When gametes form, the paired alleles separate randomly so that each gamete receives a copy of one of the two alleles. The presence of an allele does not promise that thc trait will be expressed in the individual that possesses it. In heterozygous individuals the only allele that is expressed is the dominant. The recessive allele is present but its expression is hidden (Trofim, 2011). Mendel summarized his findings in two laws, such as; the Law of Segregation (First Law) and the Law of Independent Assortment (Second Law).

2.5.1 Mendel's Law of Segregation (First Law of Mendel)

The Law of Segregation states that when any individual produces gametes, the copies of a gene separate so that each gamete reccives only one copy. A gamete will receive one allele or the other. In meiosis the paternal and maternal chromosomes get separated and the alleles with the traits of a character are segregated into two different gametes (Trofim, 2011).

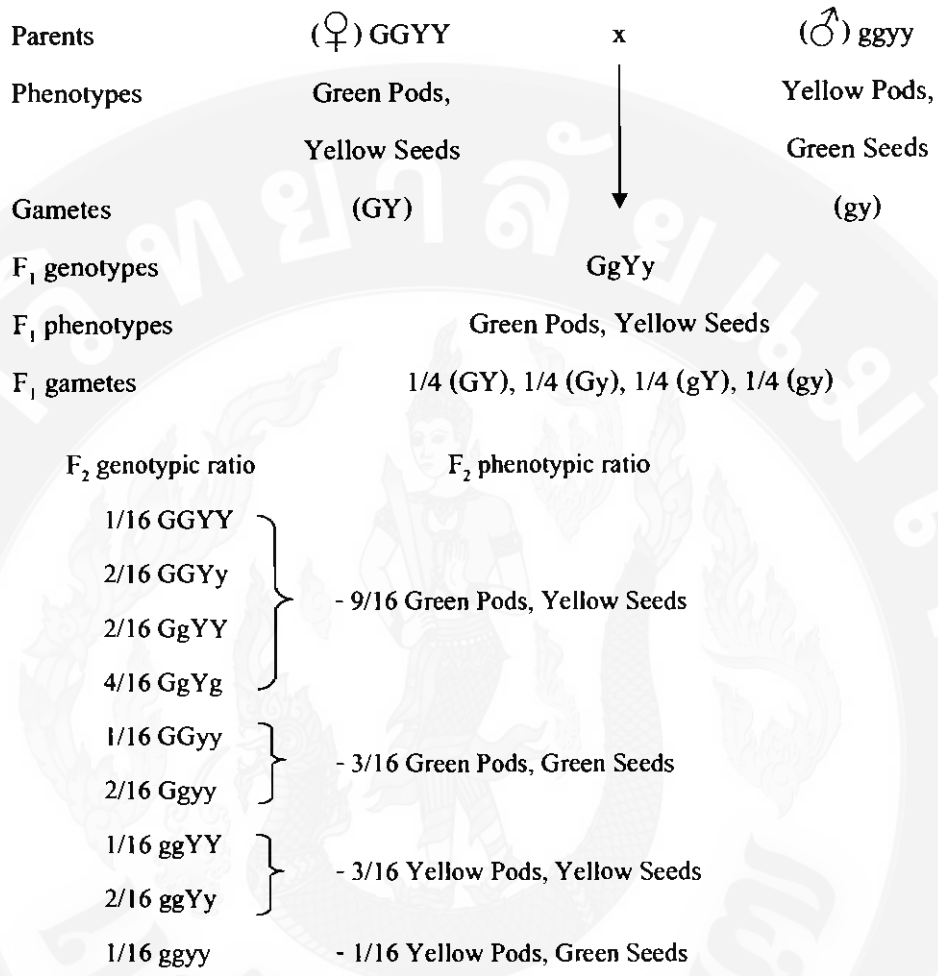
When Mendel performed cross-pollination between a true-breeding yellow pod plant and a true-breeding green pod plant, he noticed that all of the resulting offspring, F_1 generation, were green. He then allowed all of the green F_1 plants to self-pollinate. He referred to these offspring as the F_2 generation. Mendel noticed a 3:1 ratio in pod color. About 3/4 of the F_2 plants had green pods and about 1/4 had yellow pods color. From these experiments Mendel formulated what is now known as Mendel's law of segregation (Regina, 2011a).

Parents	(♀) GG	x	(♂) gg
Phenotypes	Green	↓	Yellow
Gametes	(G)	↓	(g)
F_1 genotypes		Gg	
F_1 phenotypes		Green	
F_1 gametes		1/2 (G), 1/2 (g)	
F_2 genotypic ratio		1/4 GG : 2/4 Gg : 1/4 gg	
F_2 phenotypic ratio		3/4 green : 1/4 yellow	

2.5.2 Mendel's Law of Independent Assortment (Second Law of Mendel)

The Law of Independent Assortment, also known as "Inheritance Law" states that alleles of different genes assort independently of one another during gamete formation. While Mendel's experiments with mixing one trait always resulted in a 3:1 ratio between dominant and recessive phenotypes, his experiments with mixing two traits (di-hybrid cross) showed 9:3:3:1 ratios. But the 9:3:3:1 table shows that each of the two genes is independently inherited with 3:1 phenotypic ratios. Mendel concluded that different traits are inherited independently of each other, so that there is no relation (Trofim, 2011).

Mendel performed di-hybrid crosses in plants that were true-breeding for two traits. For example, a plant that had green pod color and yellow seed color was cross-pollinated with a plant that had yellow pod color and green seeds. In this cross, the traits for green pod color (GG) and yellow seed color (YY) are dominant. Yellow pod color (gg) and green seed color (yy) are recessive. The resulting offspring or F_1 generation was all heterozygous for green pod color and yellow seeds (GgYy). After observing the results of the di-hybrid cross, Mendel allowed all of the F_1 plants to self-pollinate. He referred to these offspring as the F_2 generation. Mendel noticed a 9:3:3:1 ratios. About 9 of the F_2 plants had green pods and yellow seeds, 3 had green pods and green seeds, 3 had yellow pods and yellow seeds and 1 had a yellow pod and green seed. In Mendel's experiment with pod color and seed color we see that the genotype or genetic makeup of the F_1 plants is GgYy. The phenotypes or expressed physical traits are green pod color and yellow seed color. Both of these traits are dominant. The F_2 generation pea plants show two different phenotypes for each trait. Pod color is either green or yellow and seed color is either yellow or green. There are nine different genotypes that result from this type of experiment. The F_2 generation genotypes and phenotypes can be seen in the image (Regina, 2011b).



2.5.3 Test-crossing Multi-hybrids (The Tri-hybrid Cross)

Mendel demonstrated that the identieal proeess of segregation and independent assortment apply to three pairs of contrasting traits in what is called a tri-hybrid cross, also referred to as a three-factor cross. Although a tri-hybrid cross is somewhat more complex than a di-hybrid eross, its results are easily calculated if the principles of segregation and independent assortment are followed (William and Michanel, 1997).

In our experiment example, a plant that had photoperiod sensitivity, tall plant and non-glutinous was cross-pollinated with a plant that had non-photoperiod sensitivity, short plant and glutinous. In this cross, the traits for photoperiod sensitivity (Hd1Hd1), tall (Sd1Sd1) and non-glutinous (WxWx) are dominant. Non-photoperiod sensitivity (hd1hd1), short (sd1sd1) and glutinous (wxwx) are recessive. The resulting offspring or F_1 generations were all heterozygous for photoperiod sensitivity, tall and non-glutinous (Hd1hd1Sd1sd1Wxwx genotype). The

resulting generation produces eight gamete types, when they self-pollinated, in turn produced twenty-seven different genotypes in a ratio of sixty-fours in the F_2 generations.

Parents	(♀) Hd1Hd1Sd1Sd1WxWx	x	(♂) hd1hd1sd1sd1wxwx
Phenotypes	Photoperiod sensitivity, tall and non-glutinous		Non-photoperiod sensitivity, short and Glutinous
Gametes	(Hd1Sd1Wx)	↓	(hd1sd1wx)
F_1 genotypes	Hd1hd1Sd1sd1Wxwx		
F_1 phenotypes	Photoperiod sensitivity, tall and glutinous		
F_1 gametes	1/8 (Hd1Sd1Wx), 1/8 (Hd1Sd1wx), 1/8 (Hd1sd1Wx), 1/8 (Hd1sd1wx), 1/8 (hd1Sd1Wx), 1/8 (hd1Sd1wx), 1/8 (hd1sd1Wx), 1/8 (hd1sd1wx)		

F_2 Genotypic ratio	F_2 Phenotypic ratio
27/64 Hd1-Sd1-Wx-	Photoperiod sensitivity, tall and non-glutinous
9/64 Hd1-Sd1-wxwx	Photoperiod sensitivity, tall and glutinous
9/64 Hd1-sd1sd1Wx-	Photoperiod sensitivity, short and non-glutinous
3/64 Hd1-sd1sd1wxwx	Photoperiod sensitivity, short and glutinous
9/64 hd1hd1Sd1-Wx-	Non-photoperiod sensitivity, tall and non-glutinous
3/64 hd1hd1Sd1-wxwx	Non-photoperiod sensitivity, tall and glutinous
3/64 hd1hd1sd1sd1Wx-	Non-photoperiod sensitivity, short and non-glutinous
1/64 hd1hd1sd1sd1wxwx	Non-photoperiod sensitivity, short and glutinous

2.6 Genetics of photoperiod respond *Hd1/hd1* gene

Flowering time is a major determinant for the local adaptation of crops. *Hd1* is a key flowering-time gene in rice and is orthologous to the Arabidopsis *CONSTANS* (*CO*) gene (Fujino *et al.*, 2010). Flowering time often referred to as heading date in cereal crops, is a key agronomical determinant for adaptation to specific cropping locations and growing seasons for current varieties of cultivated rice. Developing early-flowering or photoperiod insensitive cultivars has been a major objective of rice breeding for several decades (Tsuji *et al.*, 2008). The

molecular and developmental determinants of flowering time have thus also particularly important genetic targets for domestication or for breeding new varieties of rice. Flowering time is controlled by many genes, which are expressed or suppressed in close interaction with environmental factors such as day length and temperature. Progress in the molecular genetics has provided a clearer understanding of several pivotal mechanisms regulating flowering time extermination in rice, and these studies have been summarized from genetic, molecular biological, or comparative biological perspectives (Izawa, 2007a; Lee and An, 2007). Moreover, genetic analyses of flowering time in rice have been performed on mutants and natural variants. Several genes involved in the photoperiod response (photoperiod sensitivity) have been identified. A series of nearly isogeneic lines (NILs) for several photoperiod sensitivity genes have been developed to facilitate genetic analysis of flowering time in rice. However, the nature of the quantitative inheritance of flowering time has prevented us from performing more detailed analyses, including analysis of epistatic interactions and determination of chromosome locations of genes. In the last decade, the progress in development of DNA markers made QTL analysis possible to clarify the number and the nature of the genes controlling flowering time in rice (Yano and Sasaki, 1997).

Heading date is a quantitative trait important for the adaptation of rice to various growing environments and is controlled by at least 14 QTLs (Lin *et al.*, 2000; Yamamoto *et al.*, 2000). *Hd1*, a major QTL was found to be controlling heading date in rice was cloned from the short arm of chromosome 6 (Yano *et al.*, 2000). The *Hd1* gene is a homolog of *CONSTANS* (*CO*) in Arabidopsis, which functions in the photoperiodic control of flowering in this long-day plant (Putterill *et al.*, 1995).

The functional and nonfunctional alleles of *Hd1* were shown to be associated with early and late flowering, respectively, suggesting that *Hd1* is a major determinant of variation in flowering time of cultivated rice (Takahashi *et al.*, 2009). The function of *Hd1* probably is to affect transcription activation because of the presence of a zinc finger domain. Because *Hd1* transcription itself was not greatly affected by change in length of day. A nearly isogeneic line for *Hd1*, in which the Kasalath *Hd1* chromosomal region was substituted into the Nipponbare background. Non-functional alleles of *hd1* and other genes at QTLs for photoperiod response were combined. Analysis of epistatic interactions revealed that *Hd1* is epistatic to other

genes that enhance photoperiod response, such as *Hd2* and *Hd3*. These results suggest that *Hd1* plays a central role in the expression of photoperiod response under both short-day and long-day condition. However, near-isogenic lines containing *hd1* mutant alleles exhibited not only delayed flowering under short-day conditions but also early flowering under long-day conditions, indicating that *Hd1* could repress flowering under long-day that *Hd1* function is modified depending on day length (Lin *et al.*, 2000).

Cloning and sequencc analysis of *Hd1* by Yano *et al.* (2000) along with functional complementation experiments, confirmed that Nipponbare carries the functional *Hd1* allele. The recessive Kasalath allele *hd1* was found to contain numerous deletions and one insertion in the coding region compared with the Nipponbare allele. Experiments with various rice lines that are homozygous for either the functional *Hd1* or the mutant *hd1* allele showed that the presence of a functional *Hd1* allele was associated with early heading under short days but, interestingly, significantly later heading under long days. Also, *Hd1* expression appears to be unaffected by day length, although this tentative conclusion needs to be confirmed with more detailed expression analyses.

Studying on QTLs showed that photoperiod sensitivity in rice was found to be controlled by six loci: *Hd1, Hd2, Hd3a, Hd3b, Hd5* and *Hd6*, with *Hd1* being the major photoperiod sensitivity trait locus in rice (Yamamoto *et al.,* 2000; Lin *et al.,* 2000). The *Hd1* allele works in long-day lengths by delaying inflorescence in rice but in short-day lengths, *Hd1* allele promotes inflorescence. *Hd1* allele in a photosensitive variety of Nipponbare is a functional allele while *hd1* in non-photosensitive varieties of HS66, HS110 and Kasalath is considered a non-functional allele because gene mutation occurs (Yano *et al.,* 2000).

In another study, 6 QTLs such as *hd1, hd3, hd6a, hd6b, hd8,* and *hd12* were identified using F_2 population from a cross of two *indica* varieties (Lin *et al.*, 1996). The nomenclature *hd* was different from that reported by Yano and Sasaki (1997). However, it was difficult to compare the chromosomal position prccisely among these QTLs, and the allelic relationships between QTLs and know major genes remain unknown.

A great number of plants synchronize flowering with day length. In rice (*O. sativa* L.), photoperiod is the primary environmental that triggers flowering. Long-day and short-day photoperiod sensitive plants promote flowering based on a critical threshold related to the

proportion of diurnal hours that are experienced (Andres *et al.*, 2009). The short-day promotion of rice by *Hd1* plays a role in the field in tropical regions (Izawa, 2007a).

Fujino *et al.* (2010) analyzed the DNA sequences of *Hd1* among landraces and modern rice cultivars as well as accessions of *O. rufipogon* in order to examine how this gene has evolved during rice domestication. The results strongly indicated that introgression events of certain *Hd1* alleles, including defective alleles that originated from the selection of independent mutation events, seem to have contributed to the shaping and diversification of adaptation of landraces to specific areas. Furthermore, their findings suggest that *Hd1* loss-of-function may be a major requirement for the ecological habit of the *aus* cultivar group. They showed that sequences of 14 linked regions located within the 1.1 Mb chromosomal region surrounding *Hd1* were obtained from the rice core collection accessions. The lengths of the aligned sequences of these regions ranged from 528 to 801 bp, 10 of the 14 regions were from randomly selected genes (exons and introns) and the other four were from inter-genic sequences.

The sequence of the *Hd1* core segment was highly conserved in the genic regions, with 99% of the exons, 55 - 75% of the introns, and 60 - 94% of the untranslated regions being conserved with at least 80% identity. The average percent protein similarity was greater than percent nucleotide identity, suggesting conservation of putative protein function (Sanyal *et al.*, 2010).

The *Hd1* gene was sequenced in a set of 60 genetically diverse landraces and some modern cultivars termed the rice core collection. This population was collected from 19 different countries, mainly in Asia, and was selected to represent about 90% of the wide genetic diversity among rice cultivars based on a genome-wide RFLP analysis of 332 accessions from 23 countries (Kojima *et al.*, 2005).

Hd1 was first identified as the key flowering QTL between different rice subspecies, and a positional cloning approach revealed it to encode a single ortholog of Arabidopsis *CO* (Tsuji *et al.*, 2010). The highest expression levels of *Hd1* occur around 14 h after dawn, and may coincide either with daylight or with dark, depending on day length. Flowering inhibition exerted by *Hd1* under long-day conditions can be explained by a model similar to that proposed for Arabidopsis. During long-day, highest *Hd1* expression occurs during daylight hours, and therefore coincides with the presence of light-active phytochromes that could modify *Hd1* to act as an inhibitor of *Hd3a* transcription, thus inhibiting flowering (Turck *et al.*, 2008).

The *Hd1* in *japonica* is a dual-function gene. It is a floral inducer under short-day conditions and a floral repressor under long-day conditions (Yano *et al.*, 2000; Izawa *et al.*, 2002). Sanyal *et al.* (2010) compared a 155-kb reference segment of the *O. sativa* ssp. *japonica* cv. Nipponbare genome surrounding *Hd1*, a major heading date gene in rice, with orthologous regions from nine diploid *Oryza* species that diverged over a relatively short time frame to study sequence evolution around a domestieation locus. The orthologous *Hd1* region from *Sorghum bicolor* was included to compare and contrast the evolution in a more distant relative of rice.

2.7 Genetics of plant height *Sd1/sd1* gene

Plant height is one of the biological characteristic of rice. Semi-dwarfism is one of the most important traits. The rice semidwarf-1 (*sd1*) gene is well known as the "green revolution gene". This gene has contributed to the significant increase in crop production seen in the 1960s and 1970s, especially in Asia. This gene, originally derived from the Chinese cultivar Dee-Geo-Woo-Gen (DGWG), provides rice cultivar with short, thick culms, raises the harvest index, improves lodging resistance and responsiveness to nitrogen fertilizer, resulting high yields without affecting panicle and grain quality. *Sd1* has been introduced by conventional breeding procedures, but the importance of this gene makes the identification of high yield crops via genetic engineering. Several studies have reported that *sd1* is linked to certain trait or protein loci 2 - 5 and several molecular markers on chromosome 6 - 8 (Lisa *et al.*, 2002).

The *sd1* alleles have been used in many breeding programs for both *indica* and *japonica* varieties. Compare the plant height of some isogeneic lines of *sd1*. The isogeneic line carried the *sd1* allele from DGWG lower than that had the *sd1* allele of Reimei, suggesting that the DGWG *sd1* allele is stronger than that of Reimei. In general, since native *indica* varieties are taller than *japonica* varieties, the strong *sd1* alleles have been selected for producing new *japonica* varieties, including Jikkoku, Calrose 76 and Reimei. The results of molecular analysis indicate that rice breeders recognized the variations of dwarfism among the various *sd1* alleles and used the most suitable allele for producing new varieties with the desired height (Koshio *et al.*, 2000).

In recent years, the *sd1* semi-dwarf gene has been used extensively in U.S.A. public rice (*O. sativa* L.) breeding programs largely because the semi-dwarf plant type allows for

greater yields through higher nitrogen fertilization rates while reducing the susceptibility to lodging (McClung, 2003). Because of these characteristics, semi-dwarf cultivars have increased in popularity in the southern U.S.A. rice-growing region, are planted on a large percentage of the area, and have contributed to increased grain yield in the last 20 years (Tim *et al.*, 2008).

Dwarfism is an agronomical important trait in breeding for resistance to damage by wind and rain (lodging resistance) and for stable high yields via increases in harvest index (Khush, 2001). The two well-known dwarf genes that most significantly contributed to the history of crop breeding are semi-dwarf1 (*sd1*) in rice and reduces height1 (*Rht1*) in wheat also (Hedden, 2003). A dwarfing gene (allele) *sd1* has been intensively utilized to develop short-culms *indica* varieties in Southeast Asia up to now. Before the first *sd1* carrying variety IR8 was released, rice researchers had recognized the general tendency that culms length is higher in *indica* varieties than in temperate-*japonica* ones. Inter-subspecific difference of the tall (wild-type) allele *Sd1* at the *sd1* locus was examined on the common genetic background, using five isogonies' lines developed by substituting *sd1* of the recurrent parent IR36 by *Sd1* of two *indica* varieties, two temperate-*japonica* varieties and one tropical-*japonica* variety. Morcover, non-synonymous single-nucleotide polymorphism between the two subspecies was detected at two sites in Exon 1 and Exon 3 of the *sd1* locus. It is demonstrated that the inter-subspecific differentiation of *Sd1* contributes height difference between *indica* and *japonica*. The *indica*-originating and *japonica*-originating alleles at the *sd1* locus were designated as *Sd1-in*(t) and *Sd1-ja*(t), respectively (Murai *et al.*, 2010).

The gene *Sd1* (*OsGA20 oxidase*) is a key determinant of plant stature and played a key role during the Green Revolution. Located at 38.7 Mb on riee chromosome 1, it is part of the gibberellic acid pathway. The recessive allele, *sd1*, confers semi-dwarf stature and contributed to massive yield improvements throughout most of Asia by increasing the harvest index and helping to prevent lodging (Kashiwagi and Ishimaru, 2004). The *sd1* gene has been reported to reduce plant height by 25% through approximately proportional reductions in lengths of the top five internodes; with practically no effect on panicle length (Rutger, 1984). However, Ogim *et al.* (1993) reported that the *sd1* gene did not reduce the panicle length but rather increased the panicle number, while grain length and grain width were not affeeted. On the other hand, Kinoshita and Shinbashi (1982) reported that the *sd1* gene reduced the grain size.

As many as 61 dwarfing genes designated as *d1* to *d61* have been identified (Ashikari *et al.*, 2002). Even though many different dwarf accessions of mutant and spontaneous origin have been tried as alternate sources for developing semi-dwarf varieties, none other than *sd1* locus of DGWG source proved to be of practical value. Thirty-two dwarf accessions have screened for presence of DGWG allele of *sd1* gene and GA response. The dwarf accessions exhibited both DGWG allele of *sd1* gene and moderate response to GA treatment were considered to be allelic to *sd1* gene from DGWG and those behaving otherwise as alternates to DGWG allele of *sd1* gene. Of 32 dwarf accessions, it is surprising to note that, as many as 18 dwarf accessions showed the expansion of the deletion around the *sd1* gene ranging from 59.82 kb to 783.11 kb (including the *sd1* gene region) (Chirravuri, 2009). Moreover, Kinoshita (1990) shown that at least 60 dwarfing genes have been identified in rice. They are designated *d1* to *d60.* Of these, *d47*, or *sd1*, has been most widely used in rice breeding. Most of the others have been used as phenotypic markers in genetic studies, but rarely used in plant breeding. The semi-dwarf gene *sd1* is the best characterized of these and has been extensively used to produce high-yielding semi-dwarf varieties. The other dwarf or semi-dwarf genes have not been widely utilized in breeding programs because they are associated with poor agronomic performance. In addition, Asano *et al.* (2007) showed that of the 57 semi-dwarf varieties examined, 38 carried an *sd1* allele that probably controls their semi-dwarf phenotype. In total, they found the astonishing number of 6 different *sd1* alleles in the semi-dwarf varieties. These results clearly demonstrate that the *Sd1* mutations can produce an ideal architecture in rice and have been widely used to produce semi-dwarf phenotypes in both *japonica* and *indica* rice varieties.

Wolfgang *et al.* (2002) the *sd1* dwarfing gene was show to be tightly linked to restriction fragment length polymorphism (RFLP) markers *RG109* and *RG220* on the long arm of chromosome 1. They positioned RFLP markers *RG109* and *RG220* on a physical segment of chromosome 1 covered by a BAC consign of approximately 300 bp by using the physical map of Nipphonbare available on the RGP database. These markers were positioned near the ends of this consign spanning physical distance of approximately 262 kb. Anchor markers *C10419* and *S2523*, located within this region and separated by 192 kb, were mapped 1.9 cM apart on the high-resolution linkage map. From this chromosomal segment and linkage map, they estimated that the ratio of physical to genetic distance was approximately 100 kb/eM in this region. When combined

with data from the previous mapping studies, these result indicated that the *sd1* gene was located within a 262 kb interval flanked by markers *RG109* and *RG220*.

Since the late 1970s, most California varieties have been semi-dwarf or short stature. This is the result of breeding efforts that have incorporated different forms of the *Sd1* gene, which encodes an enzyme involved in the synthesis of the hormone gibberellins. These recessive forms of *Sd1* (referred to as *sd1* alleles) confer the semi-dwarf trait that enables high yields in response to fertilizers without lodging. In California, the two main sources of *sd1* are Calrose76 and DGWG (via the variety IR8). DNA markers that can differentiate the sources of semi-dwarf are available. Analysis of all the varieties released by the RES indicates that the IR8 allele of *sd1* is present in 15 of them (M9, M-201, L-202, M-202, APROJECT NO. RB-3 301, L-203, M-204, A-201, L-204, Calmati-201, L-205, M-205, M-208, L-206, and Calmati-202). In addition, DNA sequence analysis of the *sd1* gene from M-401 (an induced semi-dwarf mutant of the variety Terso) revealed that M-401 has a unique version of the *sd1* gene that is distinguishable from all previously known sources of *sd1*. Confirmation of the identity of the *sd1* alleles in the other semi-dwarf California varieties is pending although all of these varieties most likely contain the Calrose 76 source. Whether any of the different *sd1* alleles has an advantage over the others in terms of agronomic performance in the California environment is unknown (Thomas, 2006).

2.8 Genetics of endosperm amylose *Wx/wx* gene

Rice eating and cooking qualities are mainly influenced by the physical properties of its starch. Rice starch comprises 90% of the total dry weight of milled rice and has a great impact on the eating and cooking qualities. Rice starch is composed of two classes of polymers: amylose, a lightly branched linear molecule with a degree of polymerization of 1,000 – 5,000 glucose units, and amylopectin, a much larger polymer unit containing frequent a 1, 6 branching linkages (Jiang *et al.*, 2004). The rice quality mainly refers to appearance, cooking and eating qualities, and the parameters such as amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) are usually used to present eating and cooking qualities for rice. The proportion of amylose to amylopectin influences the structure and characteristics of the starch grain, whereas amylose content is the determinant to regulate the quality of rice. It has been indicated that AC is mainly determined by the allelic variance of *Wx* gene and the single nucleotide polymorphism (G or T) at the first

nucleotide of splice donor site of *Wx* gene intron 1, which affects the expression of *Wx* gene. If the first nucleotide of intron 1 is a G, the intron could be excised for external splicing normally and the expression level of mature mRNA is high, and then the AC is higher (Wan *et al.*, 2007).

Starch is mainly composed of two types of glucanpolymer, amylose and amylopectin. The gene, which controls amylose synthesis in endosperm and pollen, is called the Waxy (*wx*) gene. Amylose content, an important determinant of rice starch quality, is primarily controlled by the *wx* gene encoding Granule-Bound Starch Synthase (GBSS). *O. rufipogon*, known as the ancestor of Asian cultivated rice (*O. sativa* L.), is the most important wild relative for rice improvement (Prathepha, 2008). The starch of wild-type endosperm tissue consist of between 15-30% amylase and 70-85% amylopectin where endosperm starch is 100% amylopectin in most waxy mutants (Sano *et al.*, 1986).

Amylose content in rice has been reported to vary from 0 to 25%. Amylose content of 0-2, 3-9, 10-19, 20-25 and more than 25% have been specified as waxy, very low, low, intermediate and high amylose, respectively (Fitzgerald, 2004). Rice grains with low amylose levels are associated with tender, cohesive, glossy grains when cooked, while grains with higher amylose levels appear dry, fluffy and separated as cooked rice. Among non-waxy (or non-glutinous) rice cultivars, amylose content which affects grain quality also varies greatly. Waxy (or glutinous) phenotypes are controlled by a single recessive gene (*wx*) and the locus is responsible not only for the grain appearance but also for differences in amylose content in the endosperm among non-waxy (*Wx*) rice grain (Mikami *et al.*, 1999). Wang *et al.*, (1995) reported that amylose content (AC) in rice endosperm was related to the post-transcriptional regulation of the *Wx* gene. Genetic studies with rice revealed that a major gene on chromosome 6 and 5 and the major gene on chromosome 6 explained 91.1% of the total variation, it should be an allele of *Wx* and QTLs qAC-5 explained 11.8% of the total variation. However, it is suggested that the *japonica* types of *O.sativa* L. has comparatively lower amylose content than the *indica* types showing some overlapping. Further examinations are needed to know if differential regulation as found between Wx^a and Wx^b is related to intraspecific difference into the *japonica* and *indica* types.

As for the recessive allele *wx* existing in glutinous rice, there is an additional 23 bp sequence insertion in exon 2, at 108 bp site downstream the translation start site, when compared to the sequences of Wx^a and Wx^b allele. This 23 bp insertion results in the translation terminated

prematurely at the region 172 bp downstream in exon 2, and subsequently failing to the synthesis of normal GBSS (Wanchana *et al.,* 2003). The Waxy (*Wx*) gene encodes a GBSS that plays a key role in the amylose synthesis of rice and other plant species. Two functional *Wx* alleles of rice exist: Wx^a, which produces a large amount of amylose, and Wx^b, which produces a smaller amount of amylose because of the mutation at the 5′ splice site of intron 1. Wx^b is largely distributed in *japonica* cultivars, and high amylose cultivars do not exist in *japonica* cultivars (Itoh *et al.,*2003). In addition, rice Waxy gene (*Wx*) encodes a GBSS necessary for the synthesis of endosperm amylose. Although, the inheritance of amylose content is rather complicated, the GBSS alleles, *Wx* protein, and *Wx* gene expression were found to be highly correlated and associated with the variation of amylose content. Genetic studies have showed that the *Wx* gene consists of 13 exon with a 1.1 kb untranslated leader intron (Wang *et al.,* 1995; Umeda *et al.,* 1991). The waxy rice (*wx*) was first reported as a loss-of-function mutation in the gene Wx^a encoding GBSS which caused a low-amylose content (Wang, *et al.,* 1995). Molecular characterization of *wx* mutation, by uses of EMS-induction and gamma-ray-induction, revealed base substitutions in several exons and introns, and base deletion, in the *japonica* traditional cultivar 'Kinishita-mochi', showed that exon 2 had 23 bp duplication in the coding sequence (Inukai *et al.*, 2000).

Moreover, Sano (1984) reported that there are two functional alleles in non-waxy (*Wx*) rice, Wx^a and Wx^b, original defined by the different levels of *Wx* protein. It was also reported that Wx^a and Wx^b were *indica*- and *japonica*- specific, respectively (Sano *et al.,* 1986). Recently, sequence data of *Wx* alleles show that Wx^a has an AG<u>G</u>T sequence within a consensus donor site in the first intron, whereas Wx^b has an alternative AG<u>T</u>T sequence. This one-base substitution was attributed to a decreased splicing efficiency and lower amount of amylose in Wx^b cultivars (Hirano and Sano, 2000).

However, QTL analysis has demonstrated that apparent amylose content and paste viscosity parameters are mainly controlled by the *Wx* gene on chromosome 6, which encodes the granule bound starch synthase (Tian *et al.*, 2004).

To obtain basic information on the diversity of *wx* microsatellite alleles and on the relationship between *wx* microsatellite alleles and amylose classes. Bligh *et al.* (1995) reported first a polymorphic microsatellite (CT repeats) in the *Wx* gene. Later then, Ayres *et al.* (1997) reported that seven *wx* microsatellite alleles (n = 8, 11, 14, 17, 18, 19 and 20) and these were found to be correlated with variation in amylose content. Six *wx* microsatellite alleles (n =

10, 11, 14, 17, 18 and 20) were found in the non-glutinous rice samples showing correlation with amylose content (Bergman *et al.*, 2001). Bao *et al.* (2002) described four *wx* microsatellite alleles (n = 16, 17, 18 and 19) in Chinese glutinous rice cultivars. Recently, Prathepha and Baimai (2004) reported that four *wx* microsatellite alleles (n = 16, 17, 18 and 19) were found in glutinous Thai rice, whereas non-glutinous Thai rice has five *wx* microsatellite alleles (n = 11, 16, 17, 18 and 19).

CHAPTER 3

Materials and Methods

3.1 Plant materials

Plant materials used in this experiment consisted of RD 15 rice variety, two improved RD 6 rice lines, Chainat 80 rice variety, Sanpatong 1 rice variety and RD 10 rice variety.

RD 15 is a rice variety which was developed from KDML 105 rice variety through mutations using gamma radiation. RD 15 variety is photoperiod sensitive, tall and non-glutinous rice which carries homozygous dominant Hd1Hd1Sd1Sd1WxWx genotype. The qualities of cooked rice are soft, fragrant, good color quality, slender and long rice grain. It was used as the recipient parent.

Improved RD 6 line is a rice line which was developed by MAB in Maejo University as derived from Taichung 65 variety which is a non-functional non-photoperiod sensitive, *hd1* gene, and semi-dwarf, *sd1* gene from RD 1 rice variety as the donor parents with photoperiod sensitive, tall and glutinous RD 6 rice variety as the recipient parent. Improved RD 6 line is non-photoperiod sensitive, short and glutinous rice and carries homozygous recessive hd1hd1sd1sd1wxwx genotype. It was used as the donor parent.

Another improved RD 6 line with non-photoperiod sensitive, tall and glutinous rice: Chainat 80 variety with non-photoperiod sensitive, short and non-glutinous rice; Sanpatong 1 variety with non-photoperiod sensitive, short and glutinous rice and RD 10 variety with non-photoperiod sensitive, short and glutinous rice were used as the standard rice.

3.2 Markers analysis

Three markers used in this experiment were: *hd1* marker, *sd1* marker and *wx* marker. Where:

hd1 marker is specific for *Hd1/hd1* gene. This gene is located on chromosome 6 and controls photoperiod response. *Hd1* gene controlled photoperiod sensitive plants while *hd1* gene controlled non-photoperiod sensitive plants.

sd1 marker is specific for *Sd1/sd1* gene. This gene is located on chromosome 1 and controls plant height. *Sd1* gene controlled tall plants while *sd1* gene controlled short plants.

wx marker is specific for *Wx/wx* gene. This gene is located on chromosome 6 and controls endosperm amylose traits. *Wx* gene controlled non-glutinous seeds while *wx* gene controlled glutinous seeds.

3.3 DNA extraction and PCR

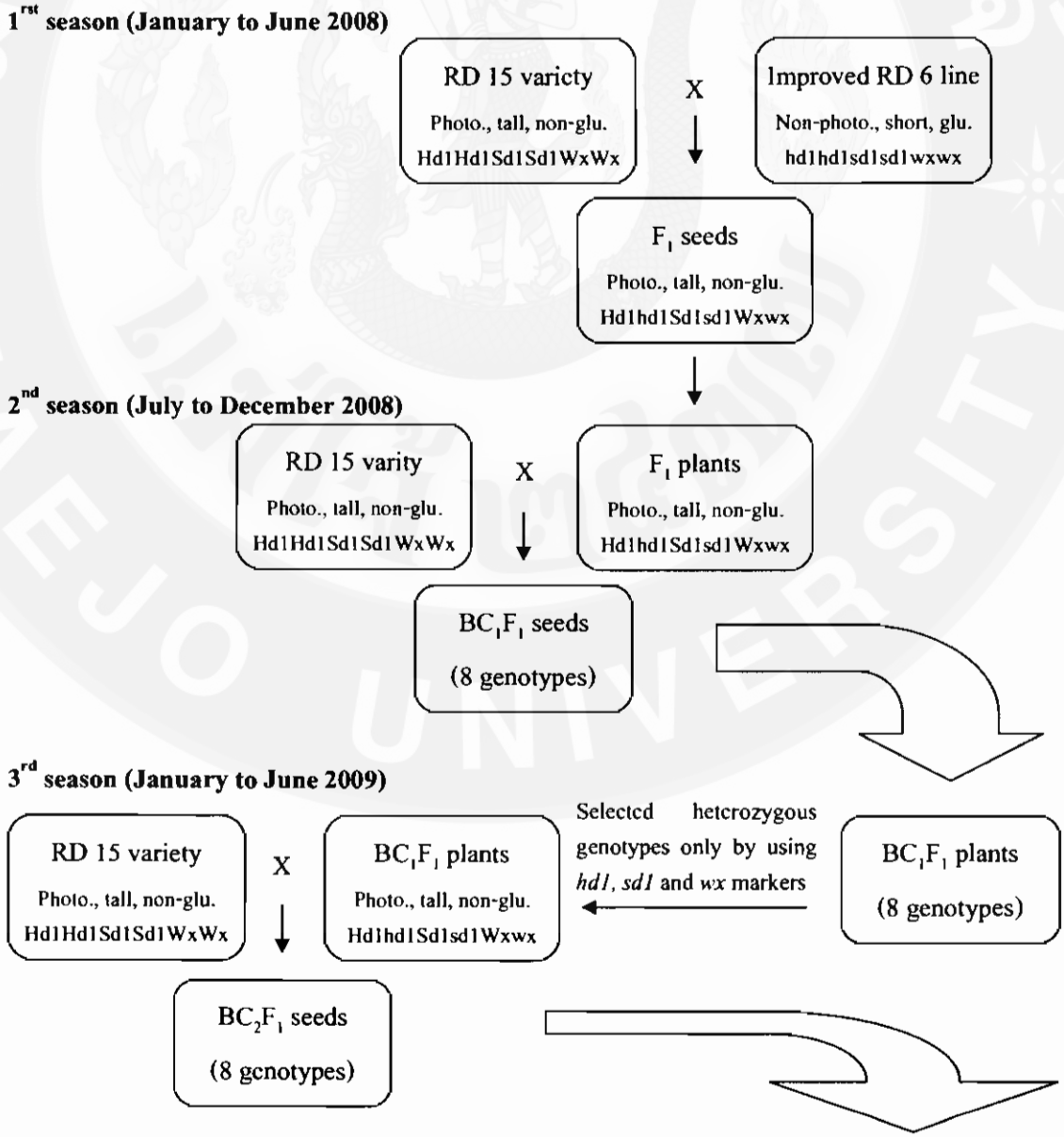
Pieces of young rice leaves were collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaves were ground after adding 75 µl of 10 mM Tris-HCL pH 8.0 and 133.3 µl of 2.5% Lysis Solution. The mixture was incubated at 65°C for at least 5 minutes (if a frozen sample is used, lysis solution should be added before thawing and incubated at 65°C for 10 minutes with occasional inverting the tube) and taken out and shaken for 20 times. After that the mixtures were incubated again in five minutes, taken out and shaken for 10 times. Immediately, 200 µl of chloroform was added, gently emulsified by inversion (3-5 times) and centrifuged the sample at 12,000 rpm for four minutes. The 120 µl of supernatant part containing DNA was transferred to a new tube. Then 266.7 µl of precipitation solutions were added and mixed gently by several inversions at room temperature for one to two minutes (shake about 80 times) and centrifuged at 12,000 rpm for five minutes. Supernatant was removed completely (do not dry) and DNA pellet was dissolved in 33.3 µl of 1.2 M NaCl solutions by gentle overtaxing (make sure that pellet is completely dissolved). Then 100 µl of absolute ethanol was added letting the DNA to precipitate (at least 20 minute at minus 20°C) and centrifuged (10,000 rpm for 3-4 minutes). The ethanol was poured off. The DNA pellet was washed once with 100 µl of 75% cold ethanol. The sample was centrifuged again at 12,000 rpm for 4 minutes. Then, it was taken out and air-dried for 20 – 30 minutes until the tubes dried up. Next, 25-35 µl of 10mM Tris-HCL pH 8.0 was added to dissolve and DNA was prepared for doing PCR (Follow Fermentas life sciences- www.fermentas.com).

The PCR reactions were performed using in the thermal cycle following profile: Pre-denature 94 °C for 3 minutes, denature 94 °C for 30 seconds, annealing 55 °C for 1 minute and extension 72 °C for 2.5 minutes. Repeat denature, annealing and extension for 35 cycles and final extension 72 °C for 5 minutes. PCR products were analyzed by electrophoresis in ethidium bromide stained 4.0% agarose gels, run 100V for 2.30 hour (for *wx* marker) and 3.0% agarose

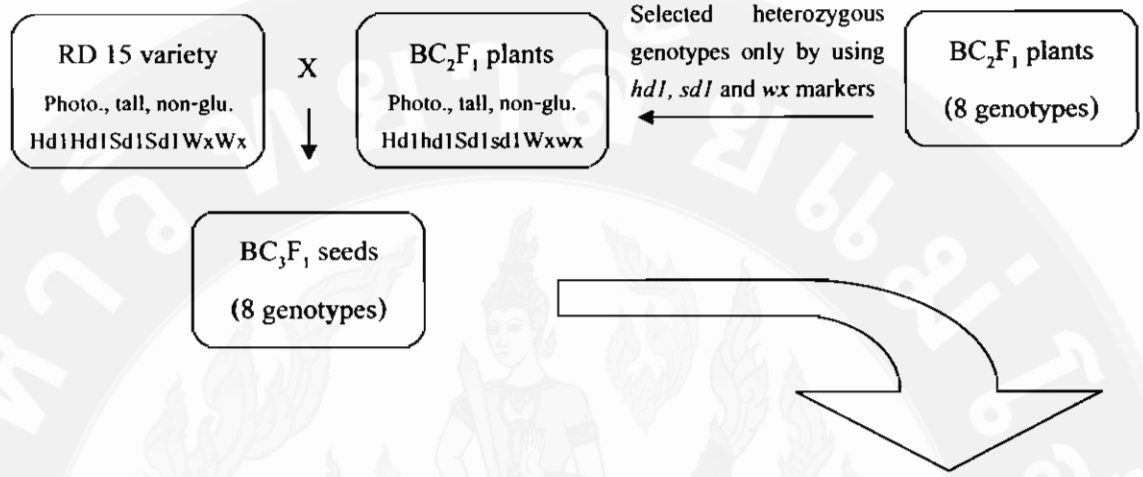
gels run 100V for 1.45 hours (for *hd1* and *sd1* markers). A 100 bp ladder molecular weight standard was used to estimate PCR fragment size.

3.4 Molecular marker assisted backcrossing (MAB)

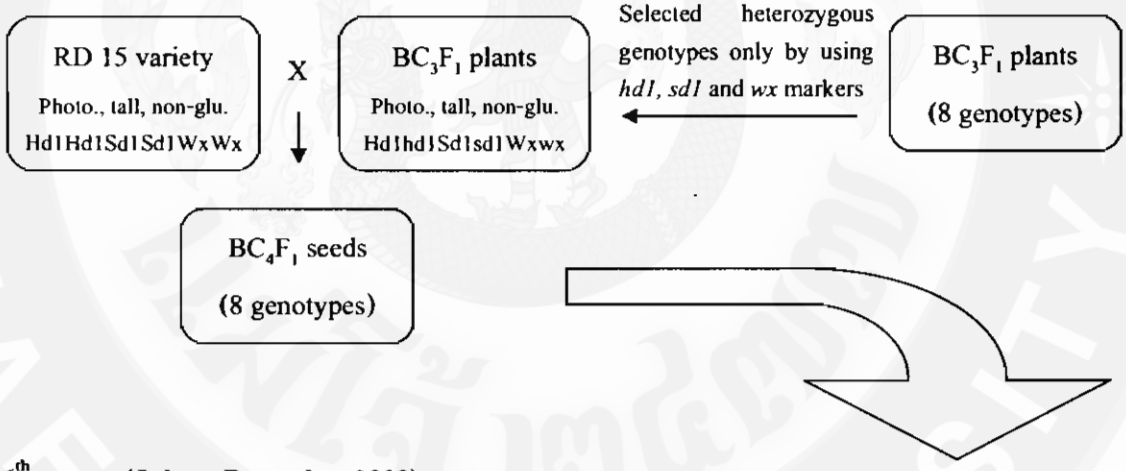
Molecular marker assisted backcrossing (MAB) was applied in this experiment by using RD 15 variety (photoperiod sensitive, tall and non-glutinous with Hd1Hd1Sd1Sd1WxWx genotype) as the recipient parent to cross with improved RD 6 line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype) as the donor parent to generate BC_5F_1 seeds through 6 seasons:



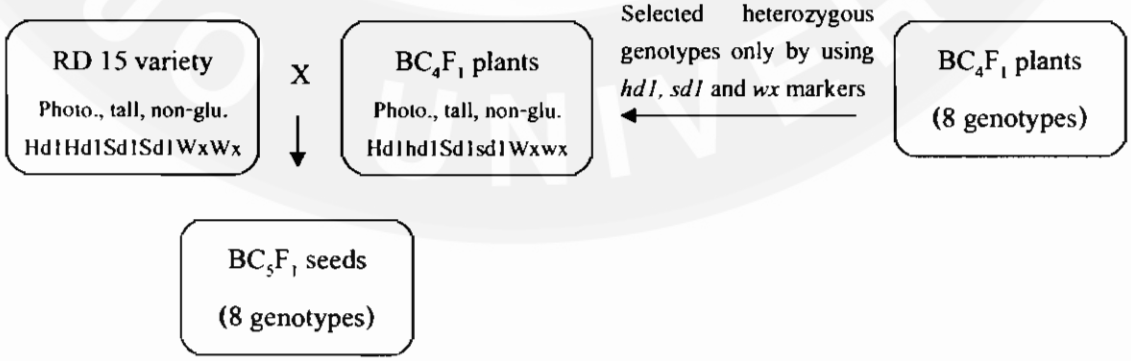
4th season (July to December 2009)



5th season (January to June 2010)



6th season (July to December 2010)



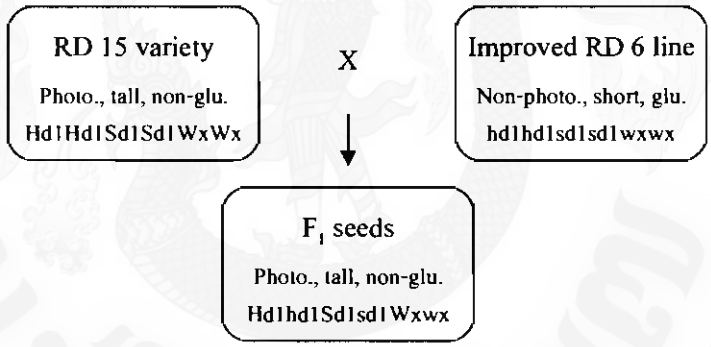
3.5 Number of experiments

This thesis was started in the rainy season from July to December 2009 (4th season) to produce BC_3F_1 seeds and BC_2F_2 seeds until BC_5F_1 seeds and BC_4F_2 seeds.

3.5.1 Experiment 1: Produced BC_3F_1 seeds to BC_5F_1 seeds in a greenhouse

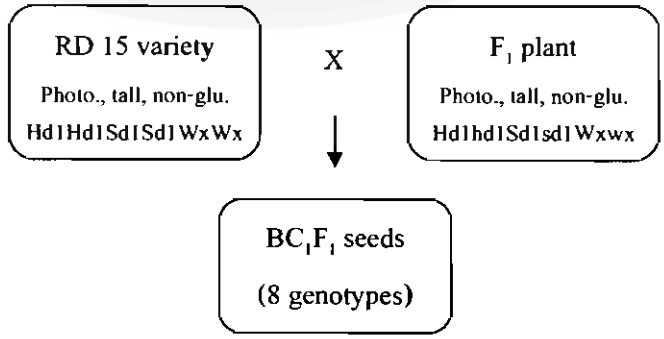
3.5.1.1 Produced F_1 seeds in a greenhouse during the dry season from January to June 2008 (1rst season)

In this season, RD 15 variety and improved RD 6 line were grown in a greenhouse, using RD 15 variety as the recipient parent to cross with improved RD 6 line as the donor parent to generate F_1 seeds.



3.5.1.2 Produced BC_1F_1 seeds in a greenhouse during the rainy season from July to December 2008 (2nd season)

In this season, F_1 seeds which carried heterozygous Hd1hd1Sd1sd1Wxwx genotype only were produced in the first season then grown in a greenhouse together with RD 15 variety. Using F_1 plants as the males parent to backcross with RD 15 plants to generate BC_1F_1 seeds.



3.5.1.3 Produced BC_2F_1 seeds and BC_1F_2 seeds in a greenhouse during the dry season from January to June 2009 (3^{rd} season)

In this season, the BC_1F_1 seeds carried eight genotypes which were produced in the second season and then grown in greenhouse together with RD 15 variety.

From eight genotypes of BC_1F_1 plants:

(1)Hd1Hd1Sd1Sd1WxWx

(2)Hd1Hd1Sd1Sd1Wxwx

(3)Hd1Hd1Sd1sd1WxWx

(4)Hd1Hd1Sd1sd1Wxwx

(5)Hd1hd1Sd1Sd1WxWx

(6)Hd1hd1Sd1Sd1Wxwx

(7)Hd1hd1Sd1sd1WxWx

(8)Hd1hd1Sd1sd1Wxwx

Four heterozygous Hd1hd1 genotypes only were detected by using *hd1* marker that specific for *Hd1/hd1* gene and they were as follows:

(1)Hd1hd1Sd1Sd1WxWx

(2)Hd1hd1Sd1Sd1Wxwx

(3)Hd1hd1Sd1sd1WxWx

(4)Hd1hd1Sd1sd1Wxwx

These four BC_1F_1 plants were then detected for heterozygous Sd1sd1 genotype only by using *sd1* marker that specific for *Sd1/sd1* gene and they were as follows:

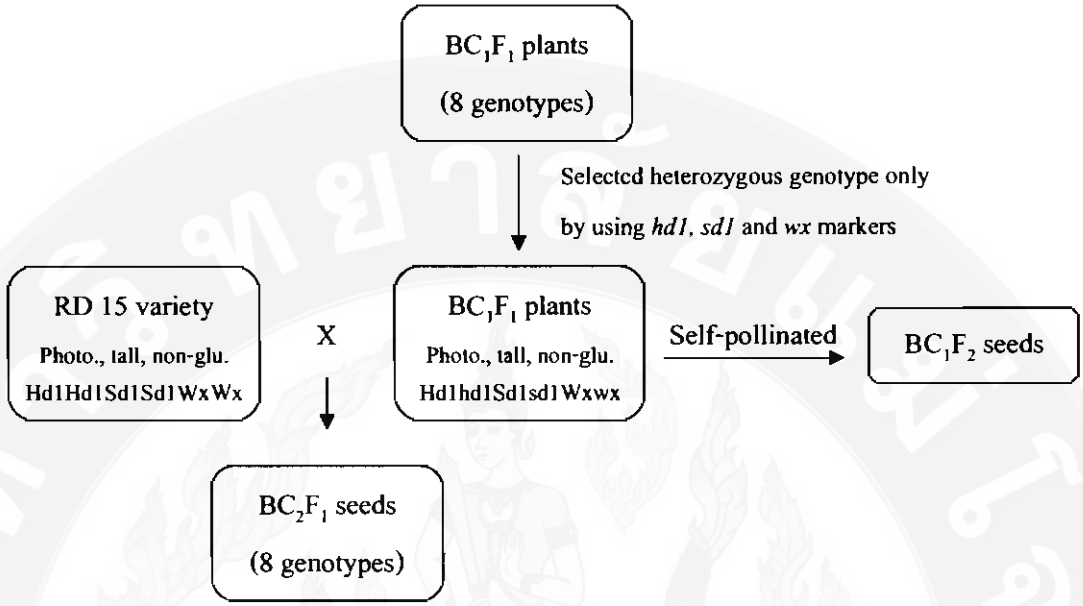
(1)Hd1hd1Sd1sd1WxWx

(2)Hd1hd1Sd1sd1Wxwx

These two BC_1F_1 plants were later detected for heterozygous Wxwx genotype only by using *wx* marker that specific for *Wx/wx* gene and it was as follows:

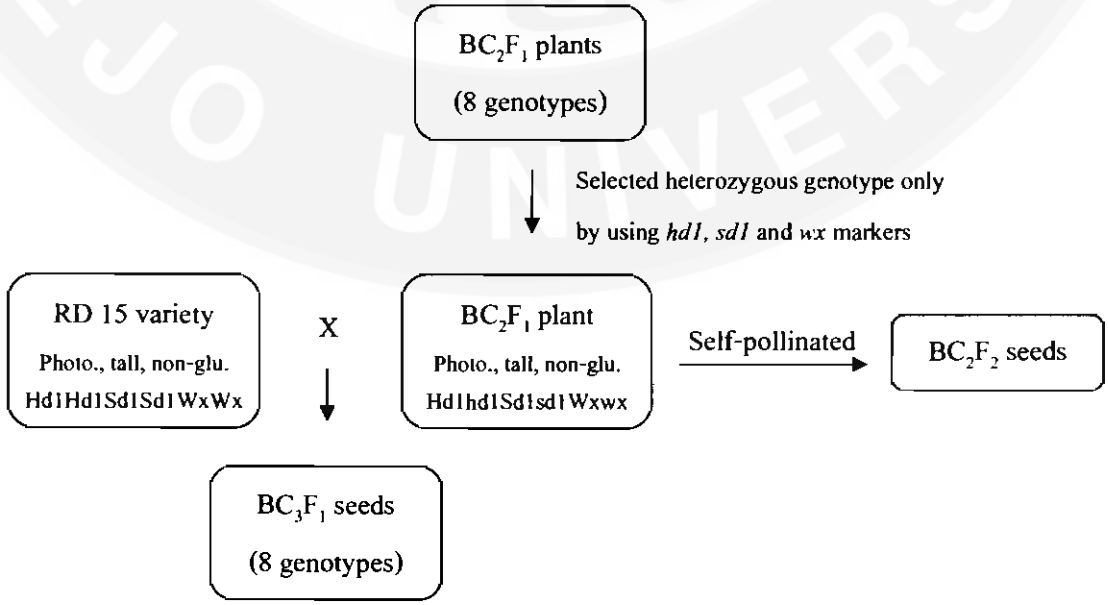
(1)Hd1hd1Sd1sd1Wxwx

The BC_1F_1 plants that carried heterozygous Hd1hd1Sd1sd1Wxwx genotype only were later used as the males parent to backcross again RD 15 variety to generate BC_2F_1 seeds. At the same time, the BC_1F_1 plants self-pollinated to BC_1F_2 seeds.



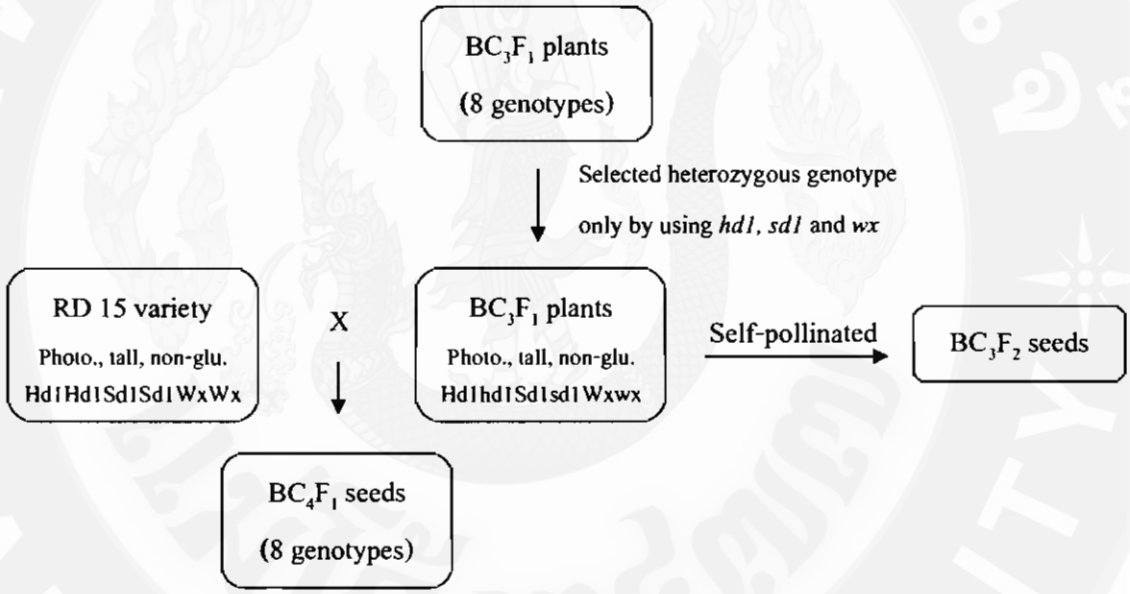
3.5.1.4 Produced BC_3F_1 seeds and BC_2F_2 seeds in a greenhouse during the rainy season from July to December 2009 (4^{th} season)

In this season, BC_2F_1 seeds which produced in the third season carried eight genotypes were grown in a greenhouse together with RD 15 variety. From eight genotypes of BC_2F_1 plants, one heterozygous Hd1hd1Sd1sd1Wxwx genotype only was detected by using *hd1*, *sd1* and wx markers. These BC_2F_1 plants were then backcrossed again to RD 15 variety to generate BC_3F_1 seeds. At the same time, the BC_2F_1 plants self-pollinated to generate BC_2F_2 seeds.



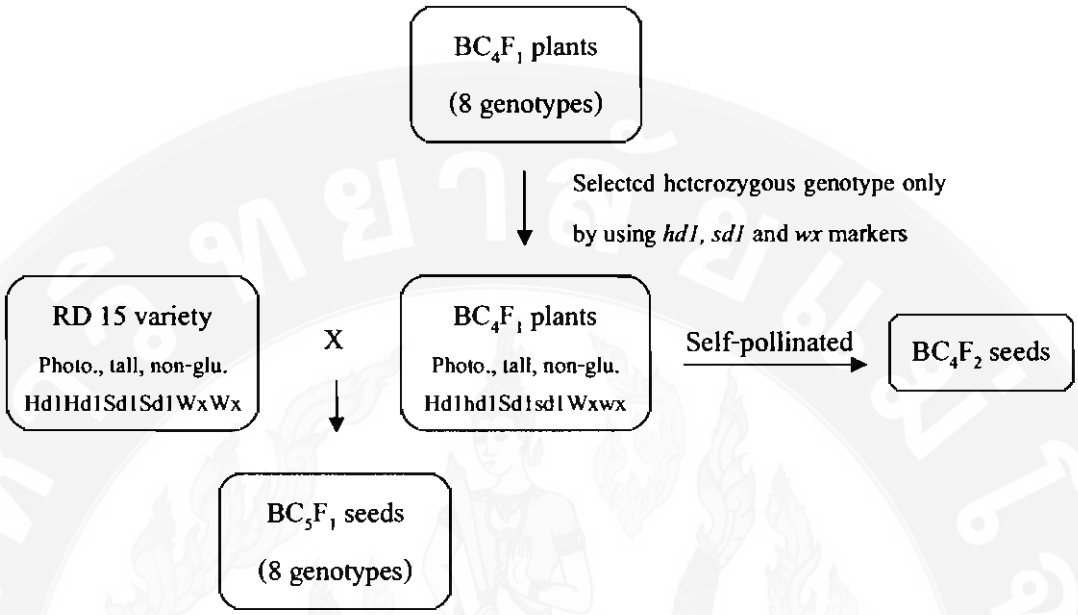
3.5.1.5 Produced BC_4F_1 seeds and BC_3F_2 seeds in a greenhouse during the dry season from January to June 2010 (5^{th} season)

In this season, BC_3F_1 seeds whieh produced in the fourth season earried eight genotypes were then grown in a greenhouse together with RD 15 variety. From eight genotypes of BC_3F_1 plants, one heterozygous Hd1hd1Sd1sd1Wxwx genotype only was deteeted by using *hd1*, *sd1* and *wx* markers. These BC_3F_1 plants were then used as the males parent to backcross again RD 15 variety to generate BC_4F_1 seeds. At the same time, the BC_3F_1 plants self-pollinated to generate BC_3F_2 seeds.



3.5.1.6 Produced BC_5F_1 seeds and BC_4F_2 seeds in a greenhouse during the rainy season from July to December 2010 (6^{th} season)

In this season, BC_4F_1 seeds which produeed in the fifth season carried eight genotypes were grown in a greenhouse together with RD 15 variety. From eight genotypes of BC_4F_1 plants, one heterozygous Hd1hd1Sd1sd1Wxwx genotype only was deteeted by using *hd1*, *sd1* and *wx* markers. These BC_4F_1 plants were later used as the males parent to baekcross again RD 15 variety to generate BC_5F_1 seeds. At the same time, the BC_4F_1 plants self-pollinated to generate BC_4F_2 seeds.


BC4F1 plants
(8 genotypes)
Selectcd hctcrozygous genotype only
by using hd1, sd1 and wx markers
RD 15 variety
Photo., tall, non-glu.
Hd1Hd1Sd1Sd1WxWx
X
BC4F1 plants
Photo., tall, non-glu.
Hd1hd1Sd1sd1Wxwx
Self-pollinated
BC4F2 seeds
BC5F1 seeds
(8 genotypes)

3.5.2 Experiment 2: Selected four best lines of RD 15 with three markers and tested them for photoperiod response under long-day condition of light exposure for 14 hours per day

3.5.2.1 Selected four best lines of RD 15 with three markers: *hd1, sd1* and *wx* in field and laboratory during the dry season from January to June 2010

To select the four best lines of RD 15 rice variety, about 2,000 BC_2F_2 rice seeds were grown in the field of Maejo University, Chiang Mai during long-day condition from January to June 2010 of the fifth season. The plants which had flowering considered non-photoperiod sensitive were then selected for 150 tall and 150 short plants. Their DNA was then extracted, PCR and gel were later done by using three markers as *hd1, sd1* and *wx* that specific for *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes, respectively to detect the plants that had homozygous hd1hd1Sd1Sd1WxWx, hd1hd1Sd1Sd1wxwx, hd1hd1sd1sd1WxWx and hd1hd1sd1sd1wxwx genotypes corresponded to theirs phenotypes of non-photoperiod sensitive, tall and non-glutinous rice; non-photoperiod sensitive, tall and glutinous rice; non-photoperiod sensitive, short and non-glutinous rice; and non-photoperiod sensitive, short and glutinous rice, respectively.

To determine the phenotypes of BC_2F_2 plants, three characters were looked as followed: non-flowering or flowering plants for photoperiod response plants; tall or short plants for plant height; and non-glutinous or glutinous seeds for rice endosperms traits.

From these detected BC_2F_2 plants, four best BC_2F_2 plants were selected with corresponding of phenotypes and genotypes as follows:

No.	Phenotypes	Genotypes
1	**NTN** (Non-photoperiod sensitive, tall and non-glutinous)	hd1hd1Sd1Sd1WxWx
2	**NTG** (Non-photoperiod scnsitive, tall and glutinous)	hd1hd1Sd1Sd1wxwx
3	**NSN** (Non-photoperiod sensitive, short and non-glutinous)	hd1hd1sd1sd1WxWx
4	**NSG** (Non-photoperiod sensitive, short and glutinous)	hd1hd1sd1sd1wxwx

These selected four best BC_2F_2 plants were then self-pollinated to generate BC_2F_3 seeds. These BC_2F_3 seeds were later used to check for photoperiod response under light exposure

of 14 hours per day and yield trial them in the field during the rainy season from July to December 2010 (6^{th} season).

BC_2F_2 plants $\xrightarrow{\text{Self-pollinated}}$ BC_2F_3 seeds

These BC_2F_3 seeds were grown in the field and became BC_2F_3 plants. These plants were now called by the name of the selected four best lines of RD 15, they were:

(1) RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype)

(2) RD 15 NTG line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype)

(3) RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype)

(4) RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype)

3.5.2.2 Tested for photoperiod response of the selected four best lines of RD 15 by growing them in a greenhouse and exposed to light for 14 hours per day from August 2010 to March 2011

To test photoperiod response of the selected four best lines of RD 15, they were grown in a greenhouse from August 2010 to March 2011 together with RD 15 variety, improved RD 6 line and Chainat 80 variety as the control rice under long-day length condition of light exposure for 14 hours per day. These lines were later checked for phenotyping by recording the inflorescence date of 50% and compared with the original RD 15 variety, improved RD 6 line and Chainat 80 variety. If the selected four best lines of RD 15 carried homozygous recessive hd1hd1 genotype were flowering under long-day condition of light exposure for 14 hours per day, meaning that they were non-photoperiod sensitive.

3.5.3 Experiment 3: Studied yield and yield components of the selected four best lines of RD 15 in the field during the rainy season from July to December 2010

This experiment was conducted in the field of Maejo University, Chiang Mai 50290 during the rainy season from July to December 2010 and using Randomized Complete Block Design (RCBD) in three replications with 10 treatments: (1) RD 15 variety (photoperiod sensitive, tall and non-glutinous with Hd1Hd1Sd1Sd1WxWx genotype), (2) RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype), (3) RD 15 NTG line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype), (4) RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype), (5) RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype), (6) improved RD 6 line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype), (7) improved RD 6 line (non-photoperiod sensitive, tall and glutinous), (8) Chainat 80 variety (non-photoperiod sensitive, short and non-glutinous), (9) Sanpatong 1 variety (non-photoperiod sensitive, short and glutinous), and (10) RD 10 variety (non-photoperiod sensitive, short and glutinous). Each plot consisted of four rows with one seedling per hill, 21 seedlings per row and had a planting density 25 cm between plants (within a row) and 25 cm between rows. In all experiments, fertilizer was applied at the rate of 16-20-0 NPK with 20 kg/rai (1 rai = 0.16 ha) at the same time of transplanting and 46-0-0 NPK with 15 kg/rai after one month of transplanting. Data were later collected for yield, yield components and seeds physically characteristics of paddy and brown rice grain.

Yield and yield components were collected for

- Date of flowering (date): Date of flowering was recorded when 50% of the individual plants in each plot had flowering.

- Age to 50% flowering (days): Age to 50% flowering was calculated from date of grow to date of 50% flowering.

- Plant height (cm): Plant height was measured at maturity stage as the distance from the soil surface to the top of the highest panicle, excluding awns.

- Number of tillers per hill (tillers): The total number of tillers per hill was counted when the plants had maximum tillers.

- Number of panicles per hill (panicles): The total number of panicles per hill was counted at maturity stage.

- Number of seeds per panicle (seeds): The total number of seeds per panicle was counted on 10 panicles of each plot and then separated for good seeds and unfilled seeds

- Fertility (%): The fertility was calculated by formula:

Fertility (%) = (good seeds x 100) / total seeds

- Grain yield (kg/rai): To determine yield in each plot, only 38 hills inner two rows were used. Two border rows and the border plants of each row were discarded. Grain yield of each plot was adjusted to 14% moisture content and extrapolated to kg/rai.

- Traits of plant height, number of tillers per hill and number of panicles per hill were measured at maturity stage and based on 10 individual plants (IRRI, 2002).

Physical characteristics of paddy and brown rice grains were measured for

- Width of rice grain (mm)

- Length of rice grain (mm)

- Thickness of rice grain (mm)

The size of paddy and brown rice grains were measured by 10 seeds per plot.

- Percentage of humidity (%): 250 grams of seeds was weighed and the percentage of humidity of seeds was determined by a moisture machine.

- Weight of 1,000 seeds (g): 1,000 seeds of each plots were counted at 14% moisture and then weighed on an electric scale in the laboratory.

Statistical analysis

The data were statistically analyzed using Sirichai 6.0 software program recommended for ANOVA of randomized complete block design (RCBD). Mean for different traits of 10 treatments were separated using least-significanee difference (LSD) test at 5% and 1% probability level by Duncan's multiple range test (DMRT).

3.5.4 Experiment 4: Studied inheritance of *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes using markers in BC_3F_2 population planting under long-day condition of light exposure for 14 hours/day

To study the inheritance of *Hd1/hd1, Sd1/sd1* and *Wx*/wx genes in BC_3F_2 population, 150 seeds of BC_3F_2 population which was produced by selfing from BC_3F_1 plants in the dry season from January to June 2010 (5^{th} season) were used to unhusk for giving phenotype of non-glutinous and glutinous seeds endosperm and then tested by chi-square test (χ^2) if they followed the Mendelian Laws of Heredity or not. These seeds were later planted in a greenhouse under long-day condition exposed to light for 14 hours per day from August 2010 to March 2011 together with RD 15 variety, improved RD 6 line and Chainat 80 variety as the control rice for determining the genotypes and phenotypes of BC_3F_2 plants.

To determine the genotypes of 150 BC_3F_2 plants, their DNA were extracted, PCR was then done by using three markers as *hd1, sd1* and *wx* that specific for *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes, respectively. Latterly, gel was run and gel doc picture was taken. The result showed that if the DNA bands of BC_3F_2 plants on the gel picture show the same size as DNA band of RD 15 variety, meaning that they were homozygous dominant Hd1Hd1Sd1Sd1WxWx genotype. By contrast, if the DNA bands of BC_3F_2 plants on the gel picture show the same size as DNA band of improved RD 6 line, meaning that they were homozygous recessive hd1hd1sd1sd1wxwx genotype, but if the DNA bands of BC_3F_2 plants on the gel picture show the same size as DNA bands of both RD 15 variety and improved RD 6 line, meaning that they were heterozygous Hd1hd1Sd1sd1Wxwx genotype. Chi-square test (χ^2) was finally applied to test whether they followed the Mendelian Laws of Heredity or not.

To determine the phenotypes of BC_3F_2 plants, 150 BC_3F_2 plants were checked for photoperiod response plants under long-day length of light 14 hours per day to give photoperiod sensitive and non-photoperiod sensitive plants, and later measured plant height for giving tall and short plants. After three months, if the plants were flowering that means they were non-photoperiod sensitive plants but the plants were not flowering that means they were photoperiod sensitive plants. Plant height was determined by measuring plant height of flowering plants as the distance from the soil surface to the top of the highest panicle and non-flowering plants as the distance from the soil surface to the top of the highest leaf with the same time. The plants were tall if theirs plant height

were more than 110 cm while the plants were short if theirs plant height were less than 110 cm. These data were then tested by chi-square (χ^2) as followed the Mendelian Laws of Heredity or not.

After six months, the electric light was turned off and all of photoperiod sensitive BC_3F_2 plants would be flowering and self-pollinated to generate BC_3F_3 seeds. When the seeds matured, 10 seeds of each individual plans were unhusked for giving non-glutinous and glutinous seeds phenotype. The seeds were non-glutinous when theirs endosperm grain appearance were translucence, while the seeds were glutinous when theirs endosperm grain appearance were chalkiness. Data was then recorded with the corresponding of phenotypes and genotypes, respectively and followed as:

Characters	Observation	Phenotypes	Genotypes
Photoperiod response	Non-flowering	Photoperiod sensitivity	Hd1Hd1 and Hd1hd1
	Flowering	Non-photoperiod sensitivity	hd1hd1
Plant height	More than 110 cm high	Tall	Sd1Sd1 and Sd1sd1
	Less than 110 cm high	Short (Semi-dwarf)	sd1sd1
Endosperm traits	Translucence	Non-glutinous	WxWx and Wxwx
	Chalkiness	Glutinous	wxwx

The chi-square formula used in these data is:

$$\chi^2 = \frac{(O - E)^2}{E}$$

Where:

- χ^2 is chi-square test.
- O is the observed frequency in each category.
- E is the expected frequency in the corresponding category.
- Degrees of freedom (df): df = (n-1) where, n is number of classes in data
- The level of significance is set at 0.05 (the standard for most science experiments).

CHAPTER 4

Results and Discussion

This study was done in four experiments:

1. Produced BC_3F_1 seeds to BC_5F_1 seeds in a greenhouse

1.1 Produced BC_3F_1 seeds and BC_2F_2 seeds in a greenhouse during the rainy season from July to December 2009

1.2 Produced BC_4F_1 seeds and BC_3F_2 seeds in a greenhouse during the dry season from January to June 2010

1.3 Produced BC_5F_1 seeds and BC_4F_2 seeds in a greenhouse during the rainy season from July to December 2010

2. Selected four best lines of RD 15 with three markers and tested them for photoperiod response under long-day condition of light exposure for 14 hours per day

2.1 Selected four best lines of RD 15 with three markers: *hd1*, *sd1* and *wx* in field and laboratory during the dry season from January to June 2010

2.2 Tested for photoperiod response of selected the four best lines of RD 15 by growing them in a greenhouse and exposed to light for 14 hours per day from August 2010 to March 2011

3. Studied yield and yield components of the selected four best lines of RD 15 in the field during the rainy season from July to December 2010

4. Studied inheritance of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes using markers in BC_3F_2 population planting under long-day condition of light exposure for 14 hours per day

4.1 Inheritance of *Hd1/hd1* gene in BC_3F_2 population by using *hd1* marker which was part of *Hd1/hd1* gene

4.2 Inheritance of *Sd1/sd1* gene in BC_3F_2 population by using *sd1* marker which was part of *Sd1/sd1* gene

4.3 Inheritance of *Wx/wx* gene in BC_3F_2 population by using *wx* marker which was part of *Wx/wx* gene

4.4 Inheritance of *Hd1/hd1* and *Sd1/sd1* genes in BC_3F_2 population by using *hd1* and *sd1* markers which were part *Hd1/hd1* and *Sd1/sd1* genes, respectively

4.5 Inheritance of *Hd1/hd1* and *Wx/wx* genes in BC_3F_2 population by using *hd1* and *wx* markers which were part of *Hd1/hd1* and *Wx/wx* gene, respectively

4.6 Inheritance of *Sd1/sd1* and *Wx/wx* genes in BC_3F_2 population by using *sd1* and *wx* markers which were part of *Sd1/sd1* and *Wx/wx* gene, respectively

4.7 Inheritance of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes in BC_3F_2 population by using *hd1*, *sd1* and *wx* markers which were part of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes, respectively

4.1 Produced BC_3F_1 seeds to BC_5F_1 seeds in the greenhouse

4.1.1 Produced BC_3F_1 seeds and BC_2F_2 seeds in the greenhouse during the rainy season from July to December 2009

RD 15 rice variety had homozygous dominant Hd1Hd1Sd1Sd1WxWx genotype, while improved RD 6 rice line had homozygous recessive hd1hd1sd1sd1wxwx genotype. Molecular marker assisted backcrossing was applied in two backcrosses by crossing RD 15 rice variety with improved RD 6 rice line, using RD 15 rice variety as a recipient parent and improved RD 6 rice line as a donor parent to generate BC_2F_1 seeds through pass generations. Three BC_2F_1 plants were selected for heterozygous Hd1hd1Sd1sd1Wxwx genotype only by using *hd1, sd1* and *wx* markers that specific for *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes, respectively (Figure 1-3).

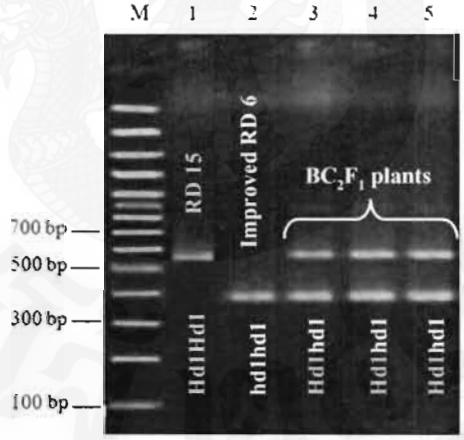


Figure 1 Shows a sample gel photograph under UV light to observe DNA banding size of BC_2F_1 plants and their parents produced from PCR product when using *hd1* marker as a primer that is specific for *Hd1/hd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Hd1Hd1 genotype, lane 2 indicated improved RD 6 line with hd1hd1 genotype and lanes 3-5 indicated BC_2F_1 plants with Hd1hd1 genotype.

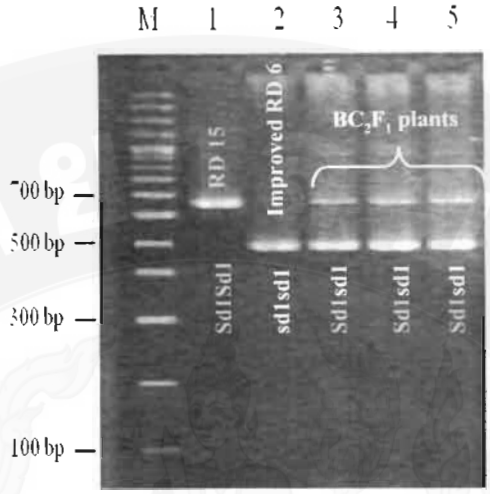


Figure 2 Shows a sample gel photograph under UV light to observe DNA banding size of BC_2F_1 plants and their parents produced from PCR product when using *sd1* marker as a primer that is specific for *Sd1/sd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Sd1Sd1 genotype, lane 2 indicated improved RD 6 line with sd1sd1 genotype and lanes 3-5 indicated BC_2F_1 plants with Sd1sd1 genotype.

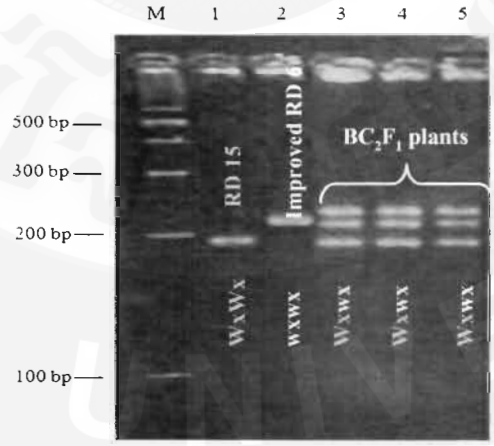


Figure 3 Shows a sample gel photograph under UV light to observe DNA banding size of BC_2F_1 and their parents produced from PCR product when using *wx* marker as a primer that is specific for *Wx/wx* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with WxWx genotype, lane 2 indicated improved RD 6 line with wxwx genotype and lanes 3-5 indicated BC_2F_1 plants with Wxwx genotype.

These three plants were then used as male parents to backcross again RD 15 variety to produce BC_3F_1 seeds. The results indicated that 74 BC_3F_1 seeds were produced with eight genotypes: Hd1Hd1Sd1Sd1WxWx, Hd1Hd1Sd1Sd1Wxwx, Hd1Hd1Sd1sd1WxWx, Hd1Hd1Sd1sd1Wxwx, Hd1hd1Sd1Sd1WxWx, Hd1hd1Sd1Sd1Wxwx, Hd1hd1Sd1sd1WxWx and Hd1hd1Sd1sd1Wxwx. At the same time, three BC_2F_1 plants self-pollinated to generate BC_2F_2 seeds (Figure 4).

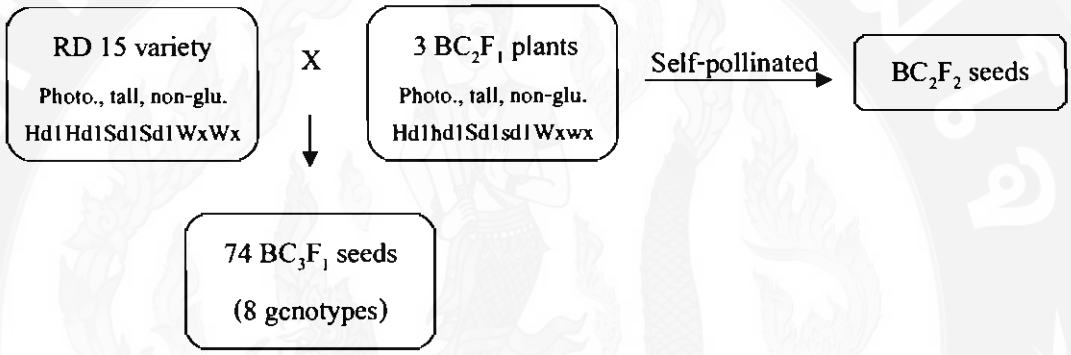


Figure 4 Schematic representation of backcrossing process between RD 15 variety and BC_2F_1 plants to produce BC_3F_1 seeds and self-pollinated to BC_2F_2 seeds from BC_2F_1 plants in the greenhouse during the rainy season from July to December 2009

4.1.2 Produced BC_4F_1 seeds and BC_3F_2 seeds in the greenhouse during the dry season from January to June 2010

RD 15 rice variety had a homozygous dominant, Hd1Hd1Sd1Sd1WxWx genotype, while improved RD 6 rice line had a homozygous recessive, hd1hd1sd1sd1wxwx genotype, thus F_1 rice plants which were derived from crossing between RD 15 variety and improved RD 6 line would be heterozygous Hd1hd1Sd1sd1Wxwx genotype. However, BC_3F_1 plants that produced in the rainy season 2010 had eight genotypes, namely: Hd1Hd1Sd1Sd1WxWx, Hd1Hd1Sd1Sd1Wxwx, Hd1Hd1Sd1sd1WxWx, Hd1Hd1Sd1sd1Wxwx, Hd1hd1Sd1Sd1WxWx, Hd1hd1Sd1Sd1Wxwx, Hd1hd1Sd1sd1WxWx, and Hd1hd1Sd1sd1Wxwx. Thus the purpose of this experiment was detected BC_3F_1 plants became one heterozygous Hd1hd1Sd1sd1Wxwx genotype only.

Firstly, by using *hd1* marker as a primer that is specific for *Hd1/hd1* gene, PCR was done by using DNA of each 74 BC_3F_1 plants as the DNA template. Gel was run to separate the size of DNA bands. The DNA bands were then detected under UV light and result showed that 28 plants were detected for heterozygous Hd1hd1 genotypes only had two DNA bands: one was same size as a DNA band of RD 15 variety and other one was same size as a DNA band of improved RD 6 line (Figure 5). These 28 plants were now carrying four genotypes, namely: Hd1hd1Sd1Sd1WxWx, Hd1hd1Sd1Sd1Wxwx, Hd1hd1Sd1sd1WxWx and Hd1hd1Sd1sd1Wxwx. They were then screened by using *sd1* marker as a primer that is specific for *Sd1/sd1* gene. The result showed that 9 plants were detected for heterozygous Sd1sd1 genotype only had two DNA bands: one was same size as DNA band of RD 15 variety and other one was same size as DNA band of improved RD 6 line (Figure 6). These 9 plants were now carrying two genotypes, namely: Hd1hd1Sd1sd1WxWx and Hd1hd1Sd1sd1Wxwx. They were later screened by using *wx* marker as a primer that is specific for *Wx/wx* gene and resulted 6 plants were detected for heterozygous Wxwx genotype only had two DNA bands: one was same size as DNA band of RD 15 variety and other one was same size as DNA band of improved RD 6 line (Figure 7). These 6 plants were now carrying one heterozygous genotype Hd1hd1Sd1sd1Wxwx only (Figure 8). In conclusion, 6 BC_3F_1 plants carrying heterozygous Hd1hd1Sd1sd1Wxwx genotype only were selected (Table 3).

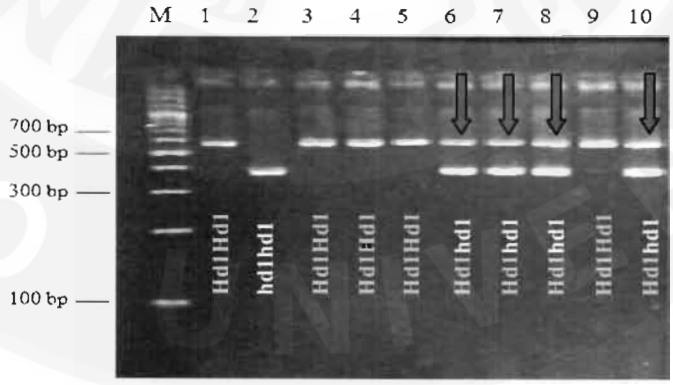


Figure 5 Shows a sample gel photograph under UV light to observe DNA banding size of BC_3F_1 plants and their parents produced from PCR product when using *hd1* marker as a primer that is specific for *Hd1/hd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Hd1Hd1 genotype, lane 2 indicated improved RD 6 line with hd1hd1 genotype, lanes 6-8 and 10 indicated BC_3F_1 plants with Hd1hd1 genotype.

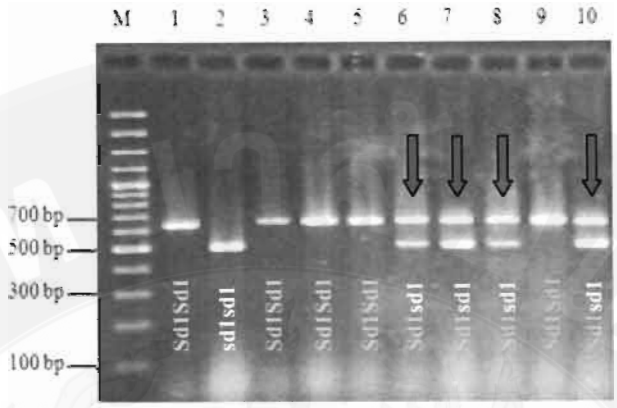


Figure 6 Shows a sample gel photograph under UV light to observe DNA banding size of BC_3F_1 plants and their parents produced from PCR product when using *sd1* marker as a primer that is specific for *Sd1/sd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Sd1Sd1 genotype, lane 2 indicated improved RD 6 line with sd1sd1 genotype, lanes 6-8 and 10 indicated BC_3F_1 plants with Sd1sd1 genotype.

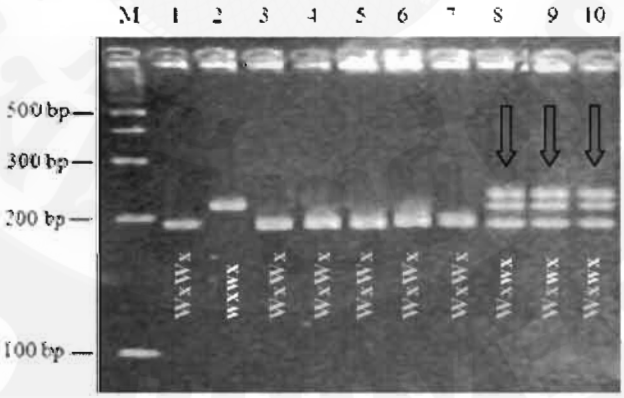


Figure 7 Shows a sample gel photograph under UV light to observe DNA banding size of BC_3F_1 plants and their parents produced from PCR product when using *wx* marker as a primer that is specific for *Wx/wx* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with WxWx genotype, lane 2 indicated improved RD 6 line with wxwx genotype and lanes 8-10 indicated BC_3F_1 plants with Wxwx genotype.

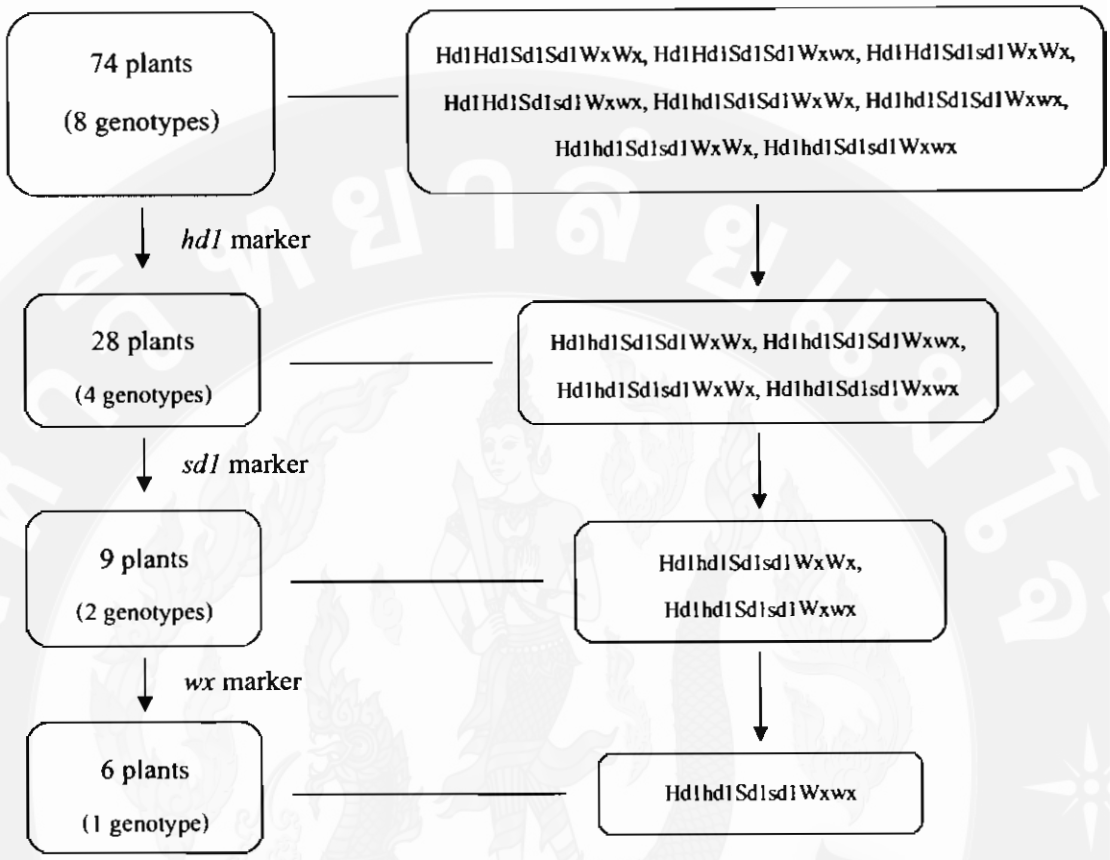


Figure 8 Schematic representation of genotypes detecting process of BC_3F_1 plants through markers which were done in the laboratory during the dry season from January to June 2010

Table 3 Phenotypes and genotypes of six BC_3F_1 plants and their parents selected in the greenhouse during the dry season from January to June 2010

No.	Lines/varieties	Phenotypes	Genotypes
1	RD 15	Photoperiod sensitive, tall and non-glutinous	Hd1Hd1Sd1Sd1WxWx
2	Improved RD 6	Non-photoperiod sensitive, short and glutinous	hd1hd1sd1sd1wxwx
3	BC_3F_1-1026-2472-1601-44	Photoperiod sensitive, tall and non-glutinous	Hd1hd1Sd1sd1Wxwx
4	BC_3F_1-1026-2472-1601-59	Photoperiod sensitive, tall and non-glutinous	Hd1hd1Sd1sd1Wxwx
5	BC_3F_1-1026-2472-1601-66	Photoperiod sensitive, tall and non-glutinous	Hd1hd1Sd1sd1Wxwx
6	BC_3F_1-1026-2480-1602-82	Photoperiod sensitive, tall and non-glutinous	Hd1hd1Sd1sd1Wxwx
7	BC_3F_1-1026-2480-1602-90	Photoperiod sensitive, tall and non-glutinous	Hd1hd1Sd1sd1Wxwx
8	BC_3F_1-1026-2483-1603-104	Photoperiod sensitive, tall and non-glutinous	Hd1hd1Sd1sd1Wxwx

Six BC_3F_1 plants were then used as the male parents to backcross again RD 15 variety to generate BC_4F_1 seeds. The result showed that 42 BC_4F_1 seeds were produced. The BC_4F_1 seeds were now also carrying eight genotypes as above. At the same time, six BC_3F_1 plants self-pollinated to generate BC_3F_2 seeds (Figure 9).

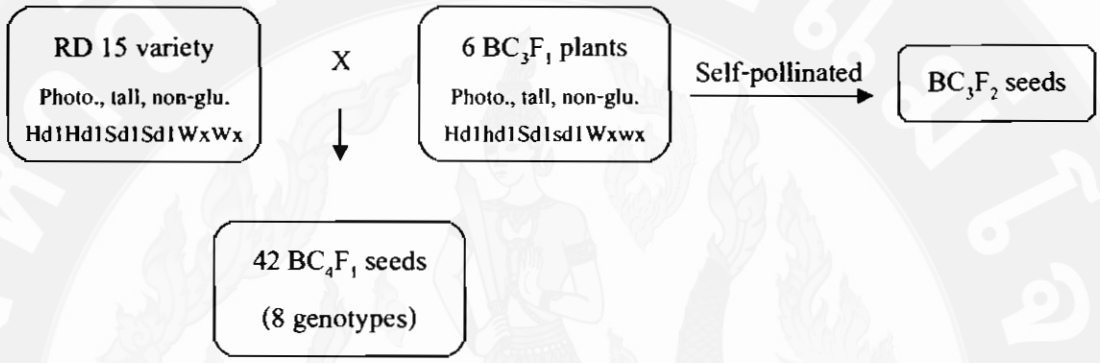


Figure 9 Schematic representation of backcrossing process between RD 15 variety and BC_3F_1 plants to produce BC_4F_1 seeds and self-pollinated to BC_3F_2 seeds from BC_3F_1 plants in the greenhouse during the dry season from January to June 2010

4.1.3 Produced BC_5F_1 seeds and BC_4F_2 seeds in the greenhouse during the rainy season from July to December 2010

From 42 BC_4F_1 seeds which were produced from the pass dry season 2010. They were grown in the greenhouse during the rainy season from July to December 2010 together with RD 15 variety and improved RD 6 line. Firstly, by using *hd1* marker for screening, 19 BC_4F_1 plants were detected for heterozygous Hd1hd1 genotype only. These 19 plants were now carrying four genotypes, namely: Hd1hd1Sd1Sd1WxWx, Hd1hd1Sd1Sd1Wxwx, Hd1hd1Sd1sd1WxWx and Hd1hd1Sd1sd1Wxwx. They were then screened by using *sd1* marker and resulted 10 plants were detected for heterozygous Sd1sd1 genotype only. These 10 plants were now carrying two genotypes, namely: Hd1hd1Sd1sd1WxWx and Hd1hd1Sd1sd1Wxwx. They were later screened by using *wx* marker and resulted 3 plants were detected for heterozygous Wxwx genotype only. These 3 plants were now carrying heterozygous genotype Hd1hd1Sd1sd1Wxwx only (Figure 10). In conclusion, three BC_4F_1 plants had one heterozygous Hd1hd1Sd1sd1Wxwx genotype only, namely:

BC_4F_1-1026-2472-1601-44-6058, BC_4F_1-1026-2472-1601-44-6060 and BC_4F_1-1026-2472-1601-59-6064 were selected (Table 4 and Figure 11).

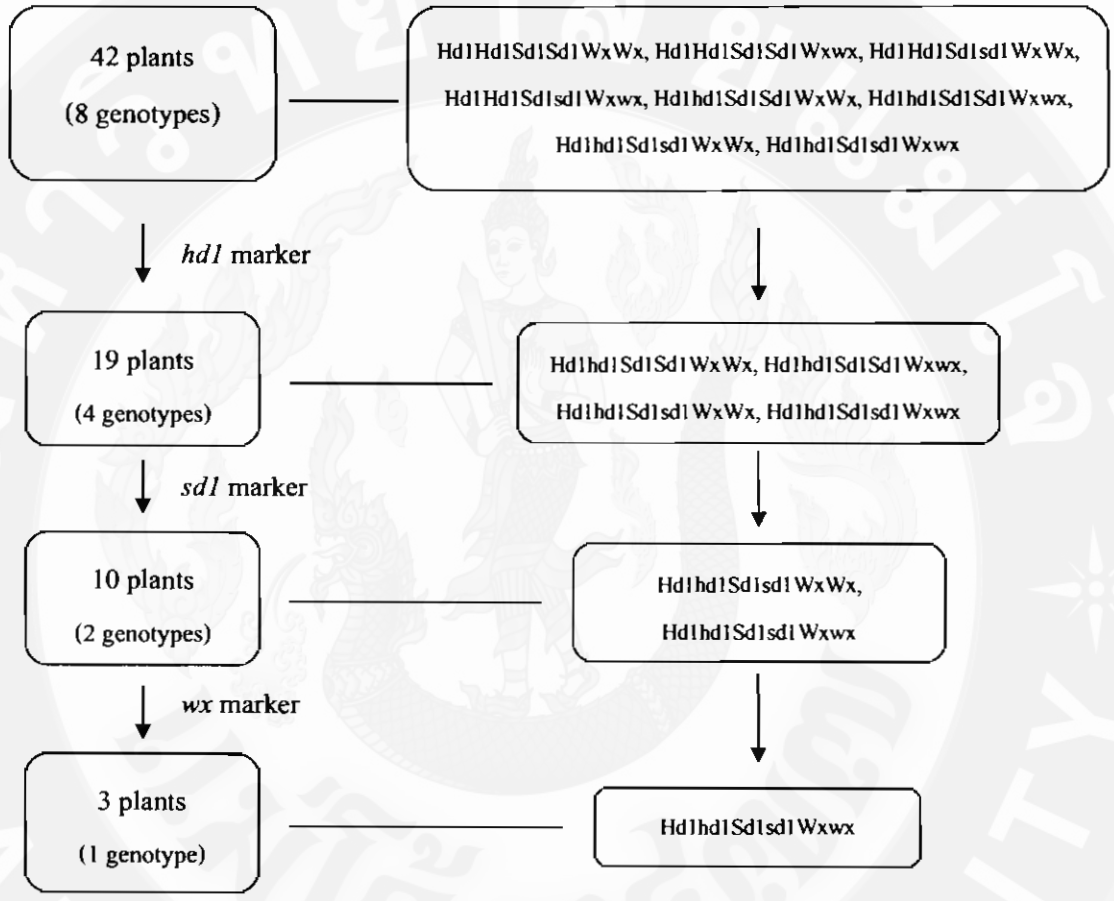


Figure 10 Schematic representation of genotypes detecting process of BC_4F_1 plants through markers which were done in the laboratory during the rainy season from July to December 2010

Table 4 Genotypes and phenotypes of three BC_4F_1 plants and their parents selected in the greenhouse during the rainy season from July to December 2010

Lines/varieties	Genotypes	Phenotypes
RD 15	Hd1Hd1Sd1Sd1WxWx	Photoperiod sensitive, tall and non-glutinous
Improved RD 6	hd1hd1sd1sd1wxwx	Non-photoperiod sensitive, short and glutinous
BC_4F_1-1026-2472-1601-44-6058	Hd1hd1Sd1sd1Wxwx	Photoperiod sensitive, tall and non-glutinous
BC_4F_1-1026-2472-1601-44-6060	Hd1hd1Sd1sd1Wxwx	Photoperiod sensitive, tall and non-glutinous
BC_4F_1-1026-2472-1601-59-6064	Hd1hd1Sd1sd1Wxwx	Photoperiod sensitive, tall and non-glutinous

(a) Hd1Hd1Sd1Sd1WxWx
(b) hd1hd1sd1sd1wxwx
(c) Hd1hd1Sd1sd1Wxwx
(d) Hd1hd1Sd1sd1Wxwx
(e) Hd1hd1Sd1sd1Wxwx

Figure 11 Shows phenotypes of BC_4F_1 plants and their parents grown in the greenhouse during the rainy season from July to December 2010 by (a) RD 15 variety with photoperiod sensitive, tall and non-glutinous; (b) improved RD 6 line with non-photoperiod sensitive, short and glutinous; (c), (d) and (e) were BC_4F_1-1026-2472-1601-44-6058 plant, BC_4F_1-1026-2472-1601-44-6060 plant and BC_4F_1-1026-2472-1601-59-6064 plant, respectively with photoperiod sensitive, tall and non-glutinous rice

Three BC_4F_1 plants selected for heterozygous Hd1hd1Sd1sd1Wxwx genotype only were then backcrossed to RD 15 rice variety to generate BC_5F_1 seeds, and resulted 100 BC_5F_1 seeds were produced. The BC_5F_1 seeds were now also carrying eight genotypes as above. Three BC_4F_1 plants were later self-pollinated to generate BC_4F_2 seeds (Figure 12).

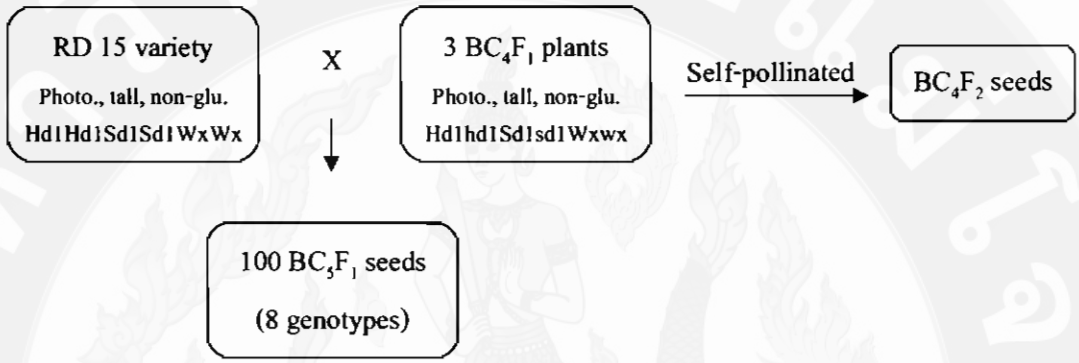


Figure 12 Schematic representation of baekerossing proeess between RD 15 variety and BC_4F_1 plants to generate BC_5F_1 seeds and self-pollinated to BC_4F_2 seeds from BC_4F_1 plants in the greenhouse during the rainy season from July to Deeember 2010

4.2 Selected four best lines of RD 15 with three markers and tested them for photoperiod response under long-day condition of light exposure for 14 hours per day

4.2.1 Selected four best lines of RD 15 with three markers: *hd1, sd1* and *wx* in the field and laboratory during the dry season from January to June 2010

From 2,000 BC_2F_2 plants whieh were grown in the field during the dry season from January to June 2010 together with RD 15 rice variety and improved RD 6 riee line, 300 flowering riee plants of BC_2F_2 population that considered non-photoperiod sensitive were selected (Figure 13). From these plants, 150 plants were then selected for tall plants (Figure 14) and other 150 plants were later selected for short plants (Figure 15).

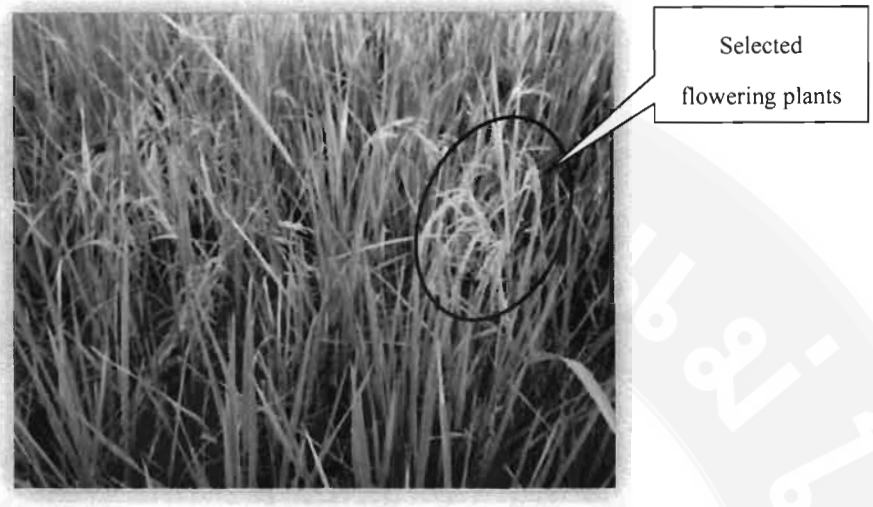


Figure 13 Shows selected flowering plants of BC_2F_2 population that were considered for non-photoperiod sensitive in the field during the dry season from January to June 2010

Figure 14 Shows rice plants that were selected for tall plants of BC_2F_2 population in the field during the dry season from January to June 2010 with plant height was more than 110 cm

Figure 15 Shows rice plants that were selected for short plants of BC_2F_2 population in the field during the dry season from January to June 2010 with plant height was less than 110 cm

DNA of these plants were then extracted, PCR and gel were later done with three markers as *hd1*, *sd1* and *wx* in laboratory. Firstly, by using *hd1* marker that is specific for *Hd1/hd1* gene, 300 BC_2F_2 plants were screened for recessive, *hd1* gene. This result indicated that 106 plants were detected for homozygous recessive, hd1hd1 genotype only that had DNA bands as same size as DNA band of improved RD 6 line (Figure 16). From these 106 plants, they were then screened for dominant, *Wx* gene and recessive, *wx* gene by using *wx* marker. The result indicated that 11 plants were detected for homozygous dominant, WxWx genotype only had DNA bands the same size as DNA band of RD 15 variety, while the other 42 plants were detected for homozygous recessive, wxwx genotype only had DNA bands the same size as DNA band of improved RD 6 line (Figure 17). From these 11 homozygous dominant WxWx genotype plants, *sd1* marker was used to screen for dominant, *Sd1* gene and recessive, *sd1* gene, and the result indicated that 3 plants were detected for homozygous dominant, Sd1Sd1 genotype only had DNA bands the same size as DNA band of RD 15 variety, while other 3 plants were detected for homozygous recessive, sd1sd1 genotype had DNA bands the same size as DNA band of improved RD 6 line. On the other hand, from 42 homozygous recessive wxwx genotype plants, result indicated that 11 plants were detected for homozygous dominant, Sd1Sd1 genotype only had DNA bands the same size as DNA band of RD 15 variety while the other 6 plants were

detected for homozygous recessive, sd1sd1 genotype only had DNA bands the same size as DNA band of improved RD 6 line (Figure 18).

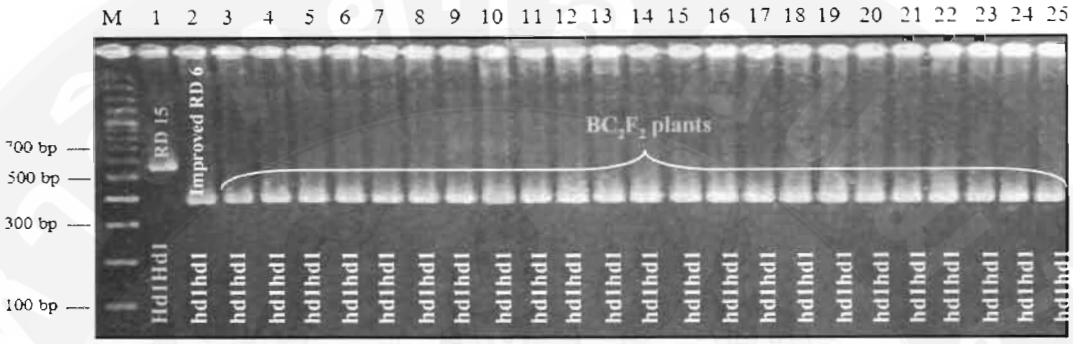


Figure 16 Shows a sample gel photograph under UV light to observe DNA banding size of BC_2F_2 plants and their parents produced from PCR product when using *hd1* marker as a primer that is specific for *Hd1/hd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Hd1Hd1 genotype; lane 2 indicated improved RD 6 line with hd1hd1 genotype; and lanes 3-25 indicated BC_2F_2 plants with hd1hd1 genotype

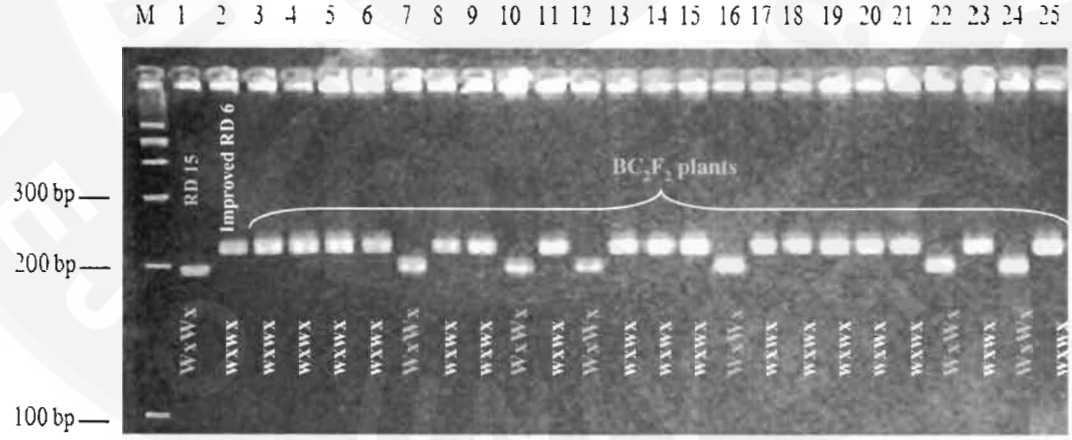


Figure 17 Shows a sample gel photograph under UV light to observe DNA banding size of BC_2F_2 plants and their parents produced from PCR product when using *wx* marker as a primer that is specific for *Wx/wx* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with WxWx genotype; lane 2 indicated improved RD 6 line with wxwx genotype; lanes 3-6, 8-9, 11, 13-15, 17-21, 23 and 25 indicated BC_2F_2 plants with wxwx genotype; and lanes 7, 10, 12, 16, 22 and 24 indicated BC_2F_2 plants with WxWx genotype.

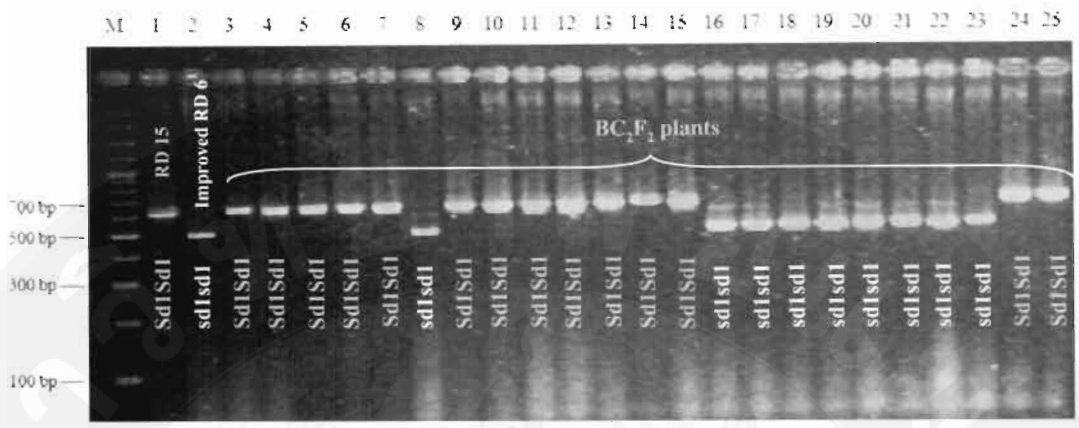


Figure 18 Shows a sample gel photograph under UV light to observe DNA banding size of BC_2F_2 plants and their parents produced from PCR product when using *sd1* marker as a primer that is specific for *Sd1/sd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Sd1Sd1 genotype; lane 2 indicated improved RD 6 line with sd1sd1 genotype; lanes 3-7, 9-15, 24 and 25 indicated BC_2F_2 plants with Sd1Sd1 genotype; and lanes 8, 16-23 indicated BC_2F_2 plants with sd1sd1 genotype.

In conclusion, the total of BC_2F_2 plants that were selected for homozygous recessive hd1hd1 genotype only were 23, where as 6 plants had homozygous dominant, WxWx genotype only, and 17 plants had homozygous recessive, wxwx genotype only. 14 plants had homozygous dominant, Sd1Sd1 genotype only, and 9 plants had homozygous recessive, sd1sd1 genotype only. From these plants, the four best lines of RD 15 were selected (Figure 19 and Table 5), namely:

1. RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype)

2. RD 15 NTG line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype)

3. RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype)

4. RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype)

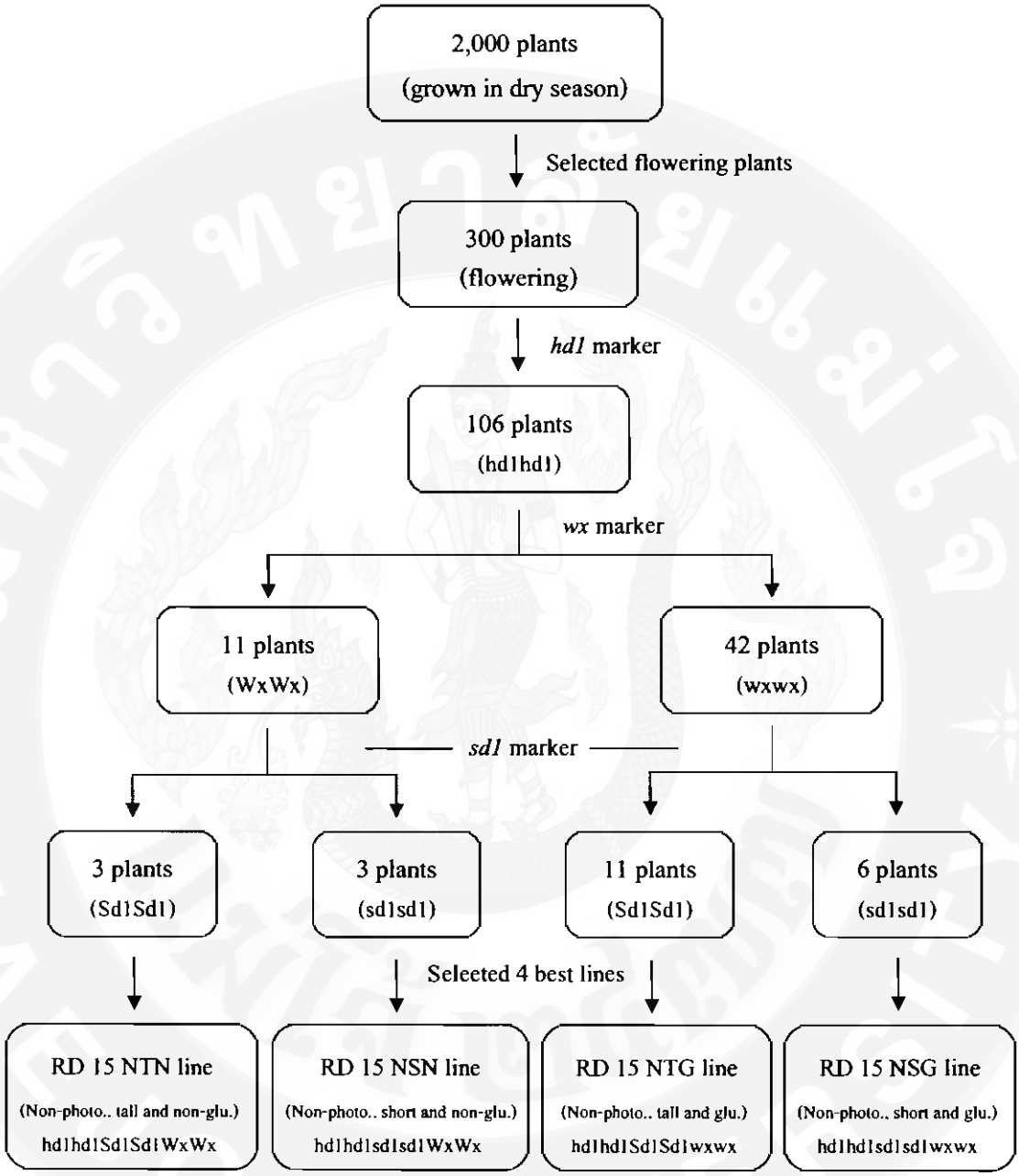


Figure 19 Schematic representation of genotypes selection process of the four best lines of RD 15 through markers were done in the field and laboratory during the dry season from January to June 2010

Table 5 Genotypes and phenotypes of the four best lines of RD 15 selected in the dry season from January to June 2010 at Maejo University, Chiang Mai

No.	Selected four best lines of RD 15	Pedigrees	Genotypes	Phenotypes	Abbreviation
1	RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous)	BC_2F_3-1026-2483-1603(139)	hd1hd1Sd1Sd1WxWx	Non-photoperiod sensitive, tall and non-glutinous	Non-photo., tall and non-glu.
2	RD 15 NTG line (non-photoperiod sensitive, tall and glutinous)	BC_2F_3-1026-2483-1603(145)	hd1hd1Sd1Sd1wxwx	Non-photoperiod sensitive, tall and glutinous	Non-photo., tall and glu.
3	RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous)	BC_2F_3-1026-2472-1601(268)	hd1hd1sd1sd1WxWx	Non-photoperiod sensitive, short and non-glutinous	Non-photo., short and non-glu.
4	RD 15 NSG line (non-photoperiod sensitive, short and glutinous)	BC_2F_3-1026-2472-1601(257)	hd1hd1sd1sd1wxwx	Non-photoperiod sensitive, short and glutinous	Non-photo., short and glu.

4.2.2 Tested for photoperiod response of the selected four best lines of RD 15 by growing them in the greenhouse and exposed to light for 14 hours per day from August 2010 to March 2011

From the four best lines of RD 15 which were selected in the dry season from January to June 2010. They were then planted in the greenhouse under long-day condition of light exposure for 14 hours per day together with RD 15 variety, improved RD 6 line and Chainat 80 variety as the control rice to check their photoperiod response. Results from this experiment showed that all of the four best lines of RD 15, namely: RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous), RD 15 NTG line (non-photoperiod sensitive, tall and glutinous), RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous) that selected for homozygous recessive hd1hd1 genotype were flowering in long-day length period (14 hours/day) as the same non-photoperiod sensitive improved RD 6 line and Chainat 80 variety. Their flowering time of 50% ranged from 108 to 115 days thus indicated their non-photoperiod sensitive, while compared to the original photoperiod sensitive RD 15 variety containing homozygous dominant Hd1Hd1 genotype, which was not flowering (Table 6 and figure 20).

Table 6 Age to 50% flowering of the selected four best lines of RD 15 and their parents which were grown in the greenhouse under long-day condition of light exposure for 14 hours per day

Lines/varieties	Genotypes	Age to 50% flowering (days)
RD 15 variety (photoperiod sensitive, tall and non-glutinous)	Hd1Hd1Sd1Sd1WxWx	-
Improved RD 6 line (non-photoperiod sensitive, short and glutinous)	hd1hd1sd1sd1wxwx	118
RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous)	hd1hd1Sd1Sd1WxWx	115
RD 15 NTG line (non-photoperiod insensitive, tall and glutinous)	hd1hd1Sd1Sd1wxwx	113
RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous)	hd1hd1sd1sd1WxWx	108
RD 15 NSG line (non-photoperiod sensitive, short and glutinous)	hd1hd1sd1sd1wxwx	111
Chainat 80 variety (non-photoperiod sensitive, short and non-glutinous)	-	100

Figure 20 Shows rice plants flowering phenotype of the selected four best lines of RD 15, their parents and Chainat 80 variety were grown in the greenhouse and exposed to light for 14 hours per day by (a) RD 15 variety with photoperiod sensitive, tall and non-glutinous) and showed by non-flowering; (b), (c), (d), (e), (f) and (g) were improved RD 6 line (non-photoperiod sensitive, short and glutinous), RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous), RD 15 NTG line (non-photoperiod sensitive, tall and glutinous), RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous), RD 15 NSG line (non-photoperiod sensitive, short and glutinous) and Chainat 80 variety (non-photoperiod sensitive, short and non-glutinous), respectively and showed by flowering

4.3 Studied yield and yield components of the selected four best lines of RD 15 in the field during the rainy season from July to December 2010

The selected four best lines of RD 15 were: RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype), RD 15 line NTG (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype), RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype)

were grown in the field together with RD 15 variety (photoperiod sensitive, tall and non-glutinous with Hd1Hd1Sd1Sd1WxWx genotype), improved RD 6 line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sdwxwx genotype), improved RD 6 line (non-photoperiod sensitive, tall and glutinous), Chainat 80 variety (non-photoperiod sensitive, short and non-glutinous), Sanpatong 1 variety (non-photoperiod sensitive, short and glutinous) and RD 10 variety (non-photoperiod sensitive, short and glutinous) as the control rice. The experiment was conducted at the field of Maejo University, Chiang Mai during the rainy season from July to December 2010 and using the Randomized Complete Block Design (RCBD) in three replications with 10 treatments. Data in this experiment were recorded for grain yield (kg/rai), age to 50% flowering (days), plant height (cm), number of tillers per hill (tillers), number of panicles per hill (panicles), number of seeds per panicle (seeds), fertility (%), weight of 1,000 seeds (g), width, length and thickness of paddy and brown rice grain (mm) and grain appearance including the calculation of the analysis of variance (ANOVA).

4.3.1 Grain yield (kg/rai)

Results in this experiment showed that from ANOVA analysis, mean of grain yield of treatments were significantly different when compared by Duncan's multiple range test (DMRT). This result indicated that grain yield of the selected four best lines of RD 15 ranged from 728 to 841 kg/rai and was not a significantly different with original RD 15 variety (771 kg/rai) (Table 7).

4.3.2 Age to 50% flowering (days)

Results in this experiment showed that from ANOVA analysis, mean of age to 50% flowering of treatments was highly significant difference when compared by DMRT. Mean of the age to 50% flowering of selected two RD 15 lines such as RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous) (96 days) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous line) (96 days) were not significantly different with original RD 15 variety (97 days). Age to 50% flowering of other selected two RD 15 lines such as RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous) (93 days) and RD 15 NTG line (non-photoperiod sensitive, tall and glutinous) (93 days) were earlier than original RD 15 varicty and showed highly significant difference (Table 7).

Table 7 Mean for grain yield and yield components of RD 15 lines improved at Maejo University, Chiang Mai during the rainy season 2010

Lines/varieties	Genotypes	Grain yield (kg/rai)	Date of flowering	Age to 50% flowering (days)	Plant height (cm)	No. of tillers/hill (tillers)	No. of panicles/ hill (panicles)	No. of seeds/ panicle (seeds)	Fertility (%)
RD 15 variety (photo., tall and non-glu.)	Hd1Hd1Sd1Sd1WxWx	772 ^{c}	10/19/ 2010	97 ^{c}	140 ^{b}	14 ^{d}	13 ^{c}	144 ^{abcd}	83
RD 15 NTN line (non-photo., tall and non-glu.)	hd1hd1Sd1Sd1WxWx	841 ^{abc}	10/15/ 2010	93 ^{d}	138 ^{b}	15 ^{cd}	14 ^{bc}	158 ^{a}	81
RD 15 NTG line (non-photo., tall and glu.)	hd1hd1Sd1Sd1wxwx	728 ^{c}	10/15/ 2010	93 ^{d}	139 ^{b}	14 ^{d}	12 ^{c}	135 ^{bcd}	84
RD 15 NSN line (non-photo., short and non-glu.)	hd1hd1sd1sd1WxWx	802 ^{bc}	10/18/ 2010	96 ^{c}	89 ^{e}	21 ^{a}	19 ^{a}	125 ^{d}	78
RD 15 NSG line (non-photo., short and glu.)	hd1hd1sd1sd1wxwx	807 ^{abc}	10/17/ 2010	96 ^{c}	89 ^{e}	19 ^{b}	16 ^{b}	133 ^{bcd}	81
Improved RD 6 line (non-photo., short and glu.)	hd1hd1sd1sd1wxwx	836 ^{abc}	10/25/ 2010	104 ^{a}	103 ^{d}	18 ^{b}	14 ^{bc}	149 ^{abc}	79
Improved RD 6 line (non-photo., tall and glu.)	hd1hd1Sd1Sd1wxwx	812 ^{abc}	10/18/ 2010	96 ^{c}	154 ^{a}	13 ^{d}	12 ^{c}	148 ^{abc}	80
Chainat 80 (non-photo., short and non-glu.)	-	932 ^{ab}	10/17/ 2010	96 ^{c}	116 ^{c}	17 ^{bc}	15 ^{b}	151 ^{ab}	79
Sanpatong 1 (non-photo., short and glu.)	-	935 ^{a}	10/27/ 2010	105 ^{a}	118 ^{c}	18 ^{b}	15 ^{b}	151 ^{ab}	80
RD 10 (non-photo., short and glu.)	-	821 ^{abc}	10/21/ 2010	100 ^{b}	112 ^{c}	15 ^{cd}	13 ^{c}	129 ^{cd}	82
Mean		829	-	98	120	16	14	142	81
F-test		*	-	**	**	**	**	**	ns
CV (%)		8.17	-	0.75	2.45	5.69	6.14	5.62	3.84

*, ** Significant at 5% and 1% level of probability, respectively; ns= non-significant difference

Within column, means followed by the same letter are not significantly different from each other at the 0.05 and 0.01 probability level based on the DMRT procedure

4.3.3 Plant height (cm)

Results in this experiment showed that from ANOVA analysis, mean plant height of treatments were a highly significant difference when compared by DMRT. The selected two best lines of RD 15 which contained homozygous dominant Sd1Sd1 genotype such as RD 15 NTN line with Sd1Sd1 genotype (138 cm) and RD 15 NTG line with Sd1Sd1 genotype (139 cm) were tall and there was not significantly different with the original RD 15 variety (Sd1Sd1 genotype) (140 cm). Other selected two best lines of RD 15 which contained homozygous recessive sd1sd1 genotype such as RD 15 NSN line with sd1sd1 genotype (89 cm) and RD 15 NSG line with sd1sd1 genotype (89 cm) were short and showed a highly significant difference with the original RD 15 variety (Sd1Sd1 genotype) (140 cm) (Table 7 and figure 21-22).

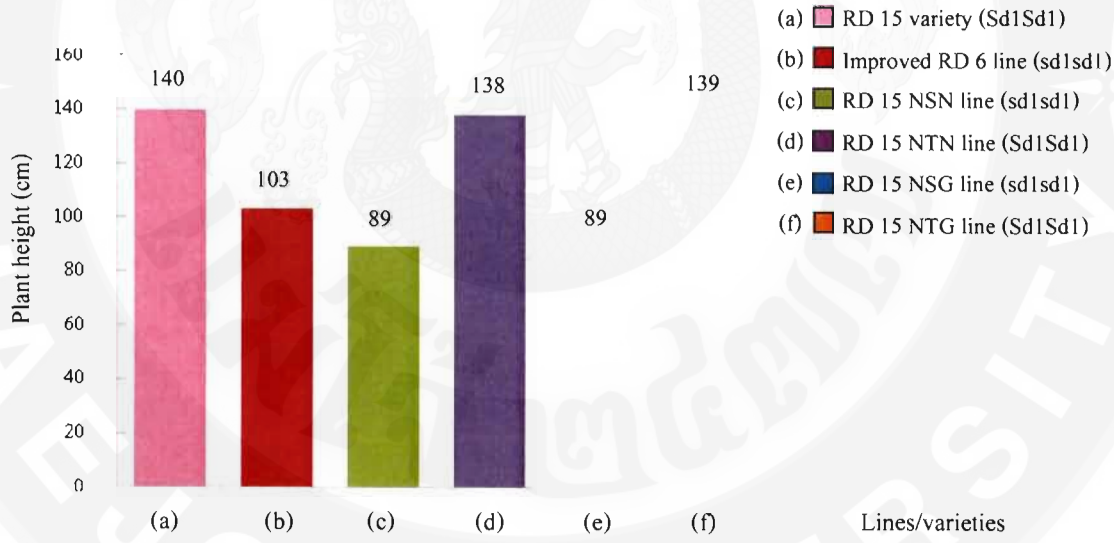


Figure 21 Shows plant height of the selected four best lines of RD 15 and their parents measured in the field during the rainy season from July to December 2010 by (a) RD 15 variety (tall plant with Sd1Sd1 genotype), (b) improved RD 6 line (short plants with sd1sd1 genotype), (c) and (e) were RD 15 NSN line and RD 15 NSG line, respectively with short plants and sd1sd1 genotype, (d) and (f) were RD 15 NTN line and RD 15 NTG line, respectively with tall plants and Sd1Sd1 genotype

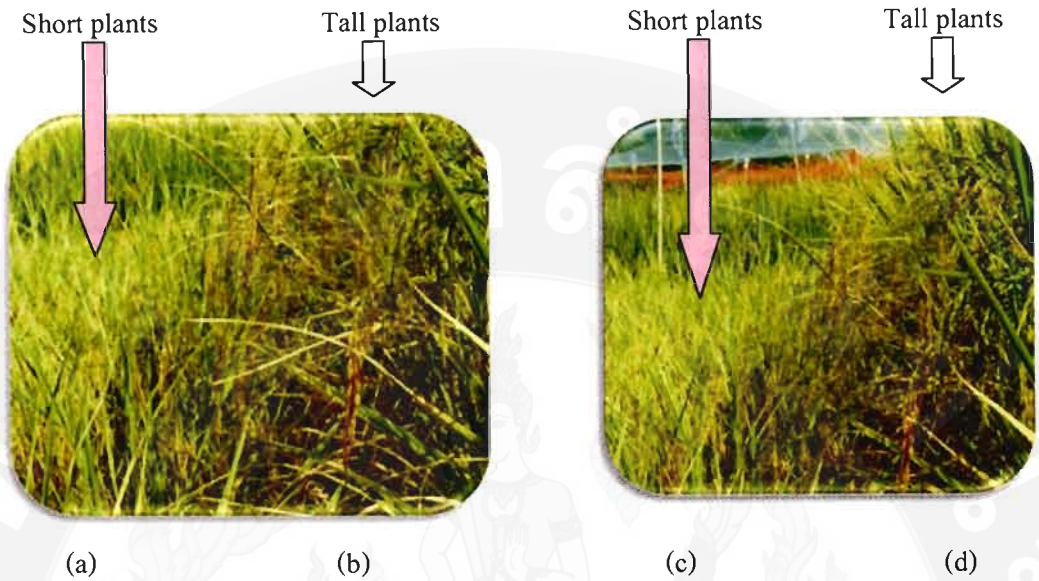


Figure 22 Shows rice plant height phenotypes of the selected four best lines of RD 15 at maturity stage grown in the field during the rainy season from July to December 2010 by (a) and (c) were RD 15 NSN line and RD 15 NSG line, respectively with short plants; (b) and (d) were RD 15 NTN line and RD 15 NTG line, respectively with tall plants

4.3.4 Number of tillers per hill (tillers)

Results in this experiment showed that from ANOVA analysis, mean number of tillers per hill of treatments were a highly significant difference when compared by DMRT. The data indicated that the number of tillers per hill of the selected two best lines of RD 15 such as RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous) (21 tillers/hill) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous) (19 tillers/hill) had more tillers than the original RD 15 variety (14 tillers/hill) and showed a highly significant difference, while the other selected two best lines such as RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous) (15 tillers/hill) and RD 15 NTG line (non-photoperiod sensitive, tall and glutinous) (14 tillers/hill) did not significantly different with the original RD 15 variety. Further, this result also showed that the short plants had more tillers than the tall plants and showed a highly significantly different (Table 7).

4.3.5 Number of panicles per hill (panicles)

Results in this experiment showed that from ANOVA analysis, the mean number of panicles per hill of treatments had a highly significant difference when compared by DMRT. The data indicated that the selected two best lines of RD 15 such as RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous) (19 panicles/hill) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous) (16 panicles/hill) had more panicles than the original RD 15 variety (photoperiod sensitive, tall and non-glutinous)) (13 panicles/hill) and showed highly significant difference, while the other selected two best lines such as RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous) (14 panicles/hill) and RD 15 NTG line (non-photoperiod sensitive, tall and glutinous) (12 panicles/hill) did not have a significantly different with the original RD 15 variety. Moreover, this result also showed that the short plants had more panicles than the tall plants as same as tillers per hill (Table 7).

4.3.6 Number of seeds per panicle (seeds)

Results in this experiment showed that from ANOVA analysis, the mean number of seeds per panicle of treatments had a highly significant difference when compared by DMRT. The data showed that the number of seeds per panicle of the selected four best lines of RD 15 ranged from 125 to 158 seeds/panicle and did not have a significantly different with the original RD 15 variety (144 seeds/panicle) (Table 7).

4.3.7 Fertility (%)

Results in this experiment showed that from ANOVA analysis, the mean fertility of treatments did not have a significant difference when compared by DMRT. The data indicated that the fertility of the selected four best lines of RD 15 ranged from 78 to 84% and were not significantly different with the original RD 15 variety (83%) (Table 7).

4.3.8 Weight of 1,000 seeds (g)

Results in this experiment showed that from ANOVA analysis, the mean weight of 1,000 seeds of treatments had a highly significant difference when compared by DMRT. The data showed that the weight of 1,000 seeds of the selected four best lines of RD 15 ranged from 23.7 to 26.4 g and were not significantly different with the original RD 15 variety (25.0 g) (Table 8).

Table 8 Mean for weight of 1,000 seeds, seed physical characteristics of paddy and brown rice grain, grain appearance and endosperm traits of RD 15 lines improved at Maejo University, Chiang Mai during the rainy season 2010

Lines/varieties	Genotypes	Weight 1,000 seeds (g)	Paddy rice grain			Brown rice grain			Grain appearance	Endosperm traits
			Width (mm)	Length (mm)	Thickness (mm)	Width (mm)	Length (mm)	Thickness (mm)		
RD 15 (photo., tall and non-glu.)	Hd1Hd1Sd1Sd1WxWx	25.0 ^{cd}	2.41 ^{d}	10.51 ^{b}	1.85 ^{c}	2.02 ^{de}	7.37 ^{b}	1.63 ^{c}	Translucence	Non-glutinous
RD 15 NTN line (non-photo., tall and non-glu.)	hd1hd1Sd1Sd1WxWx	25.7 ^{c}	2.50 ^{cd}	10.45 ^{b}	1.85 ^{c}	2.08 ^{de}	7.45 ^{ab}	1.62 ^{c}	Translucence	Non-glutinous
RD 15 NTG line (non-photo., tall and glu.)	hd1hd1Sd1Sd1wxwx	23.7 ^{d}	2.45 ^{d}	10.69 ^{ab}	1.84 ^{c}	2.00 ^{e}	7.46 ^{ab}	1.62 ^{c}	Chalkiness	Glutinous
RD 15 NSN line (non-photo., short and non-glu.)	hd1hd1sd1sd1WxWx	26.4 ^{c}	2.51 ^{cd}	10.72 ^{ab}	1.87 ^{c}	2.09 ^{d}	7.50 ^{ab}	1.64 ^{c}	Translucence	Non-glutinous
RD 15 NSG line (non-photo., short and glu.)	hd1hd1sd1sd1wxwx	25.1 ^{cd}	2.48 ^{d}	10.47 ^{b}	1.83 ^{c}	2.01 ^{e}	7.22 ^{b}	1.61 ^{c}	Chalkiness	Glutinous
Improved RD 6 line (non-photo., short and glu.)	hd1hd1sd1sd1wxwx	29.2 ^{b}	2.83 ^{b}	10.58 ^{b}	1.95 ^{b}	2.27 ^{bc}	7.39 ^{b}	1.72 ^{b}	Chalkiness	Glutinous
Improved RD 6 line (non-photo., tall and glu.)	hd1hd1Sd1Sd1wxwx	28.4 ^{b}	2.80 ^{b}	10.48 ^{b}	1.96 ^{b}	2.30 ^{b}	7.32 ^{b}	1.73 ^{b}	Chalkiness	Glutinous
Chainat 80 (non-photo., short and non-glu.)	-	29.3 ^{b}	2.60 ^{c}	9.82 ^{c}	2.03 ^{a}	2.22 ^{c}	7.23 ^{b}	1.81 ^{a}	Translucence	Non-glutinous
Sanpatong 1 (non-photo., short and glu.)	-	31.5 ^{a}	2.93 ^{a}	10.61 ^{b}	2.08 ^{a}	2.41 ^{a}	7.20 ^{b}	1.86 ^{a}	Chalkiness	Glutinous
RD 10 (non-photo., short and glu.)	-	32.8 ^{a}	2.77 ^{b}	11.09 ^{a}	2.07 ^{a}	2.25 ^{c}	7.73 ^{b}	1.87 ^{a}	Chalkiness	Glutinous
Mean		28	2.63	10.54	1.93	2.17	7.39	1.71	-	-
F-test		**	**	**	**	**	**	**	-	-
CV (%)		2.12	1. 55	1.70	1.29	1.52	1.77	1.38	-	-

** Significant at 1% probability level

Within column, means followed by the same letter are not significantly different from each other at 0.05 probability level based on the DMRT procedure

4.3.9 Width, length and thickness of paddy rice grain (mm)

Results in this experiment showed that from ANOVA analysis, means of width, length and thickness of paddy rice grain of treatments were a highly significantly different when compared by DMRT. The data showed that width, length and thickness of paddy ricc grain of the selected four best lines of RD 15 were not significantly different with the original RD 15 variety (Table 8).

4.3.10 Width, length and thickness of brown rice grain (mm)

Results in this experiment showed that from ANOVA analysis, means of width, length and thickness of brown rice grain of all treatments were a highly significantly different when compared by DMRT. Result showed that width, length and thickness of brown rice grain of selected four best lines of RD 15 were not significantly different with original RD 15 variety (Table 8).

4.3.11 Grain appearance and endosperm traits

Results from this experiment showed that non-glutinous RD 15 rice variety contained homozygous dominant WxWx genotype had a translucent endosperm grain appearance while glutinous improved RD 6 rice line contained homozygous recessive wxwx genotype had a chalky endosperms grain appearance. The two best lines of RD 15 which were selected for non-glutinous rice contained homozygous dominant WxWx genotype, such as RD 15 NTN line (non-glutinous with WxWx genotype) and RD 15 NSN line (non-glutinous with WxWx genotype) showed by a translucent endosperm grain appearance. The other two best lines of RD 15 which were selected for glutinous rice contained homozygous recessive wxwx genotype such as RD 15 NTG line (glutinous with wxwx genotype) and RD 15 NSG line (glutinous with wxwx genotype) showed by a chalky endosperm grain appearance (Table 8 and Figure 23).

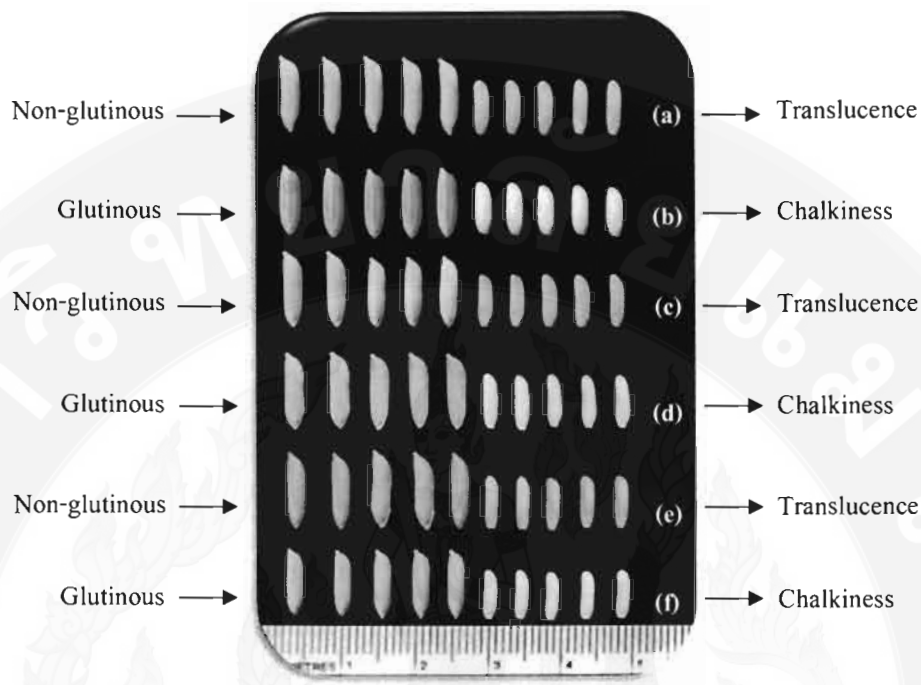


Figure 23 Shows seeds phenotype of paddy and brown rice grain of the selected four best lines of RD 15 and their parents from yield trials in the field during the rainy season from July to December 2010 by (a), (c) and (e) were RD 15 variety, RD 15 NTN line and RD 15 NSN line, respectively with non-glutinous and showed by a translucent endosperm grain appearance; (b), (d) and (f) were improved RD 6 line, RD 15 NTG line and RD 15 NSG line, respectively with glutinous and showed by a chalky endosperm grain appearance.

4.4 Studied inheritance of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes using markers in BC_3F_2 population planting under long-day condition of light exposure for 14 hours per day

RD 15 rice variety (photoperiod sensitive, tall and non-glutinous with Hd1Hd1Sd1Sd1WxWx genotype) was crossed to improved RD 6 line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype), using RD 15 variety as the recipient parent and improved RD 6 line as the donor parent to generate F_1 seeds. The F_1 plants were then used as male parent to backcross again RD 15 to generate BC_1F_1 seeds. Consecutive backcrossing was conducted until BC_3F_1 seeds were produced. The BC_3F_1 plants were then grown in the greenhouse and selected for heterozygous Hd1hd1Sd1sd1Wxwx genotype only by using *hd1, sd1* and *wx* markers. These plants were later self-pollinated to generate BC_3F_2 seeds (Figure 24). 150 BC_3F_2 seeds were used to unhusk for giving non-glutinous and glutinous seeds endosperm phenotypes and then tested by chi-square (χ^2). They were then planted in the greenhouse from August 2010 to March 2011 under long-day condition of light exposure for 14 hours per day to determine phenotypes of photoperiod response plants to produce photoperiod sensitive and non-photoperiod sensitive plants, and plant height for giving tall and short plants (Figure 25). DNA was later extracted; PCR and gel were done with three markers as *hd1, sd1* and *wx* that are specific for *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes to detect genotypes of BC_3F_2 plants. After 180 lighting days at 14 hours per day, electric light was turned off and BC_3F_3 seeds were produced 30 days later by selfing from BC_3F_2 plants. Chi-square test (χ^2) was final applied to test whether they followed the Mendelian Laws of Heredity or not.

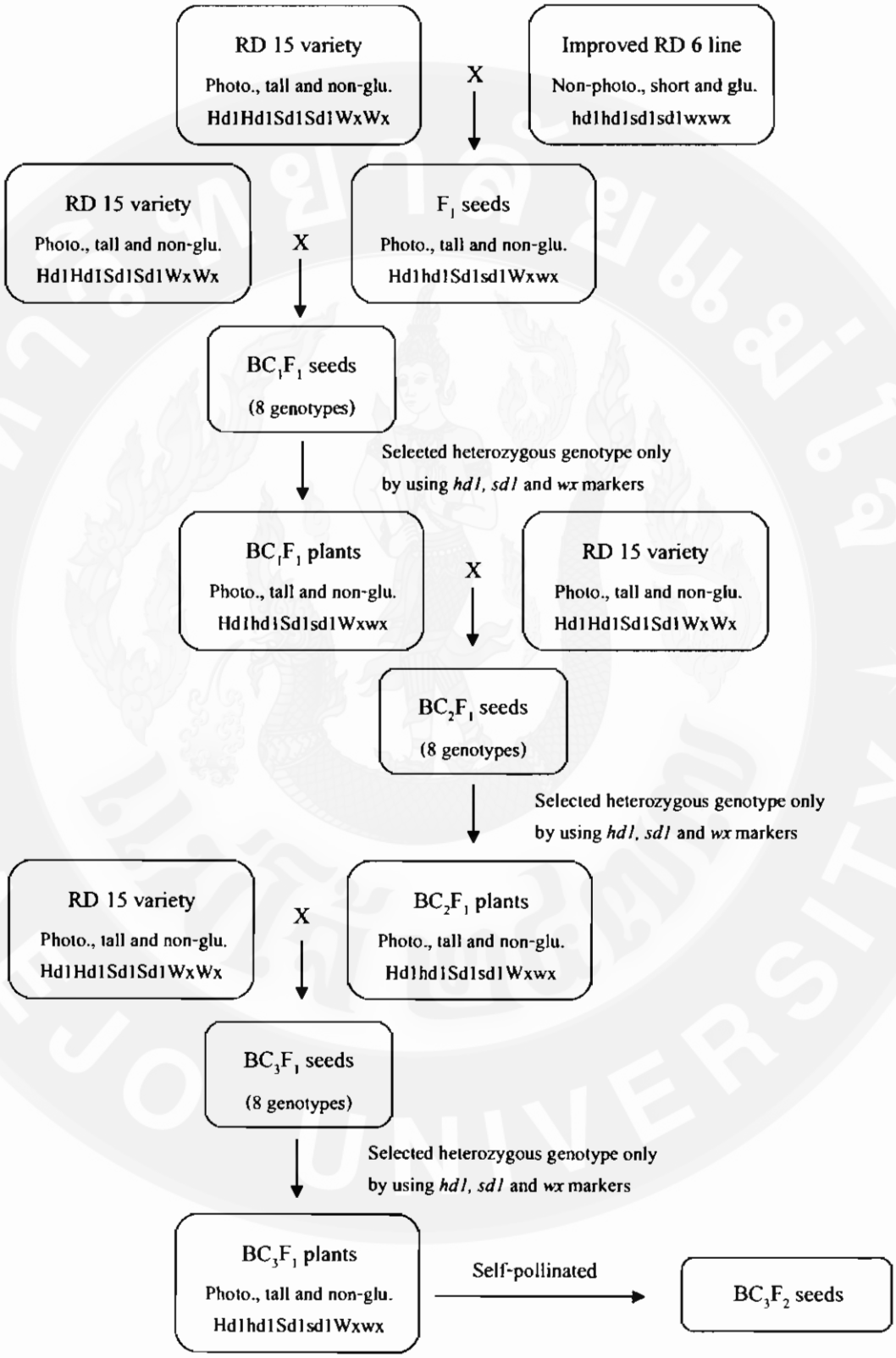


Figure 24 Schematic representation of backcrossing process between RD 15 variety and improved RD 6 line to generate BC_3F_1 seeds through three backcrosses and one selfing to get BC_3F_2 seeds

Figure 25 Shows the rice plants grown in the greenhouse under long-day condition exposure to light for 14 hours per day from August 2010 to March 2011

4.4.1 Inheritance of *Hd1/hd1* gene in BC_3F_2 population by using *hd1* marker which was part of *Hd1/hd1* gene

Genotypic ratio of *Hd1/hd1* gene in BC_3F_2 population was done by comparing the DNA banding size produced by PCR of each individual plants of BC_3F_2 population with DNA banding size produced by PCR of photoperiod sensitive RD 15 rice variety and non-photoperiod sensitive rice line, improved RD 6. RD 15 variety had homozygous dominant Hd1Hd1 genotype, while the improved RD 6 line had homozygous recessive hd1hd1 genotype. As a criterion, if BC_3F_2 plants had a DNA banding size of PCR product to be similar to a DNA banding size of PCR product of RD 15 variety, meaning that they contained homozygous dominant genotype Hd1Hd1, by contrast, if BC_3F_2 plants had a DNA banding size of PCR product to be similar to a DNA banding size of PCR product of improved RD 6 line, meaning that they contained homozygous recessive genotype hd1hd1, and if BC_3F_2 plants had two DNA bands of PCR product: one was same size as DNA band of RD 15 variety and other one was same size as DNA band of improved RD 6 line, meaning that they contained heterozygous genotype Hd1hd1 (Figure 26). Based on these comparisons, among 150 plants of BC_3F_2 population, 37 plants were detected for homozygous dominant Hd1Hd1 genotype, 70 plants were detected for heterozygous Hd1hd1 genotype and the other 43 plants were detected for homozygous recessive hd1hd1 genotype. These observed values

for each genotypes were then tested by using chi-square test to determine whether they followed the First Mendelian Law of Heredity or not under the hypothesis that the genotypic ratio of BC_3F_2 plants must be equivalent to 1/4 Hd1Hd1 : 2/4 Hd1hd1 : 1/4 hd1hd1 ratio. The results showed that the ratio of 37 : 70 : 43 was in accordance with 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 1.15 < \chi^2 (0.05, 2) = 5.99$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the First Law of Mendel (Table 9).

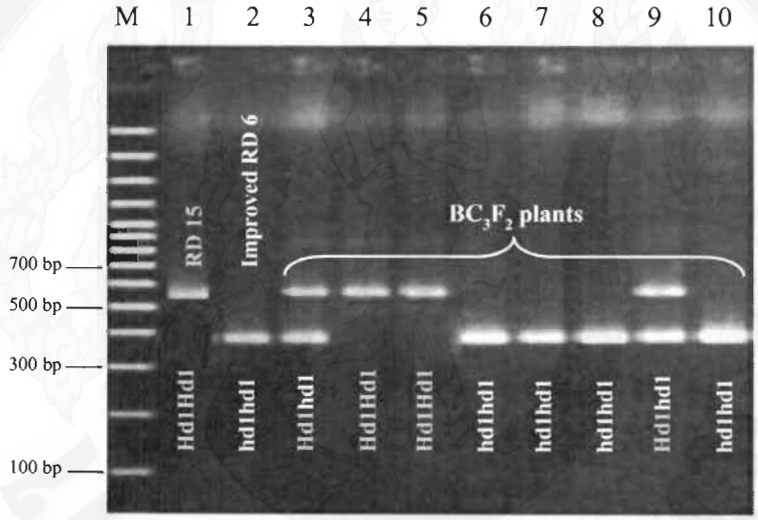


Figure 26 Shows a sample gel photograph under UV light to observe DNA banding size of BC_3F_2 plants and their parents produced from PCR product when using *hd1* marker as a primer that is specific for *Hd1/hd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Hd1Hd1 genotype; lane 2 indicated improved RD 6 line with hd1hd1 genotype; lanes 3 and 9 indicated BC_3F_2 plants with Hd1hd1 genotype; lanes 4-5 indicated BC_3F_2 plants with Hd1Hd1 genotype; lanes 6-8 and 10 indicated BC_3F_2 plants with hd1hd1 genotypes.

Table 9 Testing for genotypic ratio of *Hd1/hd1* gene and phenotypic ratio of photoperiod sensitive/non-photoperiod sensitive of 150 plants of BC_3F_2 population using chi-square test (χ^2) grown in the greenhouse under long-day condition of light exposure of 14 hours per day

Genotypes of BC_3F_2 plants[1]				**Phenotypes of BC_3F_2 plants**[2]			
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$
Hd1Hd1	37	1/4*150 = 37.5	0.01	Photoperiod sensitive (Non-flowering)	107	3/4*150 = 112.5	0.27
Hd1hd1	70	2/4*150 = 75.0	0.33				
hd1hd1	43	1/4*150 = 37.5	0.81	Non-photoperiod sensitive (Flowering)	43	1/4*150 = 37.5	0.81
Total	150	150	1.15	Total	150	150	1.08
Chi-square (χ^2)			**1.15^{ns}**	**Chi-square (χ^2)**			**1.08^{ns}**

1^{ns} with statistically significant difference when compared with tabular chi-square value of 5.99 at 95% confidence level and df = 2

2^{ns} with statistically significant difference when compared with tabular chi-square value of 3.84 at 95% confidence level and df = 1

Testing for phenotypic ratios of photoperiod sensitive/non-photoperiod sensitive plants, of the 150 plants of BC_3F_2 population were grown in the greenhouse together with RD 15 rice variety and improved RD 6 rice line during long-day length period exposed to light for 14 hours per day condition, it was found that RD 15 variety and 107 plants of BC_3F_2 population were photoperiod sensitive (non-flowering plants) while improved RD 6 line and other 43 plants of BC_3F_2 population were non-photoperiod sensitive (flowering plants) (Figure 27-28). Observation values for each phenotype of BC_3F_2 plants were later examined using chi-square test to determine if they followed the First Mendelian Law of Heredity under hypothesis that the phenotypic ratio of BC_3F_2 plants must be equivalent to 3/4 photoperiod sensitive : 1/4 non-photoperiod sensitive. Analyzing these data by chi-square test (χ^2 test), results showed that the ratio of 107 : 43 was in accordance with 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 1.08 < \chi^2 (0.05, 1) = 3.84$ (Table 9), thus indicating that the phenotypic ratio of BC_3F_2 plants followed the First Mendelian Law of Heredity. This result also showed that the phenotype of BC_3F_2 plants corresponded to their genotype. The plants which were flowering had genotype hd1hd1 only, while the plants which were not flowering had two genotypes: Hd1Hd1 and Hd1hd1.

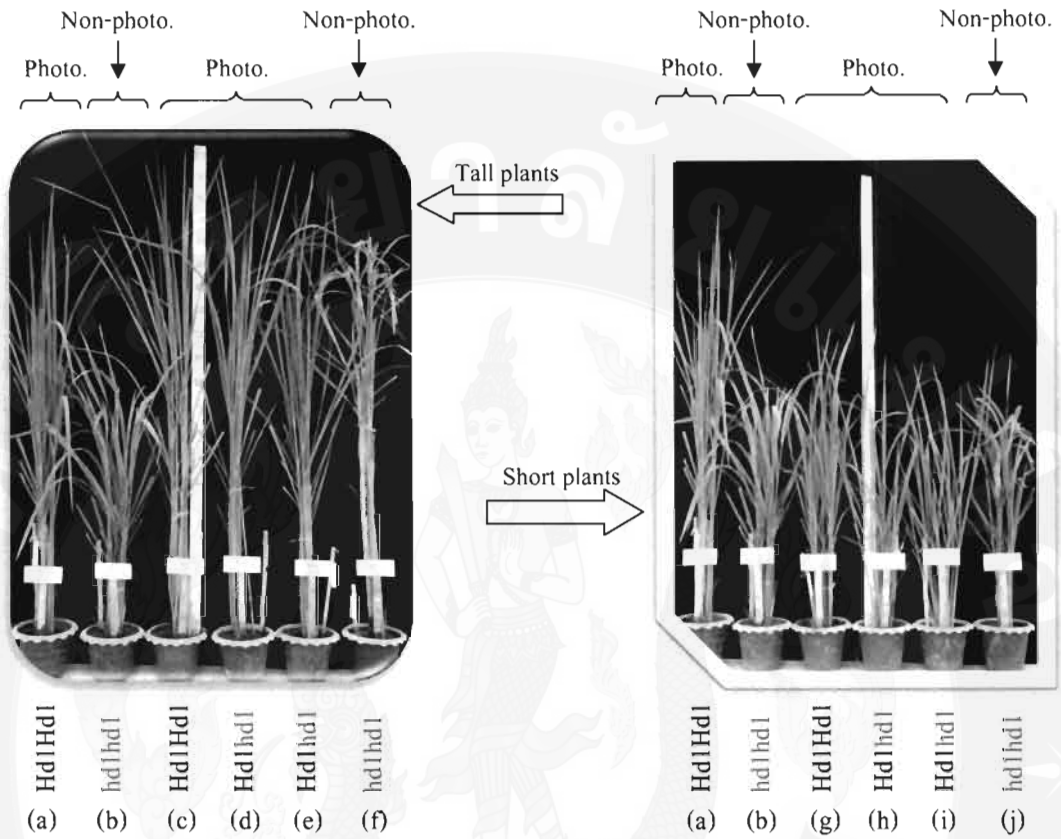


Figure 27 Shows phenotypes of photoperiod sensitive and non-photoperiod sensitive on tall and short plants of BC_3F_2 population and their parents grown under conditions of long-day condition exposed to light for 14 hours/day by (a) RD 15 variety with photosensitive plant and showed by non-flowering; (b) improved RD 6 line with non-photosensitive plant and showed by flowering; (c) and (g), (d) and (h), (e) and (i) were three tall plants and three short plants of BC_3F_2 population, respectively with photosensitive plants and showed by non-flowering; but (f) and (j) were a tall plant and a short plant of BC_3F_2 population, respectively with non-photosensitive plants and showed by flowering.

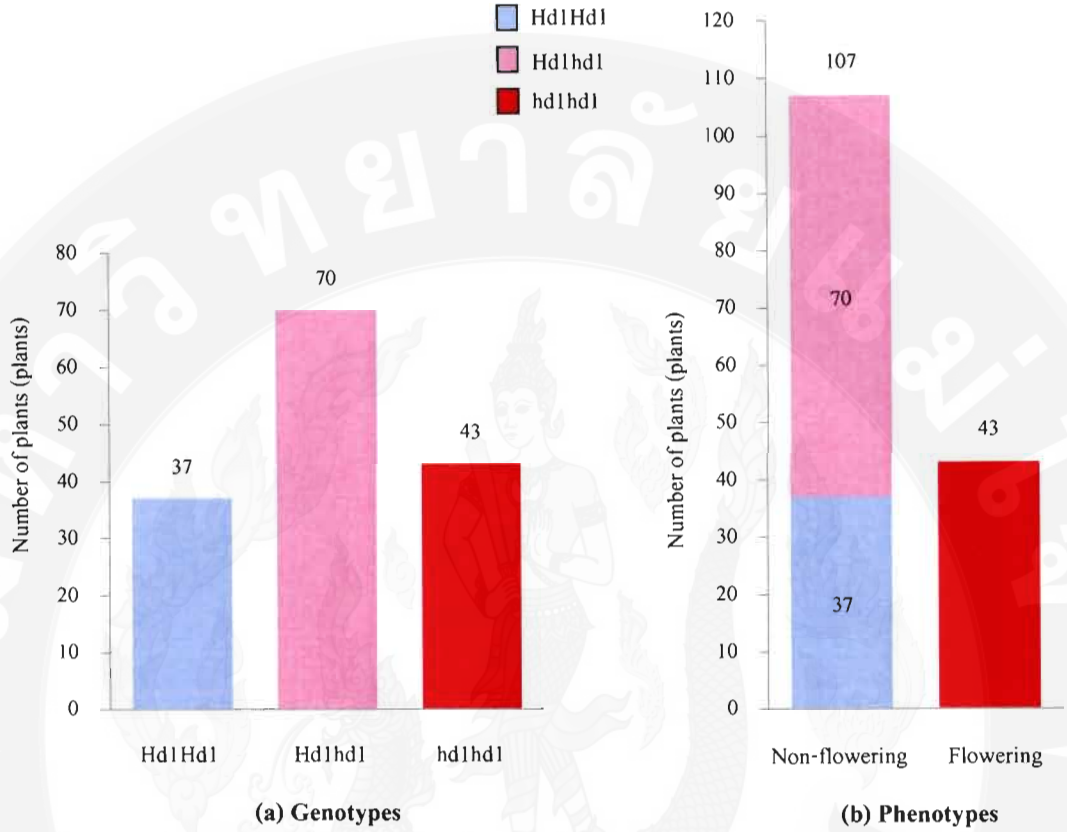


Figure 28 Shows the amount of (a) genotypes of *Hd1/hd1* gene and (b) phenotypes of photoperiod sensitive/non-photoperiod sensitive of 150 BC_3F_2 plants grown in the greenhouse under condition of long-day length period exposed to light for 14 hours per day

4.4.2 Inheritance of *Sd1/sd1* gene in BC_3F_2 population by using *sd1* marker which was part of *Sd1/sd1* gene

Genotypic ratio of *Sd1/sd1* gene in BC_3F_2 population was done by comparing the DNA banding size producted by PCR of each individual plant of BC_3F_2 population with DNA banding size produced by PCR of tall plant RD 15 rice variety and short (or semi-dwarfing) plant rice line, improved RD 6. RD 15 variety had homozygous dominant Sd1Sd1 genotype, while the improved RD 6 line had homozygous recessive sd1sd1 genotype. As a criterion, if BC_3F_2 plants had a DNA banding size of PCR product to be similar to a DNA banding size of PCR product of RD 15 variety, meaning that BC_3F_2 plants contained homozygous dominant genotype Sd1Sd1, by contrast, if BC_3F_2 plants had a DNA banding size of PCR product to be similar to a DNA banding size of

PCR product of the improved RD 6 line, meaning that BC_3F_2 plants contained homozygous recessive genotype sd1sd1, and if BC_3F_2 plants had two DNA bands: one was same size as DNA band of the RD 15 variety and other one was same size as DNA band of the improved RD 6 line, meaning that BC_3F_2 plants contained heterozygous genotype Sd1sd1 (Figure 29). Based on these comparisons, among 150 plants of BC_3F_2 population, 37 plants were detected for homozygous dominant Sd1Sd1 genotype, 73 plants were detected for heterozygous Sd1sd1 genotype and the other 40 plants were detected for homozygous recessive sd1sd1 genotype. These observed values for each genotypes were then tested by using chi-square test to determine whether they followed the First Mendelian Law of Heredity or not under the hypothesis that the genotypic ratio of BC_3F_2 plants must be equivalent to 1/4 Sd1Sd1 : 2/4 Sd1sd1 : 1/4 sd1sd1 ratio. Result showed that the ratio of 37 : 73 : 40 was in accordance with 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 0.23 < \chi^2 (0.05, 2) = 5.99$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the First Law of Mendel (Table 10).

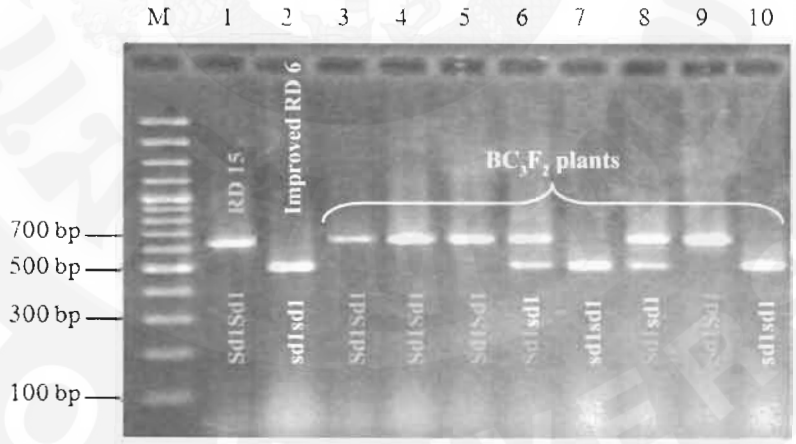


Figure 29 Shows a sample gel photograph under UV light to observe DNA banding size of BC_3F_2 plants and their parents produced from PCR product when using *sd1* marker as a primer that is specific for *Sd1/sd1* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with Sd1Sd1 genotype; lane 2 indicated improved RD 6 line with sd1sd1 genotype; lanes 3-5 and 9 indicated BC_3F_2 plants with Sd1Sd1 genotype; lanes 6 and 8 indicated BC_3F_2 plants with Sd1sd1 genotype; lanes 7 and 10 indicated BC_2F_2 plants with sd1sd1 genotype

Table 10 Testing for genotypic ratio of *Sd1/sd1* gene and phenotypic ratio of tall/short plants of 150 plants of BC_3F_2 population by using chi-square test (χ^2) grown in the greenhouse under condition of light exposure for 14 hours per day

Genotypes of BC_3F_2 plants[1]				**Phenotypes of BC_3F_2 plants[2]**			
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$
Sd1Sd1	37	1/4*150 = 37.5	0.01	Tall	110	3/4*150 = 112.5	0.06
Sd1sd1	73	2/4*150 = 75.0	0.05				
sd1sd1	40	1/4*150 = 37.5	0.17	Short	40	1/4*150 = 37.5	0.17
Total	150	150	0.23	Total	150	150	0.23
	Chi-square (χ^2)		**0.23^{ns}**		**Chi-square (χ^2)**		**0.23^{ns}**

1^{ns} with statistically significant difference when compared with tabular chi-square value of 5.99 at 95% confidence level and df = 2

2^{ns} with statistically significant difference when compared with tabular chi-square value of 3.84 at 95% confidence level and df = 1

Testing for phenotypic ratio of tall/short plants, of the 150 plants of BC_3F_2 population were measured as the distance from the soil surface to the top of the highest panicles, excluding awns for flowering plants and from the soil surface to the top of the highest leaf for non-flowering plants at the same time. Result showed that 110 plants were tall (140-210 cm), while the other 40 plants were short (78-110 cm) (Figure 30-31). Observation values for each phenotypes of BC_3F_2 plants were later examined by using chi-square test to determine if they followed the First Mendelian Law of Heredity under hypothesis that the phenotypic ratio of BC_3F_2 plants must be equivalent to 3/4 tall : 1/4 short. Analyzed these data by chi-square test (χ^2 test), it was found that the ratio of 110 : 40 was in accordance with 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 0.23 < \chi^2 (0.05, 1) = 3.84$ (Table 10), thus indicating that the phenotypic ratio of BC_3F_2 plants followed the First Mendelian Law of Heredity. From this result, it was found that the phenotype of BC_3F_2 plants corresponded to their genotype. The plants which were short had genotype sd1sd1 only, while the plants which were tall had two genotypes: Sd1Sd1 and Sd1sd1.

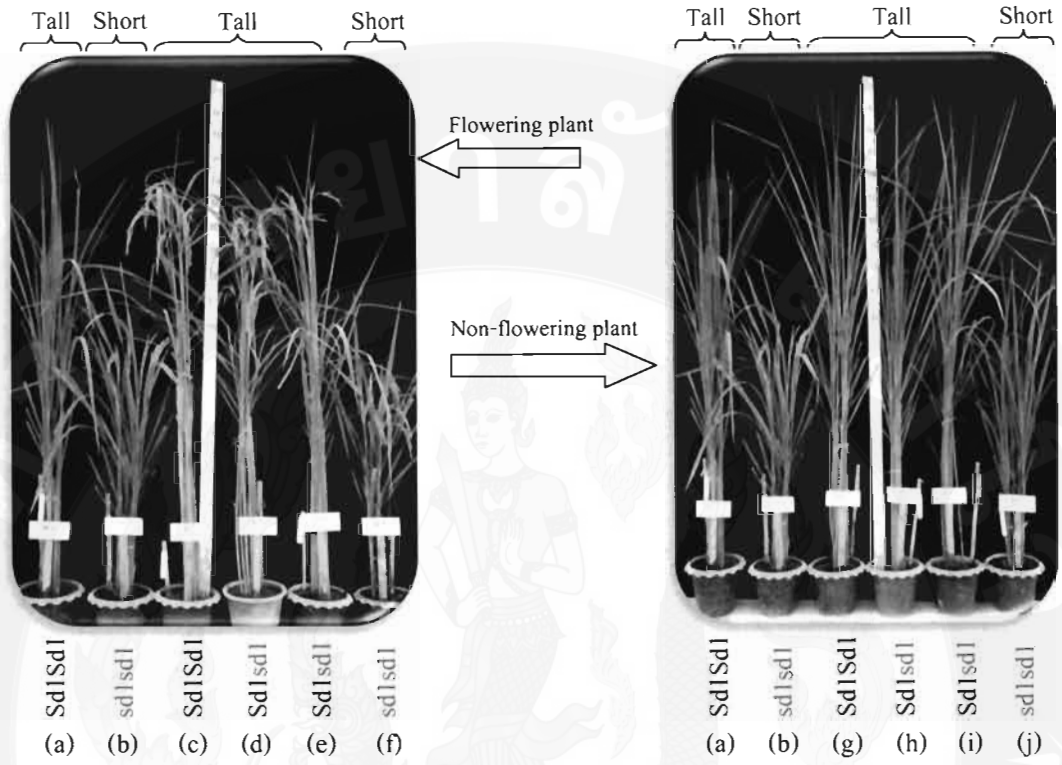


Figure 30 Shows plant height phenotype of tall and short plants on flowering and non-flowering plants of BC_3F_2 population and their parents grown under conditions of long-day lengths period exposed to light for 14 hours/day by (a) RD 15 variety with tall plant; (b) improved RD 6 line with short plant; (c) and (g), (d) and (h), (e) and (i) were three non-photosensitive plants and three photosensitive plants of BC_3F_2 population, respectively with tall plants; but (f) and (j) were a non-photosensitive plant and a photosensitive plant of BC_3F_2 population, respectively with short plants

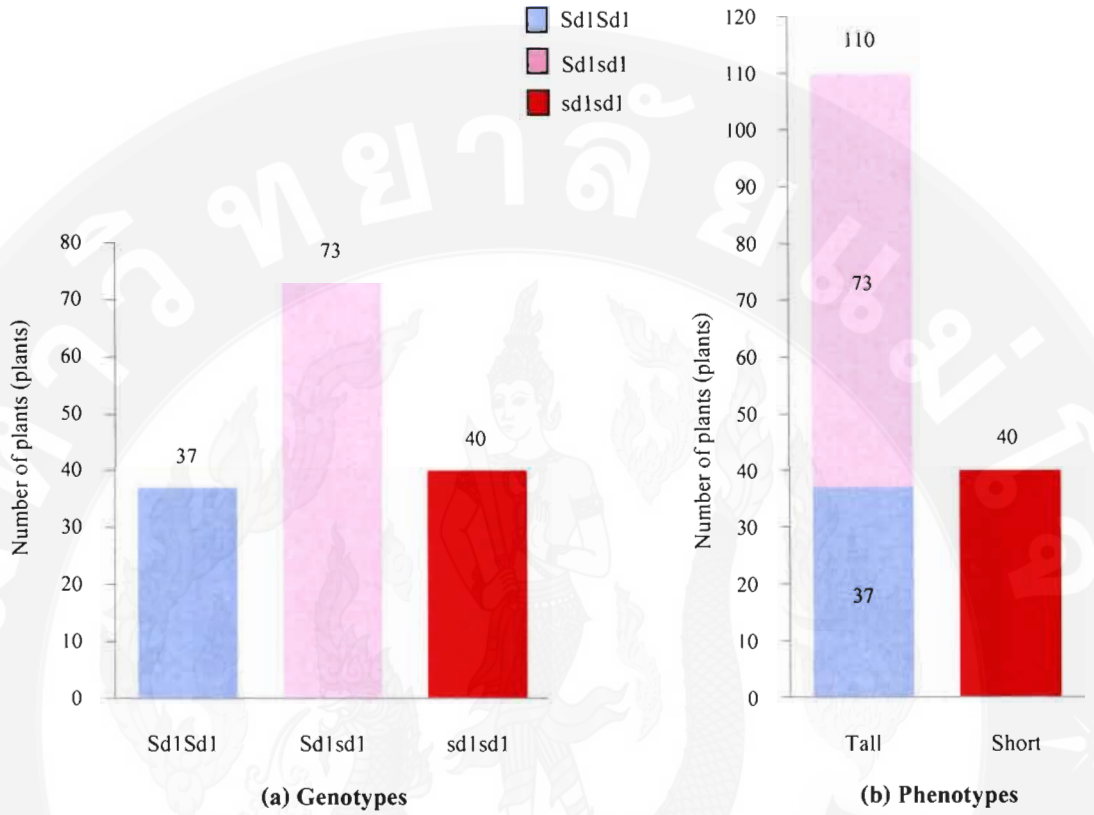


Figure 31 Shows the amount of the (a) genotypes of *Sd1/sd1* gene and (b) phenotypes of tall/short plants of 150 BC_3F_2 plants grown in the greenhouse under condition of long-day length periosd of light exposure to 14 hours per day

4.4.3 Inheritance of *Wx/wx* gene in BC_3F_2 population by using *wx* marker which was part of *Wx/wx* gene

150 BC_3F_2 seeds were unhusked for determining non-glutinous and glutinous seeds endosperm phenotypes, and then recorded phenotypes of each seeds to test chi-square (χ^2). These seeds were then grown in the greenhouse became 150 BC_3F_2 plants. DNA was later extracted; PCR and gel were done with *wx* marker to determine their genotypes; and tested by chi-square (χ^2). These BC_3F_2 plants were later self-pollinated to generate BC_3F_3 seeds. The BC_3F_2 plants carried homozygous recessive wxwx genotype and produced BC_3F_3 seeds with glutinous seed endosperm phenotype only, while the BC_3F_2 plants carried homozygous dominant WxWx genotype and produced BC_3F_3 seeds with non-glutinous seed endosperm phenotype only, but the

BC_3F_2 plants carried heterozygous Wxwx genotype producing BC_3F_3 seeds with both non-glutinous and glutinous seeds phenotypes (Figure 32).

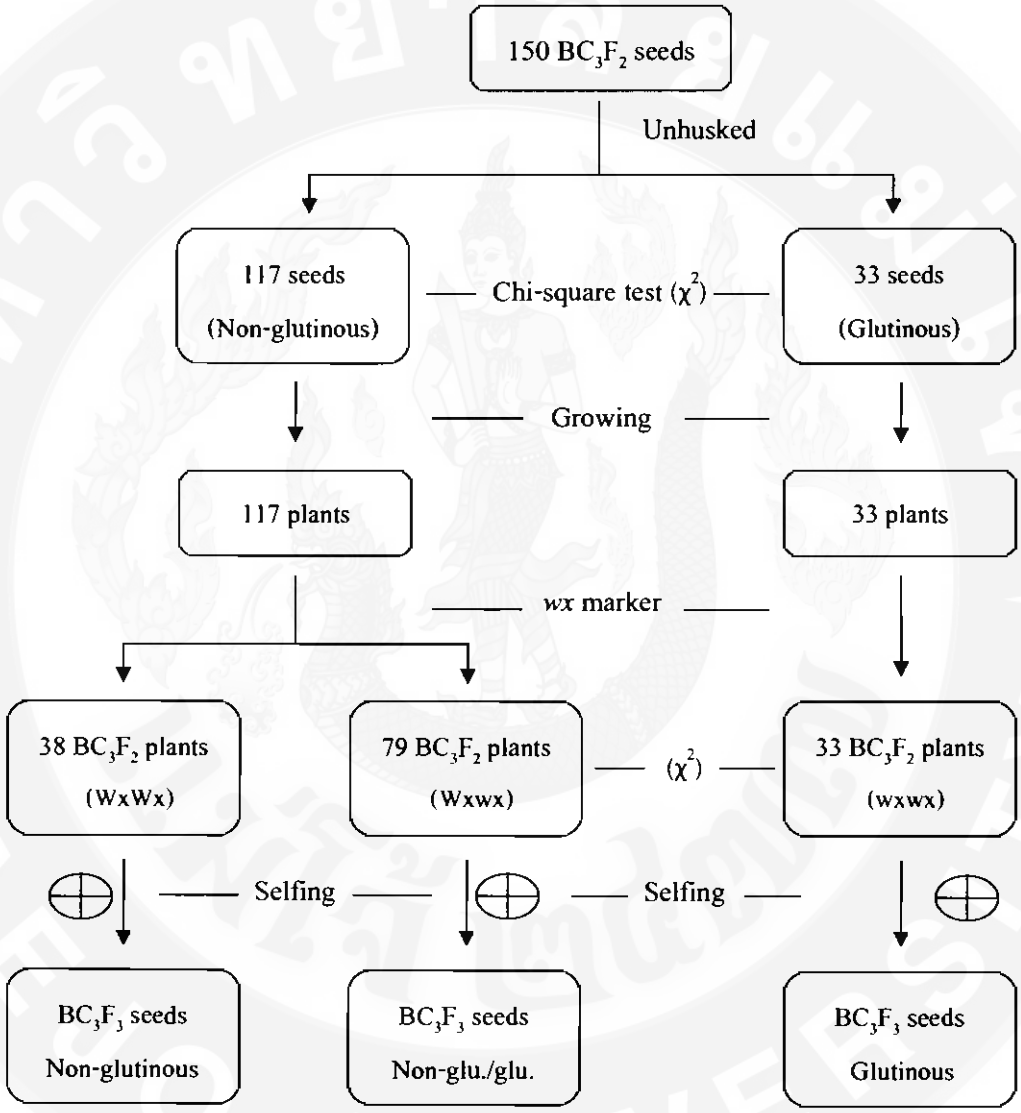


Figure 32 Schematic representation of the detecting process for phenotypes of BC_3F_2 seeds, genotypes of BC_3F_2 plants and self-pollinated to BC_3F_3 seeds by growing in the greenhouse and exposed to light for 14 hours per day

Studying phenotypie ratio of non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds, 150 BC_3F_2 seeds were unhusked and the results showed that 117 seeds were non-glutinous as shown by translueent endosperm grain appearance, while other 33 seeds were glutinous as shown by chalky endosperm grain appearance (Figure 32). Observation values for each phenotypes of BC_3F_2 seeds were later examined by using chi-square test to determine if they followed the First Mendelian Law of Heredity under hypothesis that the phenotypic ratio of BC_3F_2 seeds must be equivalent to 3/4 non-glutinous : 1/4 glutinous. Analyzing these data by chi-square test (χ^2 test), it was found that the ratio of 117 : 33 was in accordance with 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 0.72 < \chi^2 (0.05, 1) = 3.84$, thus indicating that the phenotypic ratio of BC_3F_2 seeds followed the First Mendelian Law of Heredity (Table 11).

Table 11 Testing for phenotypic ratio of non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds, genotypic ratio of *Wx/wx* gene on BC_3F_2 plants using chi-square test (χ^2) and phenotype of non-glutinous/glutinous seeds endosperm on BC_3F_3 seeds of 150 plants of BC_3F_2 population grown in the greenhouse under long-day condition of light exposure of 14 hours per day

Phenotypes of BC_3F_2 seeds[2]				**Genotypes of BC_3F_2 plants**[1]				**BC_3F_3 seeds phenotype of BC_3F_2 plants**	
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Number of plants
Non-glutinous	117	3/4*150 = 112.5	0.18	WxWx	38	1/4*150 = 37.5	0.01	Non-glutinous	38
				Wxwx	79	2/4*150 = 75.0	0.21	Non-glutinous/glutinous	79
Glutinous	33	1/4*150 = 37.5	0.54	wxwx	33	1/4*150 = 37.5	0.54	Glutinous	33
Total	150	150	0.72	Total	150	150	0.76	Total	150
Chi-square (χ^2)			**0.72^{ns}**	**Chi-square (χ^2)**			**0.76^{ns}**		

1^{ns} with statistically significant difference when compared with tabular chi-square value of 5.99 at 95% confidence level and df = 2

2^{ns} with statistically significant difference when compared with tabular chi-square value of 3.84 at 95% confidence level and df = 1

Genotypic ratio of *Wx/wx* gene in BC_3F_2 population was done by comparing the DNA banding size produced by PCR of each individual plants of BC_3F_2 population with DNA banding size produced by PCR of non-glutinous RD 15 rice variety and glutinous rice line, improved RD 6. The RD 15 variety had homozygous dominant WxWx genotype, while the improved RD 6 line had homozygous recessive wxwx genotype. As a criterion, if BC_3F_2 plants had a DNA banding size of PCR product to be similar to a DNA banding size of PCR product of RD 15 variety, meaning that BC_3F_2 plants contained homozygous dominant genotype WxWx, by contrast, if BC_3F_2 plants had a DNA banding size of PCR product to be similar to a DNA banding size of PCR product of the improved RD 6 line, meaning that BC_3F_2 plants contained the homozygous recessive genotype wxwx, and if BC_3F_2 plants had two DNA bands of PCR product: one was the same size as the DNA band RD 15 variety and the other one was the same size as the DNA band of the improved RD 6 line, meaning that BC_3F_2 plants contained heterozygous genotype Wxwx (Figure 33). Based on these comparisons, among 150 plants of BC_3F_2 population, 38 plants were detected for homozygous dominant WxWx genotype, 79 plants were detected for heterozygous Wxwx genotype and the other 33 plants were detected for homozygous recessive wxwx genotype. These observed values for each genotypes were then tested by using chi-square test to determine whether they followed the First Mendelian Law of Heredity or not under the hypothesis that the genotypic ratio of BC_3F_2 plants must be equivalent to 1/4 Sd1Sd1 : 2/4 Sd1sd1 : 1/4 sd1sd1 ratio. Results showed that the ratio of 38 : 79 : 33 was in accordance with 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 0.76 < \chi^2 (0.05, 2) = 5.99$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the First Law of Mendel (Table 11). From this result, it was found that the genotype of BC_3F_2 plants were corresponding to their BC_3F_2 seeds phenotype. The seeds which were glutinous when theirs plants had genotype wxwx only, while the seeds which were non-glutinous when theirs plants had two genotypes: WxWx and Wxwx.

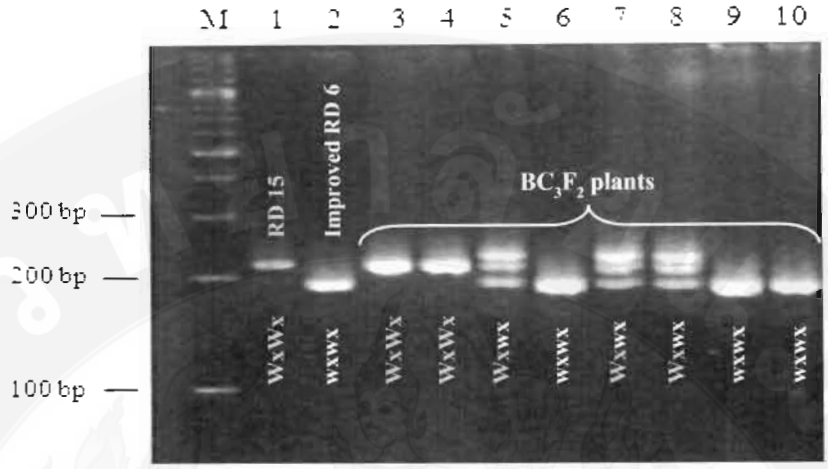


Figure 33 Shows a sample gel photograph under UV light to observe the DNA banding size of BC_3F_2 plants and their parents produced from PCR product when using *wx* marker as a primer that is specific for *Wx/wx* gene together with DNA templates of various genotypes, with M indicated the DNA size marker as standard of 100 bp ladder, lane 1 indicated RD 15 variety with WxWx genotype; lane 2 indicated improved RD 6 line with wxwx genotype; lanes 3-4 indicated BC_3F_2 plants with WxWx genotype; lanes 5 and 7-8 indicated BC_3F_2 plants with Wxwx genotypes; lanes 6, 9-10 indicated BC_3F_2 plants with wxwx genotype

On the other hand, testing for phenotyping of non-glutinous/glutinous seeds endosperm on BC_3F_3 seeds, the light was turned off after 180 lighting days at 14 hours per day, and 30 days later all of the BC_3F_2 plants were flowering and self-pollinated to get BC_3F_3 seeds (Figure 32). 10 seeds of each plant were later unhusked to check their non-glutinous or glutinous seeds endosperm phenotype. Data from this experiment showed that the phenotypes of all BC_3F_3 seeds were corresponding to their genotypes of BC_3F_2 plants, meaning that the BC_3F_2 plants had homozygous dominant WxWx genotypes showing translucent endosperm grain appearance on BC_3F_3 seeds thus giving non-glutinous seeds, while the BC_3F_2 plants had homozygous recessive wxwx genotypes showing chalky endosperm grain appearance on BC_3F_3 seeds thus giving glutinous seeds, but the BC_3F_2 plants had heterozygous Wxwx genotype showing both translucent and chalky endosperm grain appearance on BC_3F_3 seeds thus giving non-glutinous and glutinous endosperms seeds (Figure 34-35). From this result, it was found that the phenotypes of BC_3F_3

seeds were corresponding to their genotype of BC_3F_2 plants. The BC_3F_3 seeds were non-glutinous when their BC_3F_2 plants had genotype WxWx only; the BC_3F_3 seeds were glutinous when their BC_3F_2 plants had genotype wxwx only, while the BC_3F_3 seeds were both non-glutinous and glutinous when their BC_3F_2 plants had genotype Wxwx (Table 11).

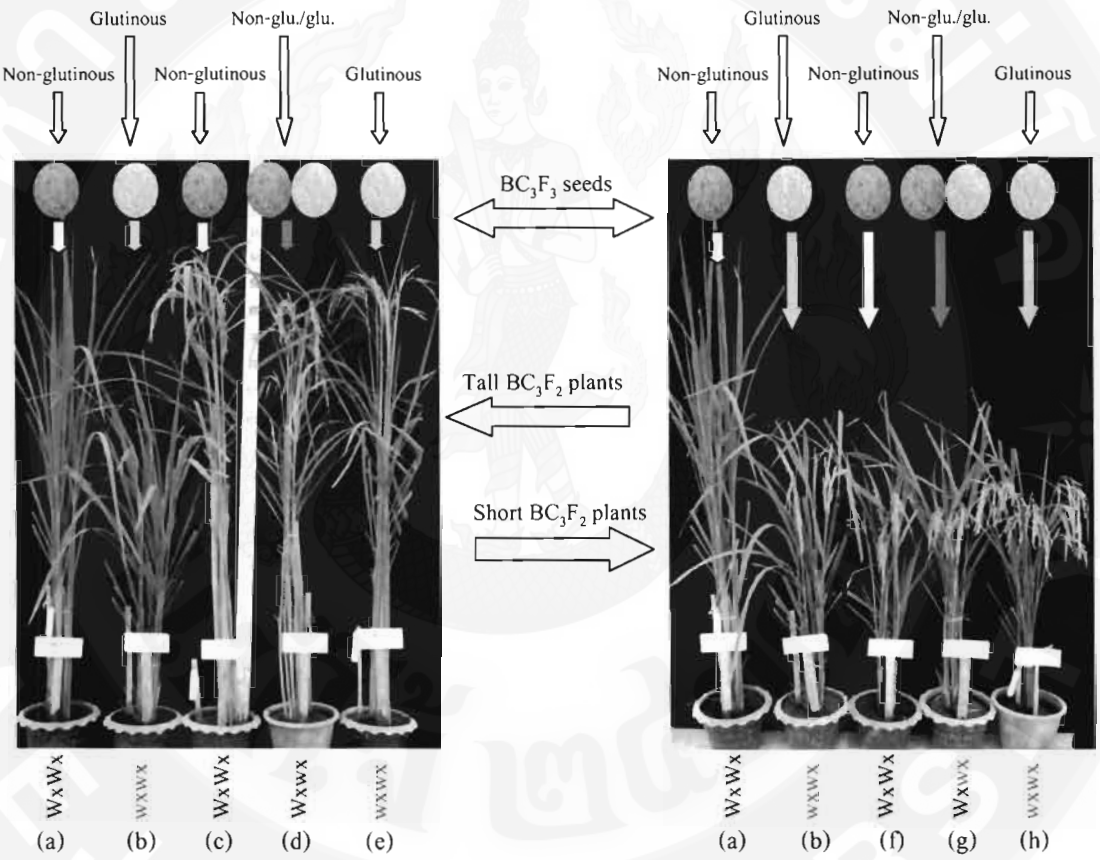
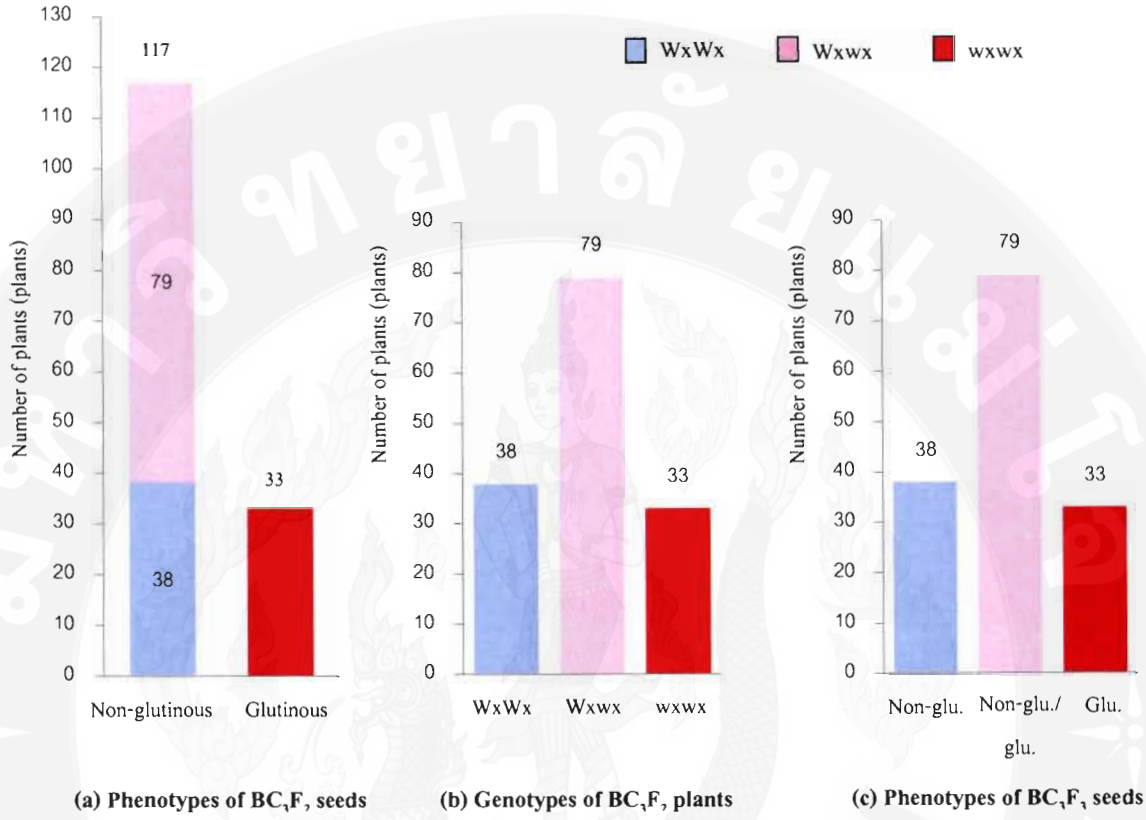


Figure 34 Shows the phenotypes of non-glutinous and glutinous seeds endosperm on BC_3F_3 seeds of tall and short BC_3F_2 plants grown under conditions of long-day lengths period exposed to light for 14 hours/day by (a) RD 15 variety with non-glutinous seed, (b) improved RD 6 line with glutinous seed, (c) and (f) were tall and short BC_3F_2 plants, respectively with non-glutinous of BC_3F_3 seeds, (d) and (g) were tall and short BC_3F_2 plants, respectively with both non-glutinous and glutinous of BC_3F_3 seeds, (e) and (h) were tall and short BC_3F_2 plants, respectively with glutinous of BC_3F_3 seeds



(a) Phenotypes of BC_3F_2 seeds **(b) Genotypes of BC_3F_2 plants** **(c) Phenotypes of BC_3F_3 seeds**

Figure 35 Shows the amount of the (a) phenotype of non-glutinous and glutinous endosperm on BC_3F_2 seeds, (b) genotype of *Wx/wx* gene on BC_3F_2 plants, and (c) phenotype of non-glutinous and glutinous seeds endosperm on BC_3F_3 seeds of 150 BC_3F_2 plants grown in the greenhouse under condition of long-day length periods exposed to light for 14 hours per day

4.3.4 Inheritance of *Hd1/hd1* and *Sd1/sd1* genes in BC_3F_2 population by using *hd1* and *sd1* markers which were part of *Hd1/hd1* and *Sd1/sd1* gene, respectively

Studied genotypic ratios of *Hd1/hd1* and *Sd1/sd1* genes on 150 BC_3F_2 plants, the result showed that, 10 plants were detected for homozygous dominant Hd1Hd1Sd1Sd1 genotype, 15 plants were detected for Hd1Hd1Sd1sd1 genotype, 16 plants were detected for Hd1hd1Sd1Sd1 genotype, 38 plants were detected for Hd1hd1Sd1sd1 genotype, 12 plants were detected for Hd1Hd1sd1sd1 genotype, 16 plants were detected for Hd1hd1sd1sd1 genotype, 11 plants were detected for hd1hd1Sd1Sd1 genotype, 20 plants were detected for hd1hd1Sd1sd1 genotype and

12 plants were detected for homozygous recessive hd1hd1sd1sd1 genotype. These observed values for each genotypes were then tested by using chi-square test to determine whether they followed the Second Mendelian Law of Heredity or not under the hypothesis that the genotypic ratios of BC_3F_2 plants must be equivalent to 1/16 Hd1Hd1Sd1Sd1 : 2/16 Hd1Hd1Sd1sd1 : 2/16 Hd1hd1Sd1Sd1 : 4/16 Hd1hd1Sd1sd1 : 1/16 Hd1Hd1sd1sd1 : 2/16 Hd1hd1sd1sd1 : 1/16 hd1hd1Sd1Sd1 : 2/16 hd1hd1Sd1sd1 : 1/16 hd1hd1sd1sd1 ratio. Result showed that the ratio of 10 : 15 : 16 : 38 : 12 : 16 : 11 : 20 : 12 was in accordance with 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 3.44 < \chi^2 (0.05, 8) = 15.51$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the Second Law of Mendel (Table 12).

Table 12 Testing for genotypic ratios of *Hd1/hd1* and *Sd1/sd1* genes and phenotypic ratios of photoperiod sensitive/non-photoperiod sensitive and tall/short plants of 150 plants of BC_3F_2 population using chi-square test (χ^2) grown in the greenhouse under long-day condition of light exposure for 14 hours per day

Genotypes of BC_3F_2 plants[1]				**Phenotypes of BC_3F_2 plants[2]**			
Class	Observed (O)	Expeeted (E)	$(O-E)^2/E$	Class	Observed (O)	Expeeted (E)	$(O-E)^2/E$
Hd1Hd1Sd1Sd1	10	1/16*150 = 9.375	0.04	Photoperiod sensitive and tall	79	9/16*150 = 84.375	0.34
Hd1Hd1Sd1sd1	15	2/16*150 = 18.750	0.75				
Hd1hd1Sd1Sd1	16	2/16*150 = 18.750	0.40				
Hd1hd1Sd1sd1	38	4/16*150 = 37.500	0.01				
Hd1Hd1sd1sd1	12	1/16*150 = 9.375	0.74	Photoperiod sensitive and short	28	3/16*150 = 28.125	0.00
Hd1hd1sd1sd1	16	2/16*150 = 18.750	0.40				
hd1hd1Sd1Sd1	11	1/16*150 = 9.375	0.28	Non-photoperiod sensitive and tall	31	3/16*150 = 28.125	0.29
hd1hd1Sd1sd1	20	2/16*150 = 18.750	0.08				
hd1hd1sd1sd1	12	1/16*150 = 9.375	0.74	Non-photoperiod sensitive and short	12	1/16*150 = 9.375	0.74
Total	150	150	3.44	Total	150	150	1.37
Chi-square (χ^2)			**3.44^{ns}**	**Chi-square (χ^2)**			**1.37^{ns}**

1^{ns} with statistically significant difference when compared with tabular chi-square value of 15.51 at 95% confidence level and df = 8

2^{ns} with statistically significant difference when compared with tabular chi-square value of 7.81 at 95% confidence level and df = 3

Testing for phenotypic ratio of photoperiod sensitive/non-photoperiod sensitive and tall/short plants of BC_3F_2 population, data from this experiment indicated that of the 150 BC_3F_2 plants which were grown under long-day length exposed to light for 14 hours per day, 79 plants were photoperiod sensitive and tall, and 28 plants were photoperiod sensitive and short; while another 31 plants were non-photoperiod sensitive and tall, and 12 plants were non-photoperiod sensitive and short (Figure 36-37). Observation values for each phenotypes of BC_3F_2 plants were later examined by using chi-square test to determine if they followed the Second Mendelian Law of Heredity under hypothesis that the phenotypic ratio of BC_3F_2 plants must be equivalent to 9/16 photoperiod sensitive and tall : 3/16 photoperiod sensitive and short : 3/16 non-photoperiod sensitive and tall : 1/16 non-photoperiod sensitive and short. Analyzing these data by chi-square test (χ^2 test), result showed that the ratio of 79 : 28 : 31 : 12 was in accordance with 9 : 3 : 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 1.37 < \chi^2 (0.05, 3) = 7.81$ (Table 12), thus indicating that the phenotypic ratio of BC_3F_2 plants followed the Second Mendelian Law of Heredity. These data also indicated that the phenotypes of BC_3F_2 plants were corresponded to their genotypes.

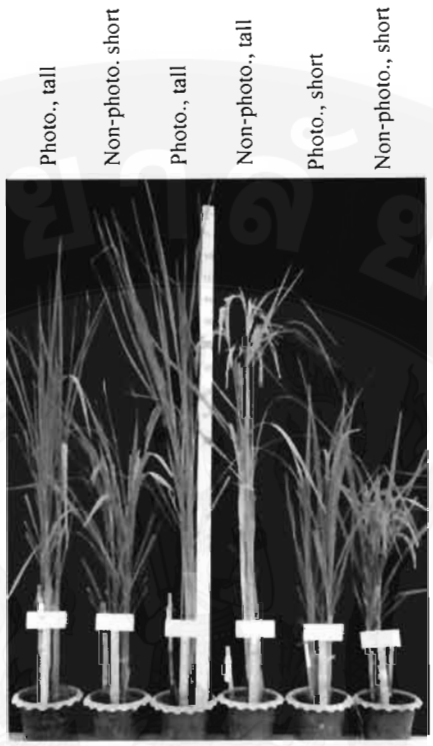


Figure 36 Shows phenotypes of photosensitive, non-photosensitive and plant height of tall and short plants of BC_3F_2 plants grown in the greenhouse under conditions of long-day lengths period exposed to light for 14 hours/day by (a) RD 15 variety with photoperiod sensitive and tall plant; (b) improved RD 6 line with non-photoperiod sensitive and short plant; (c) BC_3F_2 plant with photoperiod sensitive and tall plant; (d) BC_3F_2 plant with non-photoperiod sensitive and tall plant; (e) BC_3F_2 plant with photoperiod sensitive and short plant; and (f) BC_3F_2 plant with non-photoperiod sensitive and short plant

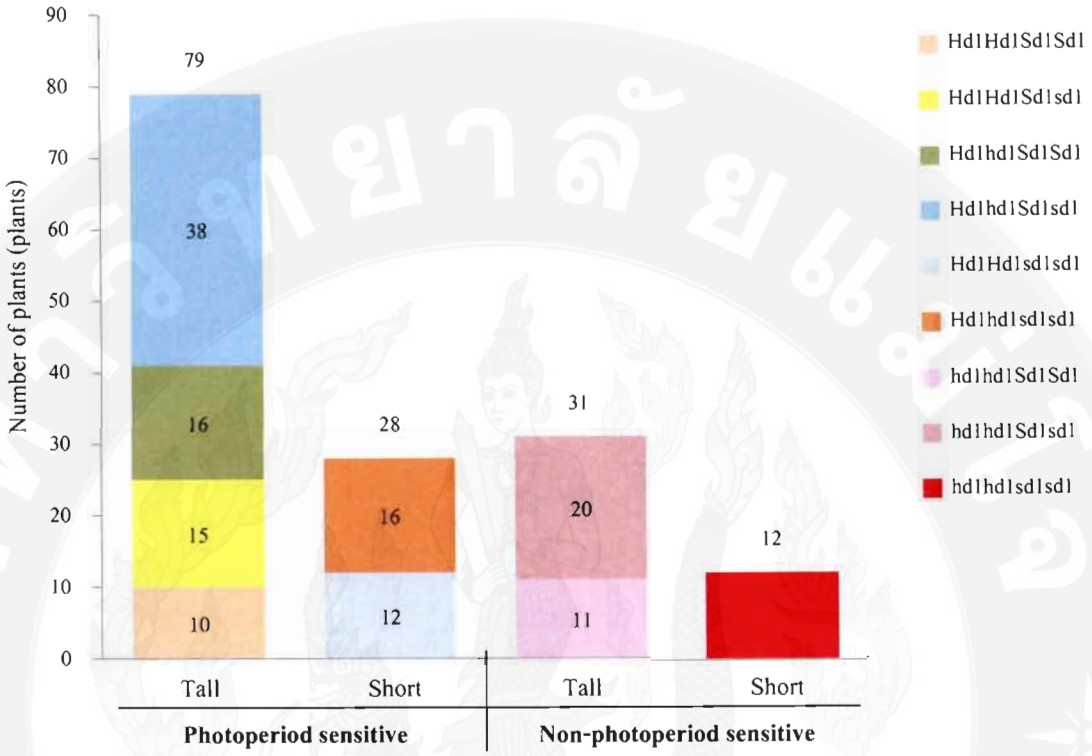


Figure 37 Shows the amount of the genotypes of *Hd1/hd1* and *Sd1/sd1* genes, and phenotypes of photoperiod sensitive/non-photoperiod sensitive plants and tall/short plants of 150 BC_3F_2 plants grown in the greenhouse under conditions of long-day lengths period exposed to light for 14 hours/day

4.3.5 Inheritance of *Hd1/hd1* and *Wx/wx* genes in BC_3F_2 population by using *hd1* and *wx* markers which were part of *Hd1/hd1* and *Wx/wx* genes, respectively

Studying the genotypic ratios of *Hd1/hd1* and *Wx/wx* genes on 150 BC_3F_2 plants, the result showed that 12 plants were detected for Hd1Hd1WxWx genotype, 16 plants were detected for Hd1hd1WxWx genotype, 18 plants were detected for Hd1Hd1Wxwx genotype, 34 plants were detected for Hd1hd1Wxwx genotype, 7 plants were detected for Hd1Hd1wxwx genotype, 20 plants were detected for Hd1hd1wxwx genotype, 5 plants were detected for hd1hd1WxWx genotype, 27 plants were detected for hd1hd1Wxwx genotype and 11 plants were detected for hd1hd1wxwx genotype. These observed values for each genotypes were then tested by using chi-square test to determine whether they followed the Second Mendelian Law of

Heredity or not, under the hypothesis that the genotypic ratio of BC_3F_2 plants must be equivalent to 1/16 Hd1Hd1WxWx : 2/16 Hd1hd1WxWx : 2/16 Hd1Hd1Wxwx : 4/16 Hd1hd1Wxwx : 1/16 Hd1Hd1wxwx : 2/16 Hd1hd1wxwx : 1/16 hd1hd1WxWx : 2/16 hd1hd1Wxwx : 1/16 hd1hd1wxwx ratio. Result showed that the ratio of 12 : 16 : 18 : 34 : 7 : 20 : 5 : 27 : 11 was accordance with 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 8.13 < \chi^2 (0.05, 8) = 15.51$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the Second Law of Mendel (Table 13).

Testing for phenotypic ratios of photopcriod sensitive/non-photoperiod sensitive on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations, result showed that 80 plants were photoperiod sensitive and non-glutinous, 27 plants were photoperiod sensitive and glutinous, 32 plants were non-photoperiod sensitive and non-glutinous, and 11 plants were non-photoperiod sensitive and glutinous. Observation values for each phenotypes of BC_3F_2 plants and seeds were later examined by using chi-square test to determine if they followed the Second Mendelian Law of Heredity under hypothesis that the phenotypic ratio of BC_3F_2 plants and seeds must be equivalent to 9/16 photoperiod sensitive and non-glutinous : 3/16 photoperiod sensitive and glutinous : 3/16 non-photoperiod sensitive and non-glutinous : 1/16 non-photoperiod sensitive and glutinous. Analyzed these data by chi-square test (χ^2 test), result showed that the ratio of 80 : 27 : 32 : 11 was in accordance with 9 : 3 : 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 1.09 < \chi^2 (0.05, 3) = 7.81$ (Table 13 and Figure 38), thus indicating that the phenotypic ratio of BC_3F_2 plants and seeds followed the Second Mendelian Law of Heredity. These data also indicated that the phenotypes of BC_3F_2 plants and seeds were corresponded to their genotypes of BC_3F_2 plants.

Table 13 Testing for genotypic ratios of *Hd1/hd1* and *Wx/wx* genes on BC_3F_2 plants and phenotypic ratios of photoperiod sensitive/non-photoperiod sensitive on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations using chi-square test (χ^2) grown in the greenhouse under long-day length condition of light exposure for 14 hours per day

Genotypes of BC_3F_2 plants[1]				Phenotypes of BC_3F_2 plants and seeds[2]			
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$
Hd1Hd1WxWx	12	1/16*150 = 9.375	0.74	Photosensitive and non-glutinous	80	9/16*150 = 84.375	0.23
Hd1hd1WxWx	16	2/16*150 = 18.750	0.40				
Hd1Hd1Wxwx	18	2/16*150 = 18.750	0.03				
Hd1hd1Wxwx	34	4/16*150 = 37.500	0.33				
Hd1Hd1wxwx	7	1/16*150 = 9.375	0.60	Photosensitive and glutinous	27	3/16*150 = 28.125	0.05
Hd1hd1wxwx	20	2/16*150 = 18.750	0.08				
hd1hd1WxWx	5	1/16*150 = 9.375	2.04	Photosensitive and non-glutinous	32	3/16*150 = 28.125	0.53
hd1hd1Wxwx	27	2/16*150 = 18.750	3.63				
hd1hd1wxwx	11	1/16*150 = 9.375	0.28	Non-photosensitive and glutinous	11	1/16*150 = 9.375	0.28
Total	150	150	8.13		150	150	1.09
	Chi-square (χ^2)		**8.13^{ns}**		**Chi-square (χ^2)**		**1.09^{ns}**

1^{ns} with statistically significant difference when compared with tabular chi-square value of 15.51 at 95% confidence level and df = 8

2^{ns} with statistically significant difference when compared with tabular chi-square value of 7.81 at 95% confidence level and df = 3

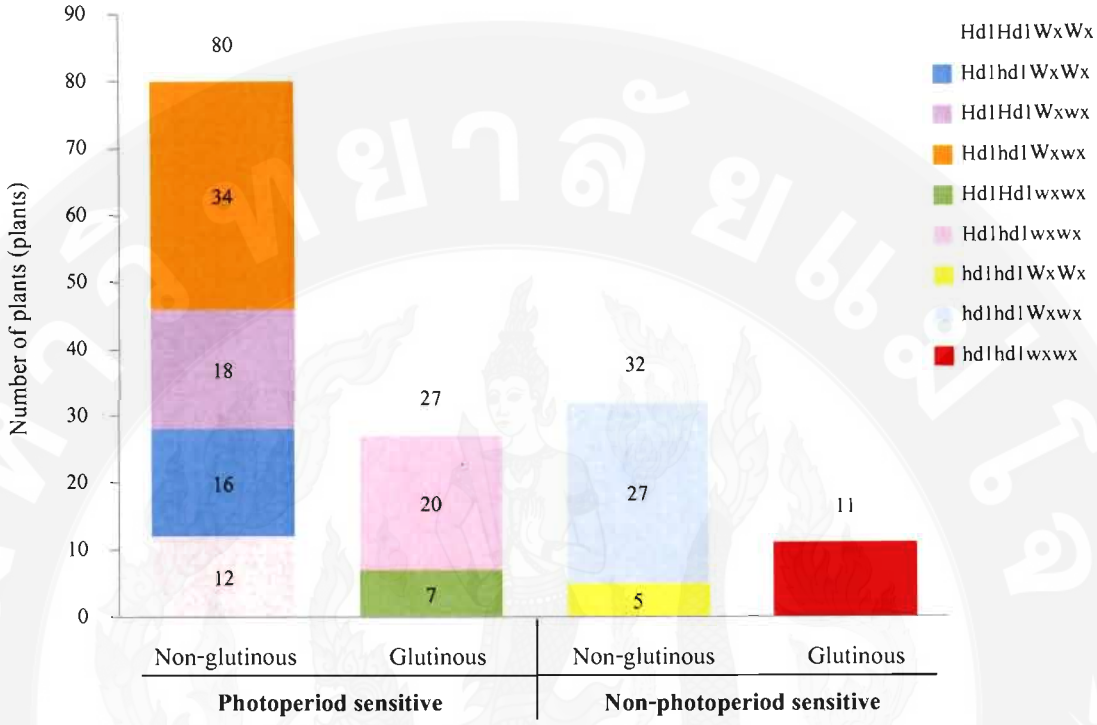


Figure 38 Shows the amount of the genotypes of *Hd1/hd1* and *Wx/wx* genes, and phenotypes of photoperiod sensitive/non-photoperiod sensitive on BC_3F_2 plants with non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations grown in the greenhouse under conditions of long-day lengths period exposed to light for 14 hours/day

4.3.6 Inheritance of *Sd1/sd1* and *Wx/wx* genes in BC_3F_2 population by using *sd1* and *wx* markers which were part of *Sd1/sd1* and *Wx/wx* gene, respectively

Studied genotypic ratios of *Sd1/sd1* and *Wx/wx* genes on 150 BC_3F_2 plants, the result showed that 4 plants were detected for Sd1Sd1WxWx genotype, 19 plants were detected for Sd1sd1WxWx genotype, 19 plants were detected for Sd1Sd1Wxwx genotype, 42 plants were detected for Sd1sd1Wxwx genotype, 14 plants were detected for Sd1Sd1wxwx genotype, 12 plants were detected for Sd1sd1wxwx genotype, 10 plants were detected for sd1sd1WxWx genotype, 18 plants were detected for sd1sd1Wxwx genotype, and 12 plants were detected for sd1sd1wxwx genotype. These observed values for each genotypes were then tested by using chi-square test to determine

whether they followed the Second Mendelian Law of Heredity or not. Under the hypothesis that the genotypic ratio of BC_3F_2 plants must be equivalent to 1/16 Sd1Sd1WxWx : 2/16 Sd1sd1WxWx : 2/16 Sd1Sd1Wxwx : 4/16 Sd1sd1Wxwx : 1/16 Sd1Sd1wxwx : 2/16 Sd1sd1wxwx : 1/16 sd1sd1WxWx : 2/16 sd1shd1Wxwx : 1/16 sd1sd1wxwx ratio. Result showed that the ratio of 4 : 19 : 19 : 42 : 14 : 12 : 10 : 18 : 12 was accordance with 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 9.15 < \chi^2 (0.05, 8) = 15.51$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the Second Law of Mendel (Table 14).

Testing for phenotypic ratio of tall/short plants on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations, the result indicated that 84 plants were tall and non-glutinous, 26 plants were tall and glutinous, 28 plants were short and non-glutinous, and 12 plants were short and glutinous. Observation values for each phenotype of BC_3F_2 plants and seeds were later examined by using chi-square test to determine if they followed the Second Mendelian Law of Heredity under hypothesis that the phenotypic ratio of BC_3F_2 plants and seeds must be equivalent to 9/16 tall and non-glutinous : 3/16 tall and glutinous : 3/16 short and non-glutinous : 1/16 short and glutinous. Analyzed these data by chi-square test (χ^2 test), result showed that the ratio of 84 : 26 : 28 : 12 was in accordance with 9 : 3 : 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 0.90 < \chi^2 (0.05, 3) = 7.81$ (Table 14 and Figure 39), thus indicating that the phenotypic ratio of BC_3F_2 plants and seeds followed the Second Mendelian Law of Heredity. These data also indicated that the phenotypes of BC_3F_2 plants and seeds were corresponded to their genotypes of BC_3F_2 plants.

Table 14 Testing for genotypic ratios of *Sd1/sd1* and *Wx/wx* genes on BC_3F_2 plants, phenotypic ratios of tall/short plants on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations using chi-square test (χ^2) grown in the greenhouse under long-day condition of light exposure for 14 hours per day

Genotypes of BC_3F_2 plants[1]				Phenotypes of BC_3F_2 plants and seeds[2]			
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$
Sd1Sd1WxWx	4	1/16*150 = 9.375	3.08	Tall and non-glutinous	84	9/16*150 = 84.375	0.00
Sd1sd1WxWx	19	2/16*150 = 18.750	0.00				
Sd1Sd1Wxwx	19	2/16*150 = 18.750	0.00				
Sd1sd1Wxwx	42	4/16*150 = 37.500	0.54				
Sd1Sd1wxwx	14	1/16*150 = 9.375	2.28	Tall and glutinous	26	3/16*150 = 28.125	0.16
Sd1sd1wxwx	12	2/16*150 = 18.750	2.43				
sd1sd1WxWx	10	1/16*150 = 9.375	0.04	Short and non-glutinous	28	3/16*150 = 28.127	0.00
sd1sd1Wxwx	18	2/16*150 = 18.750	0.03				
sd1sd1wxwx	12	1/16*150 = 9.375	0.74	Short and glutinous	12	1/16*150 = 9.375	0.74
Total	150	150	9.15		150	150	0.90
	Chi-square (χ^2)		**9.15^{ns}**		**Chi-square (χ^2)**		**0.90^{ns}**

1[ns] with statistically significant difference when compared with tabular chi-square value of 15.51 at 95% confidence level and df = 8

2[ns] with statistically significant difference when compared with tabular chi-square value of 7.81 at 95% confidence level and df = 3

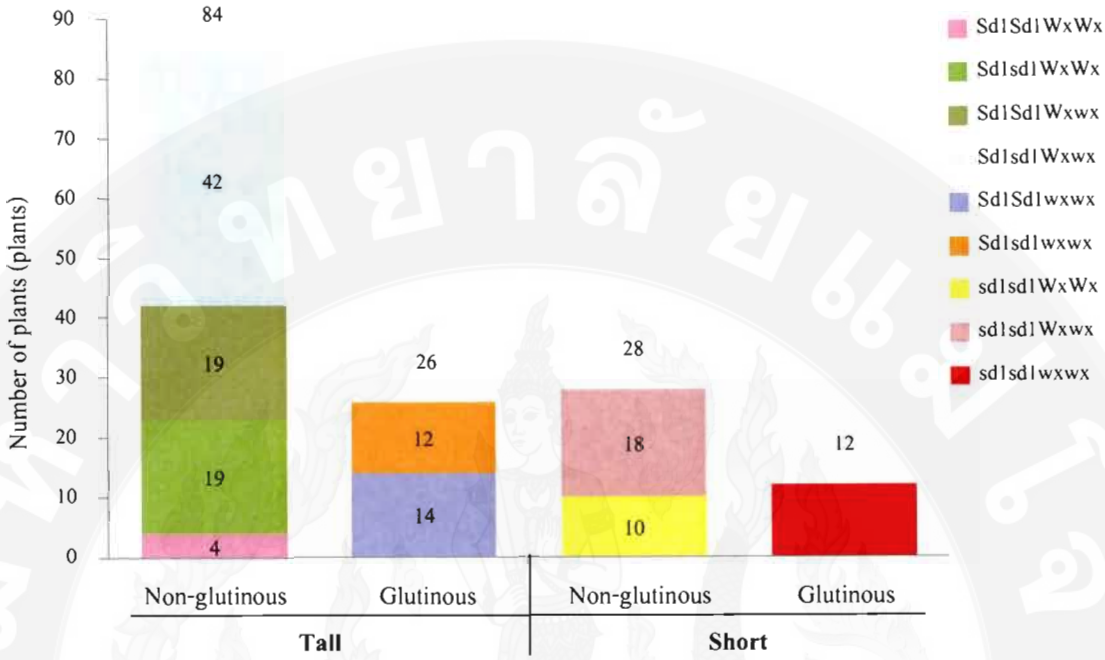


Figure 39 Shows the amount of the genotypes of *Sd1/sd1* and *Wx/wx* genes, phenotypes of tall/short plants on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations grown in the greenhouse under conditions of long-day lengths exposed to light for 14 hours/day

4.3.7 Inheritance of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes in BC_3F_2 population by using *hd1*, *sd1* and *wx* markers which were part of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes, respectively

Studied genotypic ratios of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes on 150 plants of BC_3F_2 population, results showed that two plants were detected for Hd1Hd1Sd1Sd1WxWx genotype, 6 plants were detected for Hd1Hd1Sd1sd1WxWx genotype, 1 plant was detected for Hd1hd1Sd1Sd1WxWx genotype, 10 plants were detected for Hd1hd1Sd1sd1WxWx genotype, 5 plants were detected for Hd1Hd1Sd1Sd1Wxwx genotype, 7 plants were detected for Hd1Hd1Sd1sd1Wxwx genotype, 9 plants were detected for Hd1sd1Sd1Sd1Wxwx genotype, 22 plants were detected for Hd1hd1Sd1sd1Wxwx genotype, 3 plants were detected for Hd1Hd1Sd1Sd1wxwx genotype, 2 plants were detected for Hd1Hd1Sd1sd1wxwx genotype, 6 plants were detected for Hd1hd1Sd1Sd1wxwx genotype, 6 plants were detected for Hd1hd1Sd1sd1wxwx genotype, 4 plants

were detected for Hd1Hd1sd1sd1WxWx genotype, 5 plants were detected for Hd1hd1sd1sd1WxWx genotype, 6 plants were detected for Hd1Hd1sd1sd1Wxwx genotype, 3 plants were detected for Hd1hd1sd1sd1Wxwx genotype, 2 plants were detected for Hd1Hd1sd1sd1wxwx genotype, 8 plants were detected for Hd1hd1sd1sd1wxwx genotype, 1 plants was detected for hd1hd1Sd1Sd1WxWx genotype, 3 plants were detected for hd1hd1Sd1sd1WxWx genotype, 5 plants were detected for hd1hd1Sd1Sd1Wxwx genotype, 13 plants were detected for hd1hd1Sd1sd1Wxwx genotype, 5 plants were detected for hd1hd1Sd1Sd1Wxwx genotype, 4 plants were detected for hd1hd1Sd1sd1wxwx genotype, 1 plants was detected for hd1hd1sd1sd1WxWx genotype, 9 plants were detected for hd1hd1sd1sd1Wxwx genotype, and 2 plants were detected for hd1hd1sd1sd1wxwx genotype. These observed values for each genotypes of 150 BC_3F_2 plants were then tested by using chi-square test to determine whether they followed the Tri-hybrid Cross or not under the hypothesis that the genotypic ratio of BC_3F_2 plants must be equivalent to 1/64 Hd1Hd1Sd1Sd1WxWx : 2/64 Hd1Hd1Sd1sd1WxWx : 2/64 Hd1hd1Sd1Sd1WxWx : 4/64 Hd1hd1Sd1sd1WxWx : 2/64 Hd1Hd1Sd1Sd1Wxwx : 4/64 Hd1Hd1Sd1sd1Wxwx : 4/64 Hd1sd1Sd1Sd1Wxwx : 8/64 Hd1hd1Sd1sd1Wxwx : 1/64 Hd1Hd1Sd1Sd1wxwx : 2/64 Hd1Hd1Sd1sd1wxwx : 2/64 Hd1hd1Sd1Sd1wxwx : 4/64 Hd1hd1Sd1sd1wxwx : 1/64 Hd1Hd1sd1sd1WxWx : 2/64 Hd1hd1sd1sd1WxWx : 2/64 Hd1Hd1sd1sd1Wxwx : 4/64 Hd1hd1sd1sd1Wxwx : 1/64 Hd1Hd1sd1sd1wxwx : 2/64 Hd1hd1sd1sd1wxwx : 1/64 hd1hd1Sd1Sd1WxWx : 2/64 hd1hd1Sd1sd1WxWx : 2/64 hd1hd1Sd1Sd1Wxwx : 4/64 hd1hd1Sd1sd1Wxwx : 1/64 hd1hd1Sd1Sd1Wxwx : 2/64 hd1hd1Sd1sd1wxwx : 1/64 hd1hd1sd1sd1WxWx : 2/64 hd1hd1sd1sd1Wxwx : 1/64 hd1hd1sd1sd1wxwx. The result showed that the ratio of 2 : 6 : 1 : 10 : 5 : 7 : 9 : 22 : 3 : 2 : 6 : 6 : 4 : 5 : 6 : 3 : 2 : 8 : 1 : 3 : 5 : 13 : 5 : 4 : 1 : 9 : 2 was accordance with 1 : 2 : 2 : 4 : 2 : 4 : 4 : 8 : 1 : 2 : 2 : 4 : 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 ratio segregation according to chi-square test (χ^2 test), $\chi^2 = 26.85 < \chi^2 (0.05, 26) = 38.9$, thus indicating that the genotypic ratio of BC_3F_2 plants followed the Tri-hybrid Cross (Table 15).

Table 15 Testing for genotypic ratio of *Hd1/hd1*, *Sd1/sd1* and *Wx/wx* genes, phenotypic ratios of photoperiod sensitive/non-photoperiod sensitive, tall/short plants on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 plants of BC_3F_2 population using chi-square test (χ^2) grown in the greenhouse under long-day condition of light exposure for 14 hours per day

Genotypes of BC_3F_2 plants[1]				Phenotypes of BC_3F_2 plants and seeds[2]			
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$
Hd1Hd1Sd1Sd1WxWx	2	1/64*150 = 2.344	0.05	Photosensitive, tall and non-glutinous	62	27/64*150 = 63.281	0.03
Hd1Hd1Sd1sd1WxWx	6	2/64*150 = 4.688	0.37				
Hd1hd1Sd1Sd1WxWx	1	2/64*150 = 4.688	2.90				
Hd1hd1Sd1sd1WxWx	10	4/64*150 = 9.375	0.04				
Hd1Hd1Sd1Sd1Wxwx	5	2/64*150 = 4.688	0.02				
Hd1Hd1Sd1sd1Wxwx	7	4/64*150 = 9.375	0.60				
Hd1hd1Sd1Sd1Wxwx	9	4/64*150 = 9.375	0.02				
Hd1hd1Sd1sd1Wxwx	22	8/64*150 = 18.750	0.56				
Hd1Hd1Sd1Sd1wxwx	3	1/64*150 = 2.344	0.18	Photosensitive, tall and glutinous	17	9/64*150 =21. 094	0.79
Hd1Hd1Sd1sd1wxwx	2	2/64*150 = 4.688	1.54				
Hd1hd1Sd1Sd1wxwx	6	2/64*150 = 4.688	0.37				
Hd1hd1Sd1sd1wxwx	6	4/64*150 = 9.375	1.22				
Hd1Hd1sd1sd1WxWx	4	1/64*150 = 2.344	1.17	Photosensitive, short and non-glutinous	18	9/64*150 = 21.094	0.45
Hd1hd1sd1sd1WxWx	5	2/64*150 = 4.688	0.02				
Hd1Hd1sd1sd1Wxwx	6	2/64*150 = 4.688	0.37				
Hd1hd1sd1sd1Wxwx	3	4/64*150 = 9.375	4.34				

Table 15 (continued)

Genotypes of BC_3F_2 plants[1]				Phenotypes of BC_3F_2 plants and seeds[2]			
Class	Observed (O)	Expected (E)	$(O-E)^2/E$	Class	Observed (O)	Expected (E)	$(O-E)^2/E$
Hd1Hd1sd1sd1wxwx	2	1/64*150 = 2.344	0.05	Photosensitive, short and glutinous	10	3/64*150 = 7.031	1.25
Hd1hd1sd1sd1wxwx	8	2/64*150 = 4.688	2.34				
hd1hd1Sd1Sd1WxWx	1	1/64*150 = 2.344	0.77	Non-photosensitive, tall and non-glutinous	22	9/64*150 = 21.094	0.04
hd1hd1Sd1sd1WxWx	3	2/64*150 = 4.688	0.61				
hd1hd1Sd1Sd1Wxwx	5	2/64*150 = 4.688	0.02				
hd1hd1Sd1sd1Wxwx	13	4/64*150 = 9.375	1.40				
hd1hd1Sd1Sd1wxwx	5	1/64*150 = 2.344	3.01	Non-photosensitive, tall and glutinous	9	3/64*150 = 7.031	0.55
hd1hd1Sd1sd1wxwx	4	2/64*150 = 4.688	0.10				
hd1hd1sd1sd1WxWx	1	1/64*150 = 2.344	0.77	Non-photosensitive, short and non-glutinous	10	3/64*150 = 7.031	1.25
hd1hd1sd1sd1Wxwx	9	2/64*150 = 4.688	3.97				
hd1hd1sd1sd1wxwx	2	1/64*150 = 2.344	0.05	Non-photosensitive, short and glutinous	2	1/64*150 = 2.344	0.05
Total	150	150	26.85		150	150	4.41
Chi-square (χ^2)			**26.85^{ns}**	**Chi-square (χ^2)**			**4.41^{ns}**

1^{ns} with statistically significant difference when compared with tabular chi-square value of 38.9 at 95% confidence level and df = 26

2^{ns} with statistically significant difference when compared with tabular chi-square value of 14.1 at 95% confidence level and df = 7

Testing for phenotypic ratios of photoperiod sensitive/non-photoperiod sensitive and tall/short plants on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations, result indicated that 62 plants were photoperiod sensitive, tall and non-glutinous; 17 plants were photoperiod sensitive, tall and glutinous; 18 plants were photoperiod sensitive, short and non-glutinous; 10 plants were photoperiod sensitive, short and glutinous; while 22 plants were non-photoperiod sensitive, tall and non-glutinous; 9 plants were non-photoperiod sensitive, tall and glutinous; 10 plants non-photoperiod sensitive, short and non-glutinous; and 2 plants were non-photoperiod sensitive, short and glutinous. Observation values for each phenotype of BC_3F_2 plants and seeds were later examined by using chi-square test to determine if they followed the Tri-hybrid Cross under hypothesis that the phenotypic ratios of BC_3F_2 plants and seeds must be equivalent to 27/64 photoperiod sensitive, tall and non-glutinous : 9/64 photoperiod sensitive, tall and glutinous : 9/64 photoperiod sensitive, short and non-glutinous : 3/64 photoperiod sensitive, short and glutinous : 9/64 non-photoperiod sensitive, tall and non-glutinous : 3/64 non-photoperiod sensitive, tall and glutinous : 3/64 non-photoperiod sensitive, short and non-glutinous : 1/64 non-photoperiod sensitive, short and glutinous. Analyzed these data by chi-square test (χ^2 test), result showed that the ratio of 62 : 17 : 18 : 10 : 22 : 9 : 10 : 2 was in accordance with 27 : 9 : 9 : 3 : 9 : 3 : 3 : 1 ratio segregation according to chi-square test, $\chi^2 = 4.41 < \chi^2 (0.05, 7) = 14.1$ (Table 15 and Figure 40), thus indicating that the phenotypic ratios of BC_3F_2 plants and seeds followed the Tri-hybrid Cross. These data also indicated that the phenotypes of BC_3F_2 plants and seeds were corresponded to their genotypes of BC_3F_2 plants.

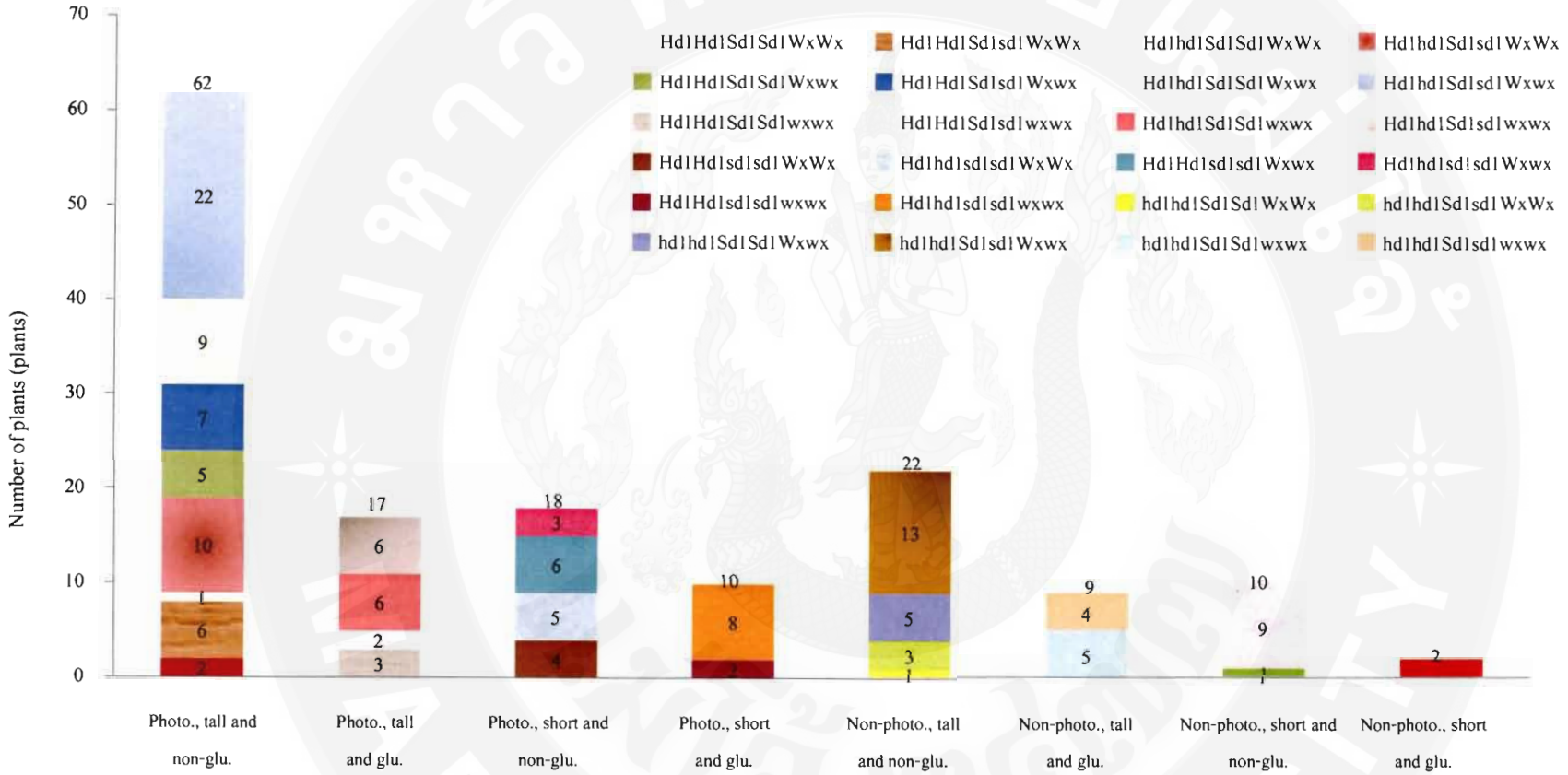


Figure 40 Shows the amount of the genotypes of *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes, phenotypes of photoperiod sensitive/photoperiod insensitive, tall/short plants on BC_3F_2 plants and non-glutinous/glutinous seeds endosperm on BC_3F_2 seeds of 150 BC_3F_2 populations grown in the greenhouse under conditions of long-day lengths exposed to light for 14 hours/day

Photoperiod sensitivity, plant height and endosperm characteristics are three of the most important traits for rice cultivar. With the help of molecular markers assisted backcrossing, we have successfully introduced non-photoperiod sensitive, semi-dwarf and glutinous rice into the RD 15 rice variety.

Flowering time, also known as heading date is an important agronomic determinant for the selection of suitable cropping location and growing seasons for cultivated rice varieties. Research addressing the molecular markers control of heading date (hd) is also important for rice breeding.

In this research, the *hd1* gene which controls non-photoperiod sensitive plant has been used and promote into effect in which improved RD 15 variety for photoperiod insensitive. Result clearly showed that all of the selected four best lines of RD 15 which carried homozygous recessive hd1hd1 genotype were flowering under long-day condition of light exposure for 14 hours per day thus indicated non-photoperiod sensitive. By contrast, photoperiod sensitive RD 15 variety contained homozygous dominant Hd1Hd1 genotype was not flowering in the same condition. This result was similar with the result of Lin *et al.* (2000) who demonstrated that near-isogenic lines containing *hd1* mutant alleles exhibited not only delayed flowering under short-day conditions but also early flowering under long-day conditions. Moreover, this result was quite similar to the result of Nishida *et al.* (2004) who presented that long-day period suppression of rice flowering became apparent when the photoperiod is longer than 13 hours per day.

On the other hand, semi-dwarfism is one of the most important traits in rice cultivar. The semi-dwarf 1 (*sd1*) gene is well known as the "green revolution gene" because of its importance in the development of high-yielding semi-dwarf rice cultivars. It has been studied intensively due to its contribution to the increase in crop production with high yield performance but without culm elongation, lodging and other problems. Aquino and Jennings (1966) first investigated the inheritance of the dwarfness originated from Dee-geo-woo-gene and reported that it was controlled by a single recessive gene, *sd1*. In this experiment, we found that the short plants were controlled by a recessive gene, *sd1*, while the tall plants were controlled by a dominant gene, *Sd1*. Results from this experiment showed that the two best lines of RD 15 which were selected for homozygous recessive sd1sd1 genotype were short at 89 cm high such as RD 15 NSN line and RD 15 NSG line (short plant with sd1sd1 genotype). This result also agreed with

the result of Negrao *et al.* (2010) who reported that plant height differed significantly between the breeding lines. When compared the F_2 mutated *sd1* generation derived from 'Allorio' versus 'Strella', they found that the 'Allorio' population, the mean height ranged from 68.1 cm (F_2 generation with mutated *sd1* in homozygosity) to 129.8 cm (F_2 generation wild type *Sd1*); while in 'Strella' population the mean ranged from 64.0 cm (F_2 generation with *sd1*) to 106.4 cm (F_2 generation wild type *Sd1*). In addition, result in this experiment also agreed with the result of Asano *et al.* (2007) who examined of the 57 semi-dwarf varieties and showed that 38 varieties carried an *sd1* allele that probably controls their semi-dwarf phenotype. Moreover, Zahid *et al.* (2005) studied 14 genotypes of basmati rice and observe high heritability couple with high genetic advance for plant height. He also reported that plant height has negative correlation with grain yield. Our result also showed that grain yield of the selected four best lines of RD 15 was not different with original RD 15, meaning that the plant height was not effective to grain yield. Moreover, result from this research showed that numbers of panicles per hill of the two short plants such as RD 15 NSN line (19 panicles) and RD 15 NSG line (16 panicles) were more than the two tall plants such as RD 15 NTN line (14 panicles) and RD 15 NTG line (12 panicles) but their seeds per panicle were not different. This result were quiet different with the result of Murai *et al.* (2004) who reported that *sd1* gene reduced spikelet's number per panicle, thus resulting in a less seeds per panicle.

Besides, glutinous rice endosperm is known to be controlled by a recessive waxy gene, *wx*. Further results of this experiment indicated that rice plants were selected for homozygous recessive wxwx genotype that showed by a chalky endosperm grain appearance, a glutinous trait such as RD 15 NTG line and RD 15 NSG line (glutinous seeds with wxwx genotype), thus in agreement with Wanchanan *et al.* (2003) who reported that rice tropical glutinous was controlled by a recessive *wx* gene. Another study of Mikami *et al.* (1999) also showed that non-glutinous rice endosperm was controlled by a dominant gene, *Wx* while glutinous rice endosperm was controlled by a recessive gene, *wx*.

Studied on genetic heritability of *Hd1/hd1* gene, a gene controlled the photoperiod response of the plants. The BC_3F_2 plants were originated from MAB of which photoperiod sensitive RD 15 variety as the recipient parent and non-photoperiod sensitive, improved RD 6 line as the donor parent. The comparisons of between genotypic and phenotypic

ratios of 150 BC_3F_2 plants by using chi-square test indicated that they followed the First Mendelian Law of Heredity as showed by 107 photoperiod sensitive (non-flowered plants) and 43 non-photoperiod sensitive (flowered plants), thus giving expected ratio of 3 photoperiod sensitive to 1 non-photoperiod sensitive. This result was similar to the result of Yamaoto *et al.* (1998) who researched on the heritability of *Hd1* gene of BC_3F_2 derived from MAB using Nipponbare, a photoperiod sensitive rice variety as the recipient parent and non-photoperiod sensitive, Ksalath as the donor parent. His results indicated that both the phenotypic and genotypic ratios of BC_3F_2 plants were 3 : 1 and 1 : 2 : 1, respectively, thus following the First Mendelian Law of Heredity since their segregation fitted the Mendelian ratio for single gene segregation.

In genetic heritability of *Sd1/sd1* gene that controlled plant height in rice showed that the BC_3F_2 plants were originated from MAB of which tall plant, RD 15 variety as the recipient parent and short plant, improved RD 6 line as the donor parent. The result indicated that the BC_3F_2 plants produced by crossing between RD 15 and improved RD 6 which were tested by using chi-square test followed the First Mendelian Law of Heredity as showed by 110 tall plants and 40 short plants, thus giving the expected ratio of 3 tall to 1 short. This result was quite similar with the result of Cho *et al.* (1994) who showed that the F_2 population derived from Shiokari/Shiokari (*sd1*) was evaluated for culm length and showed a bimodal distribution. Out of 185 plants investigated, 137 plants were tall and 48 plants were short, so giving the expected ratio of 3 tall to 1 short and providing evidence that the semi-dwarf stature in this population is conferred by a single recessive gene *sd1*, thus followed the First Mendelian Law of Heredity.

The waxy locus on the first linkage group (chromosome 6) in rice controls the production of amylose in the endosperm (Iwata and Omura, 1971). In this experiment, the genetic heritability of *Wx/wx* gene which controls endosperm showed that of 150 BC_3F_2 seeds which were originated from MAB by crossing between non-glutinous RD 15 variety and glutinous improved RD 6 line. The comparison between genotypic ratio of BC_3F_2 plants and phenotypic ratios of BC_3F_2 seeds by using chi-square test indicated that they followed the First Mendelian Law of Heredity as showed by 117 non-glutinous seeds and 33 glutinous seeds. This result was quite similar with the result of Chao (1928) who distributed F_2 populations of 14 combinations of unrelated varieties and showed that of the 57,925 rice grains, 44,043 rice grains were non-glutinous and 13,882 rice grains were glutinous, thus giving the expected ratio of 3 non-glutinous

to 1 glutinous rice. Further, Andro *et al.* (2010) who showed that F_2 seeds derived from a crossing between non-glutinous rice cultivar Hokkai287 and the glutinous rice cultivar Hakuchomochi. The F_2 seeds showed light to heavy dull endosperm and glutinous endosperm. Seeds with dull endosperm were non-glutinous. A ratio of total dull endosperm to glutinous endosperm fitted a 3 : 1 ratio, thus followed the First Law of Mendel.

CHAPTER 5

Conclusion

This thesis was conducted on studying of improvement of RD 15 rice variety for non-photoperiod sensitive, semi-dwarf and glutinous rice through molecular markers assisted backcrossing (MAB). RD 15 variety was used as the recipient parent to cross with improved RD 6 line as the donor parent to generate BC_5F_1 seeds through six seasons.

Results from this thesis showed that MAB was successfully used to produce BC_3F_1 seeds to BC_5F_1 seeds in the greenhouse. According to this result, six BC_3F_1 seeds were produced in the fourth season (rainy season of 2009) with heterozygous Hd1hd1Sd1sd1Wxwx genotype only. Three BC_4F_1 seeds were produced in the fifth season (dry season of 2010) with heterozygous Hd1hd1Sd1sd1Wxwx genotype only and 100 BC_5F_1 seeds were produced in the sixth season (rainy season of 2010).

According to the results, MAB was successfully used to select four best lines of RD 15 with corresponding of phenotypes and genotypes, namely: RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous with hd1hd1Sd1Sd1WxWx genotype), RD 15 NTG line (non-photoperiod sensitive, tall and glutinous with hd1hd1Sd1Sd1wxwx genotype), RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous with hd1hd1sd1sd1WxWx genotype) and RD 15 NSG line (non-photoperiod sensitive, short and glutinous with hd1hd1sd1sd1wxwx genotype).

Results from testing for photoperiod response plants in the greenhouse showed that the selected four best lines of RD 15 contained homozygous recessive hd1hd1 genotype were flowering under long-day condition of light exposure for 14 hours per day thus indicated non-photoperiod sensitive plants, when original RD 15 was not flowering.

Results from yield trial in the field showed that yield and other important traits of selected four best lines of RD 15 were nont significantly different with original RD 15 such as number of seeds per panicle; fertility; weight of 1,000 seeds; length, width and thickness of paddy and brown rice grain. Two lines of RD 15 which were selected for homozygous dominant Sd1Sd1 genotype were tall with 138 and 139 cm high, namely: RD 15 NTN line and RD 15 NTG line (tall

plants with Sd1Sd1 genotype), respectively. Other two lines of RD 15 which were selected for homozygous recessive sd1sd1 genotype were short with the same 89 cm high, namely: RD 15 NSN line and RD 15 NSG line (short plants with sd1sd1 genotype). Two lines of RD 15 which were selected for homozygous dominant WxWx genotype were non-glutinous as showed by translucent endosperm grain appearance, namely: RD 15 NTN line and RD 15 NSN line (non-glutinous seeds with WxWx genotype). The other two lines of RD 15 plant which were selected for homozygous recessive wxwx genotype were glutinous rice as showed by chalky endosperm grain appearance, namely: RD 15 NTG line and RD 15 NSG line (glutinous seeds with wxwx genotype).

Results from the study of inheritance of *Hd1/hd1, Sd1/sd1* and *Wx/wx* genes in BC_3F_2 population which were planted in the greenhouse under long-day condition of light exposure for 14 hours per day showed that genotypic and phenotypic ratios of 150 BC_3F_2 plants followed the Laws of Mendel and the Tri-hybrid Cross.

In summary, we successfully used molecular marker assisted backcrossing to improve photoperiod sensitive, tall and non-glutinous RD 15 rice variety to non-photoperiod sensitive, short and glutinous rice. This research was significant for rice breeders in saving time, efficiency and accuracy in selecting complex traits and in researching. Further, this method could be also applied widely for other rice varieties and useful for other ecological lands.

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APPENDICES

APPENDIX 1

Data analysis

1 Grain yield

Variable name : "grain yield"
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	Rep1	Rep2	Rep3	
1. RD 15	857.00	726.00	732.00	Mean = 771.67
2. RD 15 NTN line	718.00	928.00	877.00	Mean = 841.00
3. RD 15 NTG line	698.00	709.00	776.00	Mean = 727.67
4. Improved RD 6 line (tall)	918.00	790.00	727.00	Mean = 811.67
5. RD 15 NSN line	793.00	834.00	778.00	Mean = 801.67
6. RD 15 NSG line	713.00	814.00	893.00	Mean = 806.67
7. Improved RD 6 line (short)	810.00	877.00	822.00	Mean = 836.33
8. Chainat 80	899.00	949.00	947.00	Mean = 931.67
9. Sanpatong 1	875.00	960.00	971.00	Mean = 935.33
10.RD 10	839.00	789.00	836.00	Mean = 821.33

****

: Sirichai Statistics Version 6.00 :
12-21-2010 00:47:49
Problem Identification : Procedure : Analysis of Variance I

Table.... Analysis of Variance

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	2	4098.2000	2049.1000	0.45	3.55	6.01	0.6516
Treatment	9	111609.5000	12401.0556	2.70	2.46	3.60	0.0344
Ex.Error	18	82555.8000	4586.4333				
Total	29	198263.5000	6836.6724				

GRAND MEAN = 828.5
CV = 8.1742 %
LSD .05 = 116.176413574166
LSD .01 = 159.141227161566

****

* DUNCAN'S MULTIPLE-RANGE TEST *
*PROBLEM IDENTIFICATION= *
*NUMBER OF MEANS= 10 *
*ERROR DEGREE OF FREEDOM= 18 *
*ERROR MEAN SQUARE= 4586.43333333337 *
*STANDARD ERROR OF MEAN= 39.1000142085796 *
* *

****

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
	9	935.3	A
	8	931.6	A
	2	841.0	AB
	7	836.3	AB
	10	821.3	AB
	4	811.6	AB
	6	806.6	AB
	5	801.6	AB
	1	771.6	AB
	3	727.6	B

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
	9	935.3	A
	8	931.6	AB
	2	841.0	ABC
	7	836.3	ABC
	10	821.3	ABC
	4	811.6	ABC
	6	806.6	ABC
	5	801.6	BC
	1	771.6	C
	3	727.6	C

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

****

2 Age to 50% flowering

Variable name : age to 50% flowering
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	**Rep1**	**Rep2**	**Rep3**		
1. RD 15	96.00	98.00	97.00	Mean =	97.00
2. RD 15 NTN line	92.00	93.00	94.00	Mean =	93.00
3. RD 15 NTG line	92.00	94.00	93.00	Mean =	93.00
4. Improved RD 6 line (ta11)	95.00	97.00	97.00	Mean =	96.33
5. RD 15 NSN line	95.00	96.00	98.00	Mean =	96.33
6. RD 15 NSG line	95.00	95.00	97.00	Mean =	95.67
7. Improved RD 6 line (short)	104.00	103.00	104.00	Mean =	103.67
8. Chainat 80	95.00	95.00	97.00	Mean =	95.67
9. Sanpatong 1	104.00	106.00	106.00	Mean =	105.33
10.RD 10	99.00	100.00	100.00	Mean =	99.67

****

```
:          Sirichai Statistics Version 6.00          :
12-22-2010                                      11:35:36
Problem Identification :  Procedure : Analysis of Variance I
-----------------------------------------------------------------

Table....        Analysis of Variance

Source     df       SS          MS         F      F.05     F.01      F-Prob


Block      2        13.0667     6.5333    12.25     3.55     6.01      0.0007
Treatment  9       462.7000    51.4111    96.40     2.46     3.60      0.0000
Ex.Error   18        9.6000     0.5333
Total      29      485.3667    16.7368

         GRAND MEAN =  97.5666666666667
         CV  =             0.7485 %

         LSD .05    =  1.25279435219616
         LSD .01    =  1.71610763713496
         ************************************************************
         *                                                          *
         *          DUNCAN'S MULTIPLE-RANGE TEST                    *
         *PROBLEM IDENTIFICATION=                                   *
         *NUMBER OF MEANS= 10                                       *
         *ERROR DEGREE OF FREEDOM= 18                               *
         *ERROR MEAN SQUARE= .533333333335274                       *
         *STANDARD ERROR OF MEAN= .421637021356551                  *
         *                                                          *
         ************************************************************

       NAME     ID      MEAN         RANKED AT PROBABILITY LEVEL .01

       9                      105.3A
       7                      103.6A
       10                      99.6 B
       1                       97.0  C
       5                       96.3  C
       4                       96.3  C
       8                       95.6  C
       6                       95.6  C
       3                       93.0   D
       2                       93.0   D

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.

       NAME     ID      MEAN         RANKED AT PROBABILITY LEVEL .05

       9                      105.3A
       7                      103.6 B
       10                      99.6  C
       1                       97.0   D
       5                       96.3   D
       4                       96.3   D
       8                       95.6   D
       6                       95.6   D
       3                       93.0    E
       2                       93.0    E

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.
```

3 Plant height

Variable name : "plant height"
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	Rep1	Rep2	Rep3		
1. RD 15	141.00	139.00	141.00	Mean =	140.33
2. RD 15 NTN line	139.00	134.00	141.00	Mean =	138.00
3. RD 15 NTG line	138.00	141.00	138.00	Mean =	139.00
4. Improved RD 6 line (tall)	153.00	154.00	154.00	Mean =	153.67
5. RD 15 NSN line	89.00	90.00	87.00	Mean =	88.67
6. RD 15 NSG line	87.00	94.00	87.00	Mean =	89.33
7. Improved RD 6 line (short)	106.00	102.00	101.00	Mean =	103.00
8. Chainat 80	111.00	119.00	119.00	Mean =	116.33
9. Sanpatong 1	116.00	119.00	119.00	Mean =	118.00
10.RD 10	115.00	112.00	108.00	Mean =	111.67

****

: Sirichai Statistics Version 6.00 :
12-21-2010 00:47:48
Problem Identification : Procedure : Analysis of Variance I

Table.... Analysis of Variance

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	2	5.4000	2.7000	0.31	3.55	6.01	0.7381
Treatment	9	13588.8000	1509.8667	175.79	2.46	3.60	0.0000
Ex.Error	18	154.6000	8.5889				
Total	29	13748.8000	474.0966				

GRAND MEAN = 119.8
CV = 2.4463 %

LSD .05 = 5.02746337113995
LSD .01 = 6.88673944890089

****
* *
* DUNCAN'S MULTIPLE-RANGE TEST *
*PROBLEM IDENTIFICATION= *
*NUMBER OF MEANS= 10 *
*ERROR DEGREE OF FREEDOM= 18 *
*ERROR MEAN SQUARE= 8.58888888889083 *
*STANDARD ERROR OF MEAN= 1.69202924412186 *
* *
****

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
4		153.6	A
1		140.3	B
3		139.0	B
2		138.0	B
9		118.0	C
8		116.3	C
10		111.6	C
7		103.0	D
6		89.3	E
5		88.6	E

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
4		153.6	A
1		140.3	B
3		139.0	B
2		138.0	B
9		118.0	C
8		116.3	CD
10		111.6	D
7		103.0	E
6		89.3	F
5		88.6	F

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

****

4 Number of tiller/hill

Variable name : "tiller/hill"
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	**Rep1**	**Rep2**	**Rep3**		
1. RD 15	13.00	15.00	14.00	Mean =	14.00
2. RD 15 NTN line	13.00	15.00	17.00	Mean =	15.00
3. RD 15 NTG line	13.00	14.00	14.00	Mean =	13.67
4. Improved RD 6 line (tall)	12.00	13.00	14.00	Mean =	13.00
5. RD 15 NSN line	21.00	21.00	22.00	Mean =	21.33
6. RD 15 NSG line	19.00	19.00	18.00	Mean =	18.67
7. Improved RD 6 line (short)	18.00	18.00	17.00	Mean =	17.67
8. Chainat 80	16.00	16.00	18.00	Mean =	16.67
9. Sanpatong 1	18.00	17.00	18.00	Mean =	17.67
10.RD 10	16.00	15.00	15.00	Mean =	15.33

****

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:              Sirichai Statistics Version 6.00          :
12-21-2010                                          00:47:48
Problem Identification :  Procedure : Analysis of Variance I
-------------------------------------------------------------
Table....         Analysis of Variance

Source       df       SS        MS        F      F.05     F.01     F-Prob
Block         2        3.2000    1.6000    1.86   3.55     6.01     0.1828
Treatment     9      181.6333   20.1815   23.49   2.46     3.60     0.0000
Ex.Error     18       15.4667    0.8593
Total        29      200.3000    6.9069

          GRAND MEAN =  16.3
          CV  =              5.6869 %
          LSD .05    =  1.59016625576942
          LSD .01    =  2.17824773160609
          ************************************************************
          *          DUNCAN'S MULTIPLE-RANGE TEST                    *
          *PROBLEM IDENTIFICATION=                                    *
          *NUMBER OF MEANS= 10                                        *
          *ERROR DEGREE OF FREEDOM= 18                                *
          *ERROR MEAN SQUARE= .859259259259286                        *
          *STANDARD ERROR OF MEAN= .535181981279666                   *
          *                                                           *
          ************************************************************

        NAME      ID       MEAN       RANKED AT PROBABILITY LEVEL .01

        5                          21.3A
        6                          18.6 B
        7                          17.6 B
        9                          17.6 B
        8                          16.6 BC
        10                         15.3  CD
        2                          15.0  CD
        1                          14.0   D
        3                          13.6   D
        4                          13.0   D

           MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
           BY DUNCAN'S MULTIPLE RANGE TEST.

        NAME      ID       MEAN       RANKED AT PROBABILITY LEVEL .05

        5                          21.3A
        6                          18.6 B
        7                          17.6 BC
        9                          17.6 BC
        8                          16.6  CD
        10                         15.3   DE
        2                          15.0   DE
        1                          14.0    EF
        3                          13.6    EF
        4                          13.0     F

           MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
           BY DUNCAN'S MULTIPLE RANGE TEST.
*************************************************************
```

5 Number of panicle/hill

Variable name : "panicle/hill"
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	Rep1	Rep2	Rep3		
1. RD 15	12.00	13.00	13.00	Mean =	12.67
2. RD 15 NTN line	12.00	15.00	16.00	Mean =	14.33
3. RD 15 NTG line	12.00	12.00	13.00	Mean =	12.33
4. Improved RD 6 line (tall)	12.00	13.00	12.00	Mean =	12.33
5. RD 15 NSN line	17.00	20.00	19.00	Mean =	18.67
6. RD 15 NSG line	14.00	17.00	16.00	Mean =	15.67
7. Improved RD 6 line (short)	13.00	15.00	14.00	Mean =	14.00
8. Chainat 80	13.00	15.00	17.00	Mean =	15.00
9. Sanpatong 1	14.00	15.00	16.00	Mean =	15.00
10.RD 10	12.00	12.00	14.00	Mean =	12.67

****

: Sirichai Statistics Version 6.00 :
12-21-2010 00:47:48
Problem Identification : Procedure : Analysis of Variance I

Table.... Analysis of Variance

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	2	20.8667	10.4333	13.61	3.55	6.01	0.0004
Treatment	9	105.2000	11.6889	15.25	2.46	3.60	0.0000
Ex.Error	18	13.8000	0.7667				
Total	29	139.8667	4.8230				

GRAND MEAN = 14.2666666666667
CV = 6.1373 %

LSD .05 = 1.5020476616199
LSD .01 = 2.05754077588866

****
* *
* DUNCAN'S MULTIPLE-RANGE TEST *
*PROBLEM IDENTIFICATION= *
*NUMBER OF MEANS= 10 *
*ERROR DEGREE OF FREEDOM= 18 *
*ERROR MEAN SQUARE= .766666666666677 *
*STANDARD ERROR OF MEAN= .50552502960344 *
* *
****

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
5		18.6	A
6		15.6	B
8		15.0	B
9		15.0	B
2		14.3	BC
7		14.0	BC
10		12.6	C
1		12.6	C
4		12.3	C
3		12.3	C

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
5		18.6	A
6		15.6	B
8		15.0	BC
9		15.0	BC
2		14.3	BC
7		14.0	CD
10		12.6	DE
1		12.6	DE
4		12.3	E
3		12.3	E

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY

6 Number of seeds/panicle

Variable name : "seeds/panicle"
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	**Repl**	**Rep2**	**Rep3**		
1. RD 15	159.00	135.00	138.00	Mean =	144.00
2. RD 15 NTN line	167.00	145.00	161.00	Mean =	157.67
3. RD 15 NTG line	153.00	130.00	121.00	Mean =	134.67
4. Improved RD 6 line (tall)	149.00	155.00	140.00	Mean =	148.00
5. RD 15 NSN line	130.00	122.00	123.00	Mean =	125.00
6. RD 15 NSG line	140.00	132.00	127.00	Mean =	133.00
7. Improved RD 6 line (short)	156.00	150.00	141.00	Mean =	149.00
8. Chainat 80	146.00	156.00	150.00	Mean =	150.67
9. Sanpatong 1	151.00	160.00	143.00	Mean =	151.33
10.RD 10	130.00	124.00	132.00	Mean =	128.67

****

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:         Sirichai Statistics Version 6.00          :
12-21-2010                                    00:47:48
Problem Identification :  Procedure : Analysis of Variance I
-----------------------------------------------------------------
Table....        Analysis of Variance

Source    df       SS          MS        F      F.05    F.01     F-Prob
Block      2    576.6000    288.3000    4.51    3.55    6.01     0.0253
Treatment  9   3293.4667    365.9407    5.72    2.46    3.60     0.0011
Ex.Error  18   1150.7333     63.9296
Total     29   5020.8000    173.1310

         GRAND MEAN =  142.2
         CV  =            5.6228 %
         LSD .05    =  13.7161276129168
         LSD .01    =  18.7886793288789
         ************************************************************
         *          DUNCAN'S MULTIPLE-RANGE TEST                    *
         *PROBLEM IDENTIFICATION=                                    *
         *NUMBER OF MEANS= 10                                        *
         *ERROR DEGREE OF FREEDOM= 18                                *
         *ERROR MEAN SQUARE= 63.9296296296266                        *
         *STANDARD ERROR OF MEAN= 4.61626218310971                   *
         *                                                           *
         ************************************************************

       NAME     ID      MEAN      RANKED AT PROBABILITY LEVEL .01

       2                       157.6A
       9                       151.3AB
       8                       150.6AB
       7                       149.0ABC
       4                       148.0ABC
       1                       144.0ABCD
       3                       134.6 BCD
       6                       133.0 BCD
       10                      128.6  CD
       5                       125.0   D

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.

       NAME     ID      MEAN      RANKED AT PROBABILITY LEVEL .05

       2                       157.6A
       9                       151.3A
       8                       150.6A
       7                       149.0AB
       4                       148.0AB
       1                       144.0ABC
       3                       134.6 BCD
       6                       133.0  CD
       10                      128.6   D
       5                       125.0   D

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.
```

7 Fertility (%)

Variable name : % fertiliity
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	Rep1	Rep2	Rep3		
1. RD 15	82.00	86.00	82.00	Mean =	83.33
2. RD 15 NTN line	77.00	86.00	81.00	Mean =	81.33
3. RD 15 NTG line	76.00	89.00	86.00	Mean =	83.67
4. Improved RD 6 line (tall)	81.00	78.00	82.00	Mean =	80.33
5. RD 15 NSN line	77.00	80.00	77.00	Mean =	78.00
6. RD 15 NSG line	79.00	82.00	81.00	Mean =	80.67
7. Improved RD 6 line (short)	81.00	76.00	81.00	Mean =	79.33
8. Chainat 80	78.00	80.00	78.00	Mean =	78.67
9. Sanpatong 1	81.00	77.00	83.00	Mean =	80.33
10.RD 10	79.00	84.00	82.00	Mean =	81.67

****

: Sirichai Statistics Version 6.00 :
12-25-2010
08:41:24
Problem Identification : Procedure : Analysis of Variance I

Table.... Analysis of Variance

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	2	41.2667	20.6333	2.15	3.55	6.01	0.1440
Treatment	9	91.8667	10.2074	1.06	2.46	3.60	0.4328
Ex.Error	18	172.7333	9.5963				
Total	29	305.8667	10.5471				

GRAND MEAN = 80.7333333333333
CV = 3.8371 %
LSD .05 = 5.31413089209066
LSD .01 = 7.27942346855636

****

* *
* DUNCAN'S MULTIPLE-RANGE TEST *
*PROBLEM IDENTIFICATION= *
*NUMBER OF MEANS= 10 *
*ERROR DEGREE OF FREEDOM= 18 *
*ERROR MEAN SQUARE= 9.59629629629651 *
*STANDARD ERROR OF MEAN= 1.78850927649225 *
* *

****

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
3		83.6	A
1		83.3	A
10		81.6	A
2		81.3	A
6		80.6	A
9		80.3	A
4		80.3	A
7		79.3	A
8		78.6	A
5		78.0	A

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
3		83.6	A
1		83.3	A
10		81.6	A
2		81.3	A
6		80.6	A
9		80.3	A
4		80.3	A
7		79.3	A
8		78.6	A
5		78.0	A

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
BY DUNCAN'S MULTIPLE RANGE TEST.

****

8 Weight of1000 seed

Variable name : weight of 1000 seed
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	**Rep1**	**Rep2**	**Rep3**		
1. RD 15	25.02	25.39	24.49	Mean =	24.97
2. RD 15 NTN line	24.36	26.63	26.08	Mean =	25.69
3. RD 15 NTG line	23.39	23.65	24.13	Mean =	23.72
4. Improved RD 6 line (tall)	27.40	28.51	29.37	Mean =	28.43
5. RD 15 NSN line	25.80	26.93	26.31	Mean =	26.35
6. RD 15 NSG line	24.10	25.81	25.49	Mean =	25.13
7. Improved RD 6 line (short)	28.22	29.49	29.94	Mean =	29.22
8. Chainat 80	28.94	30.61	28.27	Mean =	29.27
9. Sanpatong 1	30.65	31.95	31.99	Mean =	31.53
10.RD 10	32.31	33.46	32.68	Mean =	32.82

****

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:              Sirichai Statistics Version 6.00          :
12-23-2010                                          11:43:10
Problem Identification :  Procedure : Analysis of Variance I
-----------------------------------------------------------
Table....        Analysis of Variance

Source      df      SS          MS       F      F.05    F.01     F-Prob
Block        2      7.8878      3.9439   11.45  3.55    6.01     0.0009
Treatment    9    245.6872     27.2986   79.26  2.46    3.60     0.0000
Ex.Error    18      6.1995      0.3444
Total       29    259.7746      8.9577

          GRAND MEAN =  27.7123332977295
          CV  =             2.1177 %
          LSD .05    =  1.00675591369287
          LSD .01    =  1.37907830538224
          ************************************************************
          *          DUNCAN'S MULTIPLE-RANGE TEST                    *
          *PROBLEM IDENTIFICATION=                                    *
          *NUMBER OF MEANS= 10                                        *
          *ERROR DEGREE OF FREEDOM= 18                                *
          *ERROR MEAN SQUARE= .344419342171629                        *
          *STANDARD ERROR OF MEAN= .338831001224125                   *
          *                                                           *
          ************************************************************

       NAME     ID      MEAN      RANKED AT PROBABILITY LEVEL .01

       10                        32.8A
       9                         31.5A
       8                         29.3 B
       7                         29.2 B
       4                         28.4 B
       5                         26.4  C
       2                         25.7  C
       6                         25.1  CD
       1                         25.0  CD
       3                         23.7   D

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.

       NAME     ID      MEAN      RANKED AT PROBABILITY LEVEL .05

       10                        32.8A
       9                         31.5 B
       8                         29.3  C
       7                         29.2  C
       4                         28.4  C
       5                         26.4   D
       2                         25.7   DE
       6                         25.1    E
       1                         25.0    E
       3                         23.7     F

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.
************************************************************************
```

9 Width of paddy rice grain

```
Variable name : width
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments =  10
Calculated Replications =  3
```

Treatments	Rep1	Rep2	Rep3		
1. RD 15	2.44	2.44	2.34	Mean =	2.41
2. RD 15 NTN line	2.51	2.52	2.47	Mean =	2.50
3. RD 15 NTG line	2.49	2.46	2.41	Mean =	2.45
4. Improved RD 6 line (tall)	2.77	2.82	2.82	Mean =	2.80
5. RD 15 NSN line	2.51	2.50	2.51	Mean =	2.51
6. RD 15 NSG line	2.50	2.52	2.41	Mean =	2.48
7. Improved RD 6 line (short)	2.91	2.83	2.75	Mean =	2.83
8. Chainat 80	2.62	2.59	2.58	Mean =	2.60
9. Sanpatong 1	2.91	2.90	2.98	Mean =	2.93
10. RD 10	2.76	2.81	2.73	Mean =	2.77

```
                    ********************
    :       Sirichai Statistics Version 6.00        :
05-23-2011                                  21:13:48
Problem Identification :  Procedure : Analysis of Variance I
-------------------------------------------------------------

Table....        Analysis of Variance

Source        df     SS         MS        F       F.05    F.01     F-Prob

Block          2     0.0110     0.0055    3.33    3.55    6.01     0.0577
Treatment      9     0.9494     0.1055    63.96   2.46    3.60     0.0000
Ex.Error      18     0.0297     0.0016
Total         29     0.9900     0.0341

         GRAND MEAN =  2.62699999809265
         CV  =            1.5459 %
         LSD .05    =  6.96666358454725E-02
         LSD .01    =  9.54310223528176E-02
         ************************************************************
         *          DUNCAN'S MULTIPLE-RANGE TEST                    *
         *PROBLEM IDENTIFICATION=                                    *
         *NUMBER OF MEANS= 10                                        *
         *ERROR DEGREE OF FREEDOM= 18                                *
         *ERROR MEAN SQUARE= 1.64925888627149E-03                    *
         *STANDARD ERROR OF MEAN= 2.34468113416408E-02               *
         *                                                          *
         ************************************************************
```

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
	9	2.93	A
	7	2.83	B
	4	2.80	B
	10	2.77	B
	8	2.60	C
	5	2.51	CD
	2	2.50	CD
	6	2.48	D
	3	2.45	D
	1	2.41	D

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
	9	2.93	A
	7	2.83	B
	4	2.83	B
	10	2.77	B
	8	2.60	C
	5	2.51	D
	2	2.50	D
	6	2.48	DE
	3	2.45	DE
	1	2.41	E

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

10 Length of paddy rice grain

Variable name : length
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatments	**Rep1**	**Rep2**	**Rep3**		
1. RD 15	10.41	10.49	10.63	Mean =	10.51
2. RD 15 NTN line	10.46	10.33	10.57	Mean =	10.45
3. RD 15 NTG line	10.55	10.80	10.72	Mean =	10.69
4. Improved RD 6 line (tall)	10.48	10.60	10.35	Mean =	10.48
5. RD 15 NSN line	10.57	10.83	10.76	Mean =	10.72
6. RD 15 NSG line	10.38	10.47	10.55	Mean =	10.47
7. Improved RD 6 line (short)	10.76	10.45	10.52	Mean =	10.58
8. Chainat 80	9.94	9.75	9.78	Mean =	9.82
9. Sanpatong 1	10.90	10.64	10.30	Mean =	10.61
10.RD 10	11.03	10.84	11.40	Mean =	11.09

****

```
:        Sirichai Statistics Version 6.00     :
05-23-2011                              21:13:48
Problem Identification :  Procedure : Analysis of Variance I
-------------------------------------------------------------
Table....        Analysis of Variance

Source       df      SS         MS        F       F.05     F.01   F-Prob
Block         2      0.0078     0.0039    0.12    3.55     6.01   0.8864
Treatment     9      2.6865     0.2985    9.30    2.46     3.60   0.0001
Ex.Error     18      0.5774     0.0321
Total        29      3.2717     0.1128

          GRAND MEAN =  10.5420000076294
          CV  =              1.6990 %
          LSD .05    =  .307253920322916
          LSD .01    =  .420883761394265
          ************************************************
          *          DUNCAN'S MULTIPLE-RANGE TEST                *
          *PROBLEM IDENTIFICATION=                                *
          *NUMBER OF MEANS= 10                                    *
          *ERROR DEGREE OF FREEDOM= 18                            *
          *ERROR MEAN SQUARE= 3.20799749106805E-02                *
          *STANDARD ERROR OF MEAN= .103408534320078               *
          *                                                       *
          *********************************************************

       NAME     ID      MEAN       RANKED AT PROBABILITY LEVEL .01

       10                        11.09A
       5                         10.72AB
       3                         10.69AB
       9                         10.61 B
       7                         10.58 B
       1                         10.51 B
       4                         10.48 B
       6                         10.47 B
       2                         10.45 B
       8                          9.82  C

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.

       NAME     ID      MEAN       RANKED AT PROBABILITY LEVEL .05

       10                        11.09A
       5                         10.72 B
       3                         10.69 B
       9                         10.61 B
       7                         10.58 B
       1                         10.51 B
       4                         10.48 B
       6                         10.47 B
       2                         10.45 B
       8                          9.82  C

          MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.
*************************************************************
```

11 Thickness of paddy rice grain

```
Variable name : thickness
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments =  10
Calculated Replications =  3
```

Treatment	Rep1	Rep2	Rep3		
1. RD 15	1.85	1.84	1.86	Mean =	1.85
2. RD 15 NTN line	1.84	1.88	1.84	Mean =	1.85
3. RD 15 NTG line	1.84	1.86	1.81	Mean =	1.84
4. Improved RD 6 line (tall)	1.95	1.95	1.97	Mean =	1.96
5. RD 15 NSN line	1.83	1.89	1.89	Mean =	1.87
6. RD 15 NSG line	1.86	1.82	1.82	Mean =	1.83
7. Improved RD 6 line (short)	1.98	1.93	1.95	Mean =	1.95
8. Chainat 80	2.04	2.04	2.00	Mean =	2.03
9. Sanpatong 1	2.11	2.07	2.07	Mean =	2.08
10. RD 10	2.11	2.05	2.04	Mean =	2.07

```
                    ********************


:              Sirichai Statistics Version 6.00          :
05-23-2011                                          21:13:48
Problem Identification :  Procedure : Analysis of Variance I
-------------------------------------------------------------

Table....        Analysis of Variance

Source      df       SS       MS       F      F.05     F.01      F-Prob

Block        2       0.0013   0.0006   1.02   3.55     6.01      0.3809
Treatment    9       0.2599   0.0289   46.19  2.46     3.60      0.0000
Ex.Error    18       0.0113   0.0006
Total       29       0.2724   0.0094


        GRAND MEAN =  1.93299999634425
        CV  =            1.2935 %

        LSD .05    =  4.28928141736709E-02
        LSD .01    =  5.87556017095787E-02
        ************************************************************
        *          DUNCAN'S MULTIPLE-RANGE TEST                    *
        *PROBLEM IDENTIFICATION=                                    *
        *NUMBER OF MEANS= 10                                        *
        *ERROR DEGREE OF FREEDOM= 18                                *
        *ERROR MEAN SQUARE= 6.25184549051028E-04                    *
        *STANDARD ERROR OF MEAN= 1.44358875613409E-02               *
        *                                                           *
        ************************************************************
```

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
9		2.08	A
10		2.07	A
8		2.03	A
4		1.96	B
7		1.95	B
5		1.87	C
2		1.85	C
1		1.85	C
3		1.84	C
6		1.83	C

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
9		2.08	A
10		2.07	AB
8		2.03	B
4		1.96	C
7		1.95	C
5		1.87	D
2		1.85	D
1		1.85	D
3		1.84	D
6		1.83	D

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

****

12 Width of brown rice grain

Variable name : width2
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatment	**Rep1**	**Rep2**	**Rep3**		
1. RD 15	2.02	2.03	2.02	Mean =	2.02
2. RD 15 NTN line	2.08	2.13	2.02	Mean =	2.08
3. RD 15 NTG line	2.04	2.00	1.97	Mean =	2.00
4. Improved RD 6 line (tall)	2.30	2.28	2.32	Mean =	2.30
5. RD 15 NSN line	2.08	2.11	2.09	Mean =	2.09
6. RD 15 NSG line	2.02	2.03	1.97	Mean =	2.01
7. Improved RD 6 line (short)	2.33	2.26	2.23	Mean =	2.27
8. Chainat 80	2.24	2.20	2.21	Mean =	2.22
9. Sanpatong 1	2.37	2.42	2.45	Mean =	2.41
10. RD 10	2.23	2.27	2.24	Mean =	2.25

****

```
:              Sirichai Statistics Version 6.00          :
05-23-2011                                          21:13:49
Problem Identification :  Procedure : Analysis of Variance I
-------------------------------------------------------------
Table....         Analysis of Variance

Source       df     SS          MS          F       F.05     F.01      F-Prob
Block         2     0.0027      0.0013      1.24    3.55     6.01      0.3134
Treatment     9     0.5555      0.0617     56.94    2.46     3.60      0.0000
Ex.Error     18     0.0195      0.0011
Total        29     0.5777      0.0199

          GRAND MEAN =  2.16533331871033
          CV  =            1.5206 %
          LSD .05    =  5.64819900768546E-02
          LSD .01    =  7.73703795531592E-02
          ************************************************************
          *          DUNCAN'S MULTIPLE-RANGE TEST                    *
          *PROBLEM IDENTIFICATION=                                    *
          *NUMBER OF MEANS= 10                                        *
          *ERROR DEGREE OF FREEDOM= 18                                *
          *ERROR MEAN SQUARE= 1.08407451417886E-03                    *
          *STANDARD ERROR OF MEAN= 1.90094232262043E-02               *
          *                                                          *
          ************************************************************

        NAME     ID      MEAN       RANKED AT PROBABILITY LEVEL .01

        9                         2.41A
        4                         2.30 B
        7                         2.27 BC
        10                        2.25 BC
        8                         2.22  C
        5                         2.09   D
        2                         2.08   DE
        1                         2.02   DE
        6                         2.01    E
        3                         2.00    E

           MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
           BY DUNCAN'S MULTIPLE RANGE TEST.

        NAME     ID      MEAN       RANKED AT PROBABILITY LEVEL .05

        9                         2.41A
        4                         2.30 B
        7                         2.27 BC
        10                        2.25 BC
        8                         2.22  C
        5                         2.09   D
        2                         2.08   DE
        1                         2.02    EF
        6                         2.01     F
        3                         2.00     F

           MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
           BY DUNCAN'S MULTIPLE RANGE TEST.
*************************************************************
```

13 Length of brown rice grain

Variable name : length2
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatment	Rep1	Rep2	Rep3		
1. RD 15	7.30	7.35	7.46	Mean =	7.37
2. RD 15 NTN line	7.53	7.36	7.46	Mean =	7.45
3. RD 15 NTG line	7.29	7.65	7.45	Mean =	7.46
4. Improved RD 6 line (tall)	7.36	7.30	7.30	Mean =	7.32
5. RD 15 NSN line	7.35	7.59	7.55	Mean =	7.50
6. RD 15 NSG line	7.14	7.16	7.36	Mean =	7.22
7. Improved RD 6 line (short)	7.54	7.30	7.32	Mean =	7.39
8. Chainat 80	7.34	7.16	7.19	Mean =	7.23
9. Sanpatong 1	7.32	7.27	7.00	Mean =	7.20
10. Rd 10	7.82	7.56	7.80	Mean =	7.73

****

: Sirichai Statistics Version 6.00 :
05-23-2011 21:13:49
Problem Identification : Procedure : Analysis of Variance I

Table.... Analysis of Variance

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	2	0.0043	0.0022	0.13	3.55	6.01	0.8817
Treatment	9	0.6922	0.0769	4.48	2.46	3.60	0.0036
Ex.Error	18	0.3090	0.0172				
Total	29	1.0055	0.0347				

GRAND MEAN = 7.38600006103516
CV = 1.7739 %
LSD .05 = .224759948945166
LSD .01 = .30788154834088

****
* DUNCAN'S MULTIPLE-RANGE TEST *
*PROBLEM IDENTIFICATION= *
*NUMBER OF MEANS= 10 *
*ERROR DEGREE OF FREEDOM= 18 *
*ERROR MEAN SQUARE= 1.71663120856414E-02 *
*STANDARD ERROR OF MEAN= 7.56445902133599E-02 *
* *
****

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .01
10		7.73	A
5		7.50	AB
3		7.46	AB
2		7.445	AB
7		7.39	B
1		7.37	B
4		7.32	B
8		7.23	B
6		7.22	B
9		7.20	B

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

NAME	ID	MEAN	RANKED AT PROBABILITY LEVEL .05
10		7.73	A
5		7.50	B
3		7.46	BC
2		7.45	BCD
7		7.39	BCD
1		7.37	BCD
4		7.32	BCD
8		7.23	CD
6		7.22	CD
9		7.20	D

MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY BY DUNCAN'S MULTIPLE RANGE TEST.

****

14 Thickness of brown rice grain

Variable name : thickness2
Total cases = 30
Total Relications = 3
Total Treatments = 10
Calculated Treatments = 10
Calculated Replications = 3

Treatment	Rep1	Rep2	Rep3		
1. RD 15	1.62	1.63	1.65	Mean =	1.63
2. RD 15 NTN line	1.61	1.65	1.61	Mean =	1.62
3. RD 15 NTG line	1.63	1.64	1.60	Mean =	1.62
4. Improved RD 6 line (tall)	1.73	1.73	1.74	Mean =	1.73
5. RD 15 NSN line	1.60	1.65	1.67	Mean =	1.64
6. RD 15 NSG line	1.62	1.61	1.60	Mean =	1.61
7. Improved RD 6 line (short)	1.74	1.69	1.74	Mean =	1.72
8. Chainat 80	1.82	1.83	1.79	Mean =	1.81
9. Sanpatong 1	1.88	1.87	1.84	Mean =	1.86
10. RD 10	1.90	1.85	1.85	Mean =	1.87

****

```
:              Sirichai Statistics Version 6.00              :
05-23-2011                                                  21:13:49
Problem Identification :  Procedure : Analysis of Variance I
-------------------------------------------------------------
Table....        Analysis of Variance

Source      df       SS         MS         F       F.05     F.01      F-Prob
Block       2       0.0002     0.0001     0.21    3.55     6.01      0.8111
Treatment   9       0.2855     0.0317     56.57   2.46     3.60      0.0000
Ex.Error    18      0.0101     0.0006
Total       29      0.2958     0.0102

          GRAND MEAN =  1.71300000747045
          CV  =           1.3824 %
          LSD .05    =  4.06219870497127E-02
          LSD .01    =  5.56449684574361E-02
          ************************************************************
          *          DUNCAN'S MULTIPLE-RANGE TEST                    *
          *PROBLEM IDENTIFICATION=                                    *
          *NUMBER OF MEANS= 10                                        *
          *ERROR DEGREE OF FREEDOM= 18                                *
          *ERROR MEAN SQUARE= 5.60739927293873E-04                    *
          *STANDARD ERROR OF MEAN= 1.36716242304255E-02               *
          *                                                          *
          ************************************************************

        NAME     ID      MEAN       RANKED AT PROBABILITY LEVEL .01

        10                        1.87A
        9                         1.86A
        8                         1.81A
        4                         1.73 B
        7                         1.72 B
        5                         1.64  C
        1                         1.63  C
        2                         1.62  C
        3                         1.62  C
        6                         1.61  C

           MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
           BY DUNCAN'S MULTIPLE RANGE TEST.

        NAME     ID      MEAN       RANKED AT PROBABILITY LEVEL .05

        10                        1.87A
        9                         1.86A
        8                         1.81 B
        4                         1.73  C
        7                         1.72  C
        5                         1.64   D
        1                         1.63   D
        2                         1.62   D
        3                         1.62   D
        6                         1.61   D

    MEANS NOT SHARING LETTER IN COMMON DIFFER SIGNIFICANTLY
          BY DUNCAN'S MULTIPLE RANGE TEST.
       ***************************************************
```

APPENDIX 2

Table for chi-square test (χ^2)

Table 16 Genotypes and phenotypes of 150 plants of BC_3F_2 population planting under long-day condition of light exposure for 14 hours per day in the greenhouse

No.	Plot No.	Lines/varieties	*Hd1/hd1*			*Sd1/sd1*			*Wx/wx*		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
	-	RD 15	-	Photo.	Hd1Hd1	166	Tall	Sd1Sd1	Translucence	Non-glu.	WxWx
	-	Improved RD 6	118	Non-photo.	hd1hd1	92	Short	sd1sd1	Chalkiness	Glu.	wxwx
1	6359	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6359	108	Non-photo.	hd1hd1	190	Tall	Sd1Sd1	Translucence	Non-glu.	WxWx
2	6269	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6269	107	Non-photo.	hd1hd1	174	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
3	6339	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6339	113	Non-photo.	hd1hd1	188	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
4	6341	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6341	107	Non-photo.	hd1hd1	160	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
5	6342	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6342	111	Non-photo.	hd1hd1	171	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
6	6348	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6348	112	Non-photo.	hd1hd1	185	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
7	6289	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6289	108	Non-photo.	hd1hd1	180	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
8	6292	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6292	105	Non-photo.	hd1hd1	177	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
9	6353	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6353	112	Non-photo.	hd1hd1	171	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
10	6362	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6362	108	Non-photo.	hd1hd1	192	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
11	6364	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6364	115	Non-photo.	hd1hd1	178	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
12	6262	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6262	114	Non-photo.	hd1hd1	150	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
13	6307	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6307	109	Non-photo.	hd1hd1	152	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
14	6315	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6315	108	Non-photo.	hd1hd1	152	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
15	6249	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6249	108	Non-photo.	hd1hd1	140	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx

Table 16 (continued)

No.	Plot No.	Line	*Hd1/hd1*			*Sd1/sd1*			*Wx/wx*		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
16	6256	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6256	107	Non-photo.	hd1hd1	155	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
17	6293	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6293	107	Non-photo.	hd1hd1	160	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
18	6303	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6303	109	Non-photo.	hd1hd1	154	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
19	6323	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6323	111	Non-photo.	hd1hd1	168	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
20	6328	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6328	108	Non-photo.	hd1hd1	154	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
21	6298	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6298	107	Non-photo.	hd1hd1	144	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
22	6344	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6344	107	Non-photo.	hd1hd1	135	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
23	6347	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6347	107	Non-photo.	hd1hd1	158	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
24	6355	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6355	110	Non-photo.	hd1hd1	144	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
25	6367	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6367	107	Non-photo.	hd1hd1	147	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
26	6375	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6375	108	Non-photo.	hd1hd1	163	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
27	6331	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6331	107	Non-photo.	hd1hd1	158	Tall	Sd1sd1	T/C	Non-glu./glu.	WxWx
28	6253	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6253	103	Non-photo.	hd1hd1	154	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
29	6254	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6254	110	Non-photo.	hd1hd1	160	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
30	6270	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6270	109	Non-photo.	hd1hd1	150	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
31	6287	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6287	104	Non-photo.	hd1hd1	157	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
32	6345	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6345	110	Non-photo.	hd1hd1	85	Short	sd1sd1	Translucence	Non-glu.	WxWx

Table 16 (continued)

No.	Plot No.	Line	*Hd1/hd1*			*Sd1/sd1*			*Wx/wx*		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
33	6263	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6263	109	Non-photo.	hd1hd1	95	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
34	6268	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6268	107	Non-photo.	hd1hd1	93	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
35	6290	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6290	107	Non-photo.	hd1hd1	94	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
36	6302	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6302	106	Non-photo.	hd1hd1	92	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
37	6316	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6316	109	Non-photo.	hd1hd1	102	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
38	6324	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6324	111	Non-photo.	hd1hd1	92	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
39	6357	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6357	104	Non-photo.	hd1hd1	87	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
40	6361	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6361	114	Non-photo.	hd1hd1	78	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
41	6381	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6381	108	Non-photo.	hd1hd1	93	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
42	6371	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6371	108	Non-photo.	hd1hd1	79	Short	sd1sd1	Chalkiness	Glu.	wxwx
43	6388	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6388	108	Non-photo.	hd1hd1	91	Short	sd1sd1	Chalkiness	Glu.	wxwx
44	6304	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6304	-	Photo.	Hd1Hd1	180	Tall	Sd1Sd1	Translucence	Non-glu.	WxWx
45	6322	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6322	-	Photo.	Hd1Hd1	192	Tall	Sd1Sd1	Translucence	Non-glu.	WxWx
46	6384	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6384	-	Photo.	Hd1Hd1	195	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
47	6279	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6279	-	Photo.	Hd1Hd1	190	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
48	6285	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6285	-	Photo.	Hd1Hd1	170	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
49	6294	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6294	-	Photo.	Hd1Hd1	182	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx

Table 16 (continued)

No.	Plot No.	Line	Hd1/hd1			Sd1/sd1			Wx/wx		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
50	6297	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6297	-	Photo.	Hd1Hd1	170	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
51	6382	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6382	-	Photo.	Hd1Hd1	195	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
52	6271	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6271	-	Photo.	Hd1Hd1	183	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
53	6319	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6319	-	Photo.	Hd1Hd1	185	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
54	6266	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6266	-	Photo.	Hd1Hd1	175	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
55	6329	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6329	-	Photo.	Hd1Hd1	175	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
56	6352	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6352	-	Photo.	Hd1Hd1	175	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
57	6366	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6366	-	Photo.	Hd1Hd1	142	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
58	6369	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6369	-	Photo.	Hd1Hd1	174	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
59	6389	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6389	-	Photo.	Hd1Hd1	141	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
60	6258	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6258	-	Photo.	Hd1Hd1	154	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
61	6276	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6276	-	Photo.	Hd1Hd1	172	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
62	6296	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6296	-	Photo.	Hd1Hd1	155	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
63	6330	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6330	-	Photo.	Hd1Hd1	180	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
64	6343	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6343	-	Photo.	Hd1Hd1	143	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
65	6363	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6363	-	Photo.	Hd1Hd1	171	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
66	6386	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6386	-	Photo.	Hd1Hd1	172	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx

Table 16 (continued)

No.	Plot No.	Line	Hd1/hd1			Sd1/sd1			Wx/wx		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
67	6374	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6374	-	Photo.	Hd1Hd1	187	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
68	6377	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6377	-	Photo.	Hd1Hd1	153	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
69	6251	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6251	-	Photo.	Hd1Hd1	110	Short	sd1sd1	Translucence	Non-glu.	WxWx
70	6260	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6260	-	Photo.	Hd1Hd1	110	Short	sd1sd1	Translucence	Non-glu.	WxWx
71	6300	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6300	-	Photo.	Hd1Hd1	98	Short	sd1sd1	Translucence	Non-glu.	WxWx
72	6332	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6332	-	Photo.	Hd1Hd1	99	Short	sd1sd1	Translucence	Non-glu.	WxWx
73	6246	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6246	-	Photo.	Hd1Hd1	100	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
74	6259	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6259	-	Photo.	Hd1Hd1	105	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
75	6306	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6306	-	Photo.	Hd1Hd1	105	Short	sd1sd1	T/C	Non-glu/glu.	Wxwx
76	6326	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6326	-	Photo.	Hd1Hd1	110	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
77	6333	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6333	-	Photo.	Hd1Hd1	106	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
78	6261	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6261	-	Photo.	Hd1Hd1	100	Short	sd1sd1	Chalkiness	Glu.	wxwx
79	6272	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6272	-	Photo.	Hd1Hd1	100	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
80	6281	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6281	-	Photo.	Hd1Hd1	110	Short	sd1sd1	Chalkiness	Glu.	wxwx
81	6313	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6313	-	Photo.	Hd1hd1	192	Tall	Sd1Sd1	Translucence	Non-glu.	WxWx
82	6241	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6241	-	Photo.	Hd1hd1	187	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx
83	6252	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6252	-	Photo.	Hd1hd1	194	Tall	Sd1Sd1	T/C	Non-glu./glu.	Wxwx

Table 16 (continued)

No.	Plot No.	Line	Hd1/hd1			Sd1/sd1			Wx/wx		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
84	6351	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6351	-	Photo.	Hd1hd1	190	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
85	6267	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6267	-	Photo.	Hd1hd1	185	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
86	6273	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6273	-	Photo.	Hd1hd1	183	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
87	6278	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6278	-	Photo.	Hd1hd1	190	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
88	6282	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6282	-	Photo.	Hd1hd1	190	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
89	6312	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6312	-	Photo.	Hd1hd1	182	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
90	6340	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6340	-	Photo.	Hd1hd1	182	Tall	Sd1Sd1	T/C	Non-glu./glu.	WxWx
91	6265	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6265	-	Photo.	Hd1hd1	210	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
92	6334	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6334	-	Photo.	Hd1hd1	185	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
93	6373	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6373	-	Photo.	Hd1hd1	210	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
94	6378	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6378	-	Photo.	Hd1hd1	180	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
95	6379	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6379	-	Photo.	Hd1hd1	170	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
96	6380	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6380	-	Photo.	Hd1hd1	182	Tall	Sd1Sd1	Chalkiness	Glu.	wxwx
97	6244	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6244	-	Photo.	Hd1hd1	164	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
98	6247	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6247	-	Photo.	Hd1hd1	150	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
99	6248	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6248	-	Photo.	Hd1hd1	143	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
100	6255	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6255	-	Photo.	Hd1hd1	157	Tall	Sd1sd1	Translucence	Non-glu.	WxWx

Table 16 (continued)

No.	Plot No.	Line	Hd1/hd1			Sd1/sd1			Wx/wx		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
101	6310	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6310	-	Photo.	Hd1hd1	170	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
102	6311	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6311	-	Photo.	Hd1hd1	172	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
103	6325	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6325	-	Photo.	Hd1hd1	175	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
104	6358	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6358	-	Photo.	Hd1hd1	180	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
105	6372	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6372	-	Photo.	Hd1hd1	175	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
106	6383	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6383	-	Photo.	Hd1hd1	190	Tall	Sd1sd1	Translucence	Non-glu.	WxWx
107	6242	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6242	-	Photo.	Hd1hd1	195	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
108	6243	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6243	-	Photo.	Hd1hd1	175	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
109	6277	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6277	-	Photo.	Hd1hd1	185	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
110	6286	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6286	-	Photo.	Hd1hd1	174	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
111	6295	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6295	-	Photo.	Hd1hd1	160	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
112	6305	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6305	-	Photo.	Hd1hd1	195	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
113	6318	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6318	-	Photo.	Hd1hd1	158	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
114	6321	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6321	-	Photo.	Hd1hd1	144	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
115	6327	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6327	-	Photo.	Hd1hd1	181	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
116	6335	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6335	-	Photo.	Hd1hd1	165	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
117	6336	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6336	-	Photo.	Hd1hd1	163	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx

Table 16 (continued)

No.	Plot No.	Line	Hd1/hd1			Sd1/sd1			Wx/wx		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
118	6337	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6337	-	Photo.	Hd1hd1	168	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
119	6350	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6350	-	Photo.	Hd1hd1	175	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
120	6360	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6360	-	Photo.	Hd1hd1	187	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
121	6365	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6365	-	Photo.	Hd1hd1	167	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
122	6368	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6368	-	Photo.	Hd1hd1	165	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
123	6370	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6370	-	Photo.	Hd1hd1	180	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
124	6385	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6385	-	Photo.	Hd1hd1	175	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
125	6387	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6387	-	Photo.	Hd1hd1	157	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
126	6250	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6250	-	Photo.	Hd1hd1	147	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
127	6264	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6264	-	Photo.	Hd1hd1	170	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
128	6280	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6280	-	Photo.	Hd1hd1	180	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
129	6283	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6283	-	Photo.	Hd1hd1	175	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
130	6288	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6288	-	Photo.	Hd1hd1	180	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
131	6308	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6308	-	Photo.	Hd1hd1	140	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
132	6356	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6356	-	Photo.	Hd1hd1	183	Tall	Sd1sd1	T/C	Non-glu./glu.	Wxwx
133	6346	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6346	-	Photo.	Hd1hd1	170	Tall	Sd1sd1	Chalkiness	Glu.	wxwx
134	6376	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6376	-	Photo.	Hd1hd1	171	Tall	Sd1sd1	Chalkiness	Glu.	wxwx

Table 16 (continued)

No.	Plot No.	Line	Hd1/hd1			Sd1/sd1			Wx/wx		
			Flowering day of 50%	Phenotypes	Genotypes	Plant height (cm)	Phenotypes	Genotypes	Observation	Phenotypes	Genotypes
135	6257	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6257	-	Photo.	Hd1hd1	108	Short	sd1sd1	Translucence	Non-glu.	WxWx
136	6274	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6274	-	Photo.	Hd1hd1	110	Short	sd1sd1	Translucence	Non-glu.	WxWx
137	6275	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6275	-	Photo.	Hd1hd1	110	Short	sd1sd1	Translucence	Non-glu.	WxWx
138	6301	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6301	-	Photo.	Hd1hd1	100	Short	sd1sd1	Translucence	Non-glu.	WxWx
139	6320	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6320	-	Photo.	Hd1hd1	109	Short	sd1sd1	Translucence	Non-glu.	WxWx
140	6284	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6284	-	Photo.	Hd1hd1	110	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
141	6317	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6317	-	Photo.	Hd1hd1	110	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx
142	6245	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6245	-	Photo.	Hd1hd1	100	Short	sd1sd1	Chalkiness	Glu.	wxwx
143	6291	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6291	-	Photo.	Hd1hd1	110	Short	sd1sd1	Chalkiness	Glu.	wxwx
144	6299	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6299	-	Photo.	Hd1hd1	105	Short	sd1sd1	Chalkiness	Glu.	wxwx
145	6309	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6309	-	Photo.	Hd1hd1	110	Short	sd1sd1	Chalkiness	Glu.	wxwx
146	6314	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6314	-	Photo.	Hd1hd1	100	Short	sd1sd1	Chalkiness	Glu.	wxwx
147	6338	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6338	-	Photo.	Hd1hd1	98	Short	sd1sd1	Chalkiness	Glu.	wxwx
148	6349	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6349	-	Photo.	Hd1hd1	110	Short	sd1sd1	Chalkiness	Glu.	wxwx
149	6354	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6354	-	Photo.	Hd1hd1	100	Short	sd1sd1	Chalkiness	Glu.	wxwx
150	6390	(RD15 x F_2-1196) BC_3F_2-1026-2483-125-6390	-	Photo.	Hd1hd1	100	Short	sd1sd1	T/C	Non-glu./glu.	Wxwx

APPENDIX 3

Selected four best lines of RD 15 at maturity stage

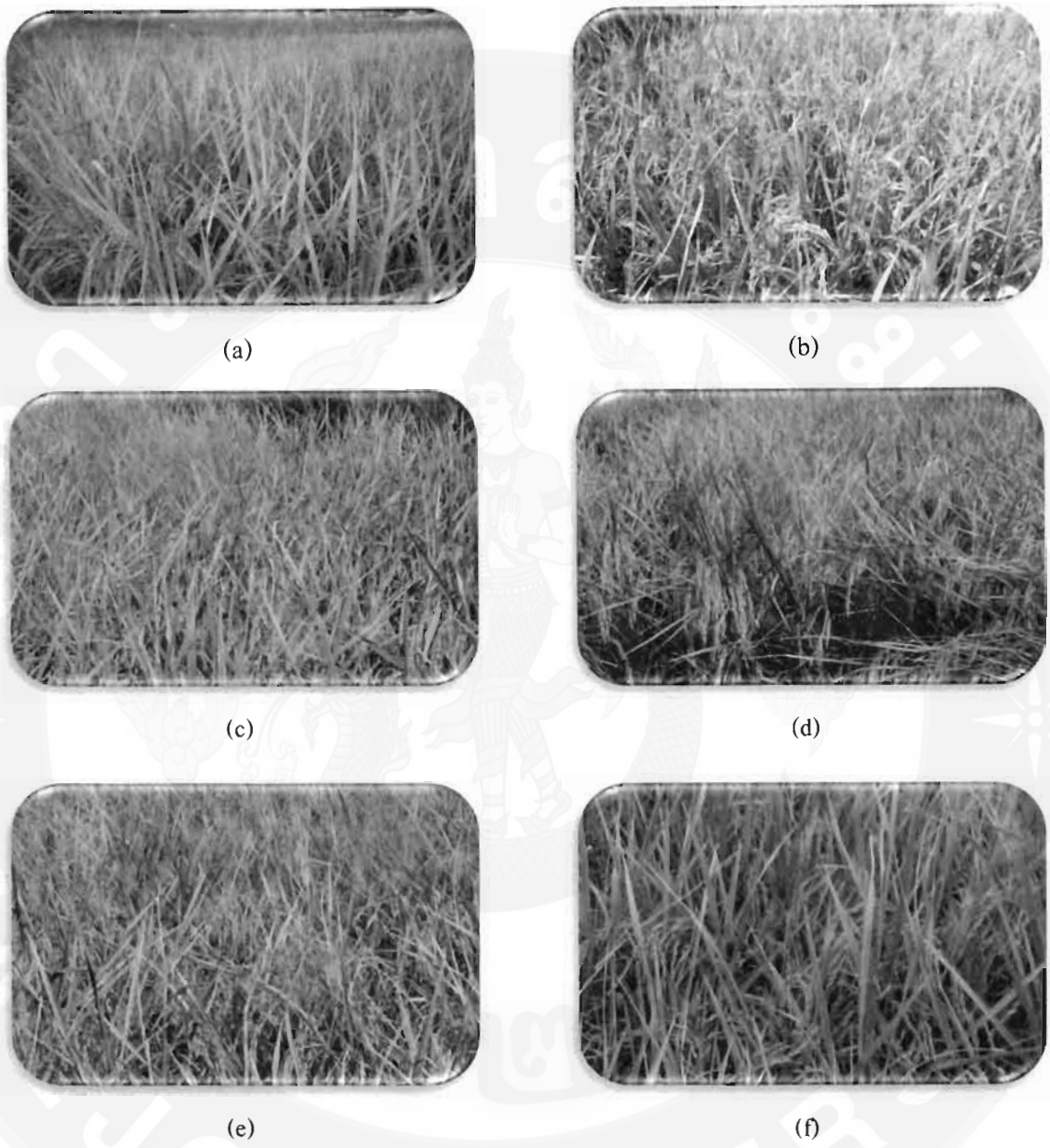


Figure 41 Shows phenotypes of the selected four best lines of RD 15 and their parents at maturity stage in the field during rainy season from July to December 2010 by (a) RD 15 variety (photoperiod sensitive, tall and non-glutinous), (b) improved RD 6 line (non-photoperiod sensitive, short and glutinous), (c) RD 15 NTN line (non-photoperiod sensitive, tall and non-glutinous), (d) RD 15 NTG line (non-photoperiod sensitive, tall and glutinous), (e) RD 15 NSN line (non-photoperiod sensitive, short and non-glutinous) and (f) RD 15 NSG line (non-photoperiod sensitive, short and glutinous)

APPENDIX 4

Pictures in the processing of studying

Rice germination in greenhouse

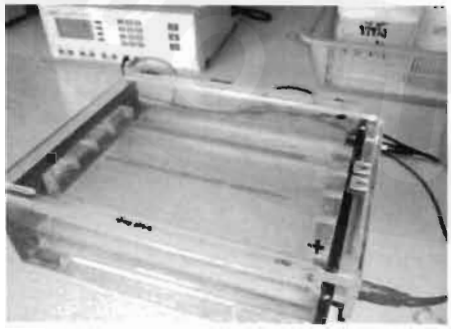
Pollination

Rice growing under light 14 hours/day

Leaf selection for DNA extraction

DNA extraction

PCR machine



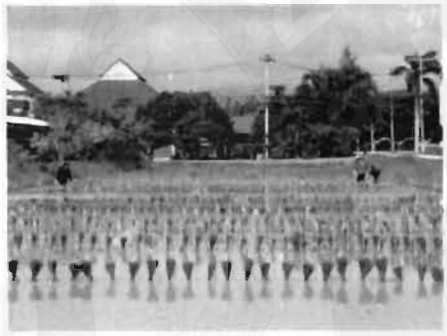
Running gel

Seeds preparation

Soil preparation

Pre-sowing rice

Sowing rice



Pre-planting rice

Rice planting

Yield trial with RCBD

Recording flowering date

Plant height measurement

CURRICULUM VITAE

Name:	Vo Van Trung	
Date of Birth:	04th November, 1983	
Address:	Nghe An, Vietnam	
Educational Background:	2002-2006	Bachelor degree of Agricultural Science (Agronomy) Agriculture and Forestry of Hue University, Vietnam
	2009-2011	Master of Science (Biotechnology) Maejo University, Chiang Mai, Thailand
Work Experience:	2007-2009	Rice research (Researcher) Food Crop Research Department, Agricultural Science Institute of Northern Central Vietnam., Vietnam
Publication	23-24th June, 2011	9th National Postharvest Technology Conference (Oral presentation and proceeding) Pattaya Park Beach Resort, Agricultural Science Journal (In press), Thailand.