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THESIS

ASSESSMENT OF STEPWISE MARKER ASSISTED SELECTION IN COMBINING SUBMERGENCE TOLERANCE, BACTERIAL BLIGHT RESISTANCE AND COOKING QUALITY TRAITS IN RICE (*Oryza sativa* L.)

JUTARUT JANTABOON

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The aim of plant breeding is to develop superior genotypes by combining the desirable alleles from different sources. In the present study, submergence tolerance, bacterial blight resistance and cooking quality traits including fragrance, amylose content, gel consistency and gelatinization temperature are combined by marker assisted selection (MAS) to develop new rice varieties for rainfed and irrigated lowland ecosystems in the Mekong region. Stepwise selection approach (SSA) was used to minimize number of plants to be genotyped by DNA markers. Two breeding program were conducted including the cross between rice varieties IR57514 (IR) and KDML105 (KD) and the cross between RD6 and KDIII (KDML-*Sub1-Xa21-Bph*). In the first cross, a large population consisted of 2,037 recombinant inbred lines (RILs) was developed by single seed descent. Then MAS was conducted to identify the uniform offsprings that combined the desirable alleles from IR57514 and KDML105. Two ideotypes, ideotype1 (ID1; $Sub1^{IR}/badh2^{KD}/Wx^{KD}/SSIIa^{KD}$) and ideotype2 (ID2; $Sub1^{IR}/badh2^{KD}/Wx^{KD}/SSIIa^{IR}$) were selected as breeding target and selected lines were tested for trait performances by compared with those of the parents. All of the ID1 lines exhibited submergence tolerance and jasmine-like cooking quality and displayed a low amylose content, a fragrance and a high alkali spreading value, whereas the ID2 showed the same characteristics as ID1, except for a low alkali spreading value. A wide range of agronomic characteristics was observed in both of the ID groups, and some of the IDs were superior in the yield component, as compared to their parents. In the second cross, SSA was used for MAS since the F_2 generation until the F_6 generation. Two ideotypes, ideotype3 (ID3; $Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$) and ideotype4 (ID4; $Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$) were selected as breeding target and selected lines were tested for trait performances by compared with those of the parents. All of the ID3 lines exhibited glutinous grain type, submergence tolerance and BB resistance, whereas the ID4 lines shown the same characteristic but susceptible to BB. This study provides further support that the precision of markers used in MAS can enhance the development of superior rice genotypes in which they combined all desirable alleles from the parents.

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Jutarut Jantaboon

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

2AP	=	2-acetyl-1-pyrroline
AC	=	amylose Content
ANOVA	=	analysis of variance
ASV	=	alkali spreading value
badh2	=	betaine aldehyde dehydrogenase-2
BB	=	bacterial blight
Bp	=	base pair
Chr	=	chromosome
Cm	=	centimeter
cM	=	centimorgan
DF	=	days to flowering
DNA	=	deoxyribonucleic acid
dNTP	=	deoxy-nucleotide triphosphate
F1	=	first filial
FR	=	fragrance
GBSS	=	granule-bound starch syntheses
GC	=	gel-consistency
GC-MS	=	gas-chromatography/mass-spectrometry
GT	=	gelatinization temperature
ha	=	hectare
IDs	=	ideotypes
INDEL	=	insertion deletion
kb	=	kilo base pair
KOH	=	potassium hydroxide
LL	=	lesion length
LSD	=	least significant difference
MAP	=	marker assisted QTL pyramiding
MAS	=	marker assisted selection
ml	=	milliliter
mm	=	millimeter
mM	=	milli Molar

LIST OF ABBREVIATIONS (Continued)

mRNA	=	messenger RNA
NFGP	=	numbers of filled grains per panicle
ng	=	nanogram
nm	=	nanometer
NPP	=	numbers of panicle per plant
NTP	=	numbers of tiller per plant
ºC	=	degree Celsius
PCR	=	polymerase chain reaction
PH	=	plant height
PSE	=	percentage of seedling elongation
PSF	=	percent of spikelet fertility
PSS	=	percentage of surviving seedlings
PTS	=	plant type selection
QTL	=	quantitative traits loci
RCBD	=	randomized complete block design
RFLP	=	restriction fragment length polymorphism
RILs	=	recombinant inbred lines
SNP	=	single nucleotide polymorphism
SSA	=	stepwise selection approach
SSD	=	single seed descent
SSR	=	simple sequence repeat
STS	=	sequence tagged site
SUB	=	submergence tolerance
TGW	=	thousand grains weight
ul	=	micro liter
uM	=	micro Molarity

ASSESSMENT OF STEPWISE MARKER ASSISTED SELECTION IN COMBINING SUBMERGENCE TOLERANCE, BACTERIAL BLIGHT RESISTANCE AND COOKING QUALITY TRAITS IN RICE (*Oryza sativa* L.)

INTRODUCTION

Breeding methodology in the first half of the 20th century was a simple process in which crosses were made based on the criteria of the best phenotypes. Genetic advances, therefore, depend upon how to select the best, the genetic complexity of the trait and the accuracy of the phenotypic screening techniques. Although conventional selection is still in use, few breeders can quantify the genetic advances they produce; however, advances in molecular marker technology have proven to be a powerful tool for the genetic manipulation of a genotype (Toojinda *et al.*, 2005; Wan *et al.*, 2005; Jena and Mackill, 2008).

In most rice breeding programs, a new genotype can be developed through a single cross or backcross (Liu *et al.*, 2006; Neeraja *et al.*, 2007; Yi *et al.*, 2009; Zhou *et al.*, 2009). In a single cross, the success of generating a new genotype that simultaneously expresses many beneficial characteristics depends upon the selection of the parental lines for the cross and the efficiency and accuracy of the selection and validation (Toojinda *et al.*, 2003; Xu *et al.*, 2004; Liu *et al.*, 2006). The accuracy of selecting proper genotypes that contain many traits by conventional phenotypic selection is very low; this is mainly due to low heritability of certain traits and the time-consuming process of trait selection (Jansen *et al.*, 2003). Since the last decade, Quantitative trait loci (QTL) analysis has provided targets for marker assisted selection (MAS) and has been touted as a means to improve the efficiency, accuracy and speed of the selection process (Mackill *et al.*, 1999; Jena and Mackill, 2008).

In rainfed lowland areas, abiotic and biotic stresses have been reported as a limiting factor for rice production. Their effect or damage on rice may range from

minor or complete loss in yield. Important abiotic stresses include submergence, drought and salinity while important biotic stresses include blast and bacterial blight diseases and brown planthopper. Of these stresses, submergence and bacterial blight disease are two most important stress in irrigated and rainfed lowland areas in Thailand. Submergence is one of natural occurrences devastating rice crop in irrigated and rainfed lowland areas in Asia during monsoon season. Transient submergence up to four weeks is common in these areas. Twenty two million hectares of rainfed lowlands in South and Southeast Asia were reportedly affected by this type of flooding at various stages of growth (Setter *et al.*, 1997; Ram *et al.*, 2002; Jackson and Ram, 2003). Bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases in rice production worldwide. Rice yield losses in severely infected fields generally range from 20 to 30% and in some areas are reportedly up to 80% (Singh *et al.*, 1977).

In Mekong region, high-quality rice varieties with fragrance and soft texture are preferred. All of current growing varieties are intolerant to submergence and susceptible to bacterial blight disease. Jasmine rice cultivars including Kao Dawk Mali 105 (non-glutinous) and RD6 (glutinous) are known for their excellent grain and cooking qualities, such as a long grain, rich fragrance, nutty flavor, soft and fluffy texture and light piles of slightly chewy and well-formed grains after cooking (Lanceras *et al.*, 2000; Jairin *et al.*, 2009). Thus, a jasmine-like cooking quality has become a top priority of many rice breeding programs in the Mekong region (Toojinda *et al.*, 2005).

The investigation of the genetic determinant of submergence tolerance, bacterial blight resistance and cooking quality traits such as fragrance (FR), amylose content (AC), gel consistency (GC) and gelatinization temperature (GT), has been well documented in various studies using QTL mapping and map-based cloning approaches (Kumar and Khush, 1986; He *et al.*, 1999; Tan *et al.*, 1999; Li *et al.*, 2003a; Zhou *et al.*, 2003). The *Sub1* QTL has been identified as a major determinant of submergence tolerance in rice cultivar FR13A and its derived progenies such as IR49830 and IR57514 (Xu and Mackill, 1996; Siangliw *et al.*, 2003; Jantaboon *et al.*,

2011). More recently, *Sub1* has been fine-mapped and cloned, yielding three candidate genes, *Sub1A, Sub1B* and *Sub1C* (Fukao *et al.*, 2006; Xu *et al.*, 2006; Ruanjaichon *et al.*, 2008). Accordingly, precise gene-based markers have been developed and used for MAS in development of submergence tolerance of the popular rice varieties (Siangliw *et al.*, 2003; Toojinda *et al.*, 2003; Xu *et al.*, 2004; Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009; Singh *et al.*, 2009). A dominant gene *Xa21* for bacterial blight resistance was identified in a wild species *O. longistaminata* and it was introgressed into *O. sativa* background (Khush *et al.*, 1989). Rice genotypes carrying *Xa21* confer resistance to a broad range of *Xoo* strains. *Xa21* was the first BB resistance gene successfully cloned. It was isolated using map-based cloning. *Xa21* is a member of a complex locus located on the long arm of chromosome 11 (Song *et al.*, 1995). A major QTL associated with fragrance was identified and mapped on chromosome 8 (Ahn *et al.*, 1992; Lorieux *et al.*, 1996). Later it was found that the recessive gene, *badh2*, was responsible for this trait (Bradbury *et al.*, 2005b). Gene-based markers have been developed for *badh2* and have been successfully used in the introgression of the fragrance allele into non-fragrant genotypes (Garland *et al.*, 2000; Cordeiro *et al.*, 2002; Chen *et al.*, 2006; Shi *et al.*, 2008; Yi *et al.*, 2009). The *Wx* gene, encoding a granule-bound starch synthase, is reported to be a major locus determining the AC and GC (Sano, 1984) and it was reported to determine GT in some rice genotype (Umemoto *et al.*, 2002). GT can be indirectly tested by the alkali spreading value (ASV). ASV is reportedly determined by starch synthase IIa (*SSIIa*) (He *et al.*, 1999; Lanceras *et al.*, 2000; Umemoto *et al.*, 2002; Li *et al.*, 2003a; Umemoto and Aoki, 2005). Gene-based and tightly linked markers for *SSIIa* have also been designed (Lanceras *et al.*, 2000). Hence, MAS for cooking quality trait has been practiced using these markers in line conversions, and great success has been made in turning poor cooking-quality genotypes into desirable cooking-quality genotypes (Zhou *et al.*, 2003; Joseph *et al.*, 2004; Toojinda *et al.*, 2005; Zhang *et al.*, 2005; Liu *et al.*, 2006; Zhang, 2007; Shi *et al.*, 2008; Zheng *et al.*, 2008; Jairin *et al.*, 2009; Yi *et al.*, 2009).

In this study, gene-specific markers for *Sub1, Xa21, Wx, badh2* and *SSIIa* were used to facilitate the selection of ideotypes carrying the pozsitive alleles of these

genes in two single-crossed populations, IR57514-PMI-5-B-1-2 (IR57514) x Khao Dawk Mali 105 (KDML105) and RD6 x KDIII (KDML-*Sub1-Xa21-Bph*). The IR57514 x KDML105 cross was hybridized with the goal of combining the submergence tolerance inherited from IR57514 and the jasmine-like cooking quality (i.e., low amylose content, fragrance, high gel consistency and low gelatinization temperature) inherited from KDML105 into the same rice genotype. The RD6 x KDIII cross was hybridized with the goal of combining the submergence tolerance and BB resistance inherited from KDIII (*Sub1* derived from the ancestor FR13A and *Xa21* derived from the ancestor IR1188) and appearance of glutinous inherited from RD6 into the same rice genotype (glutinous). Here, we described the use of MAS to identify these specific ideotypes and their performance for submergence tolerance, bacterial blight resistance and cooking quality. In each population, we will present the result in to four parts: 1) the outcome of MAS in identifying target ideotypes; 2) the evaluation of the selected genotypes (ideotypes) for the traits being selected by MAS; 3) the field evaluation for important agronomic characteristics of the selected ideotypes; and 4) the effect of genetic backgrounds on the submergence tolerance, bacterial blight resistance and cooking quality traits through genotypic-phenotypic association.

OBJECTIVES

1. Use marker assisted selection (MAS) to identify the offsprings that combined submergence tolerance and cooking quality traits in the cross between IR57514 and KDML105

2. Use marker assisted selection (MAS) to identify the offsprings that combined submergence tolerance, bacterial blight resistance and RD6 cooking quality traits in the cross between RD6 and KDIII

3. Validate the accuracy and efficiency of MAS for submergence tolerance, bacterial blight resistance and cooking quality

LITERATURE REVIEW

Rainfed lowland ecosystems

Rainfed lowland ecosystems are defined as areas where rice is grown in unirrigated, leveled and bunded fields that have shallow flooding with rain water (Mackill *et al.*, 1996). Approximately 76% of the total 9.2 million ha of rice growing areas in Thailand are under rainfed conditions. Of the three rainfed rice ecosystems, upland, lowland and deep water, rainfed lowland occupies 6.8 million ha, covering 75% of the total rice growing areas. The majority of the rainfed lowland areas are found in the Northeast (4.8 million ha) and North (1.4 million ha) of Thailand. Rice yield in these regions is low and fluctuates from year to year, ranging between 1.5 and 2.2 ton/ha. There has been little improvement in yield from plant breeding (Jongdee *et al.*, 2006). In these areas, moisture deficit is common and droughts may occur anytime during the cropping season, but late season and early drought are the most common. Floods may completely submerge crop for periods of up to10 days in low-lying areas and in river deltas. It is very difficult to estimate the area affected by water deficit and/or excess because of spatial and temporal variability. Depending on the environmental conditions, rainfed lowlands may be classified into favourable and unfavourable ecosystems. As slightly of the area is estimated to be favorable.

Flooding is one of the major problems on rice of Mekong region because excess water in their surroundings can deprive them of certain basic needs, notably of oxygen and of carbon dioxide and light for photosynthesis. Submergence tolerant rice may survive complete submersion by water for 10 days or more, depending on water conditions, and resume growth after the water has subsided. However, the effects are highly dependent on the growth stage. Young plants are least tolerant. Submergence at the seedling stage kills weak plants, drastically reduces growth and inhibits tiller formation. Submerged rice has a limited supply of carbohydrates (energy) for survival and it rapidly declines (Setter *et al.*, 1997). Traditional varieties adapted to these environments are low yield due to their low tillering ability, long droopy leaves, susceptibility to lodging and poor grain quality. Several biotic and abiotic stresses, the

lack of tolerance for submergence, insect pests such as brown planthopper and diseases such as bacterial blight are likely to be important obstacles inhibiting adoption in the rainfed lowland rice ecosystem.

Submergence Tolerance

Flooding is a natural disaster which causes growth inhibition and great reduction of yield. Common rice does not have complete tolerance to flooding, and if it is completely submerged or flooded for long periods, plants die due to oxygen starvation and energy depletion. Flooding levels vary, depending on the amount and duration of rain, underlying geological formations and distance from the water. Two forms of flooding, one is short duration over a few weeks and not very deep, termed a 'flash flood' and the other is deep flooding that lasts for a long time, a 'deepwater flood'. In this study, we focus only on flash flood.

Tolerance to flash flood that is submergence tolerance, defined as the ability of rice plant to survive 10-14 days of complete submergence and renew its growth when the water subsides (Adkins *et al.*, 1990). Flash floods are unexpected and uncontrollable in the monsoon season, and the water level can reach to complete submerge in the rainfed lowland areas. In these areas, flash floods at the seedling stage of rice cause severely to survive. However, some rice stains show submergence tolerance and survive such complete submergence. Several studies revealed that submergence tolerance has relatively high heritability, controlled by one or a few genes with major effect and minor modifiers. Genetic linkage between submergence tolerance and shoot elongation was clearly shown by QTL analysis in segregating recombinant inbred line. Both submergence tolerance and suppression of elongation were coincidentally mapped on chromosome 9 (Nandi *et al.*, 1997; Xu *et al.*, 2000; Toojinda *et al.*, 2003). Quantitative Trait Loci (QTLs) analysis for submergence tolerance had been identified "*Sub1*" as a major QTL. When this locus from FR13A is introgressed by back crossing into a normally elongating rice variety such as Thai jasmine rice (KDML105), the leaf elongation response to submergence is lost (Siangliw *et al.*, 2003).

The genetics of submergence tolerance is controlled by few genes that are partially or completely dominant. Analyses of submergence tolerance confirmed that the QTL on chromosome 9 has the strongest effect, accounting for 69% of the phenotypia variation (Jackson and Ram, 2003). QTL analysis and positional cloning allowed the identification of the *Submergence1* (*Sub1*) locus on chromosome 9, which confers submergence tolerance. In this locus, there are three similar genes that encode an ethylene response factor (ERF)-type transcription factor domain: *Sub1A*, *Sub1B* and *Sub1C* (Figure 1). These three genes are located in tandem with submergence tolerant varieties. Two alleles of *Sub1A* (*Sub1A-1* and *Sub1A-2*) have been recognized, which are distinguished by a single amino acid substitution within the coding region. The transcripts of *Sub1A-1* and *Sub1A-2* are highly and poorly induced under submergence, respectively. Interestingly, only submergence tolerant accessions possess the *Sub1A-1* allele, whereas accessions that contain the less highly expressed *Sub1A-2* are submergence intolerant. Moreover, *Sub1A* is absent from all *japonica* and some *indica* accessions, all of which are intolerant to submergence. In support of *Sub1A-1* as the key determinant of submergence tolerance, the over expression of the allele in the intolerant *japonica* Liaogeng was confirmed to be sufficient to markedly enhance submergence tolerance (Fukao *et al.*, 2006; Xu *et al.*, 2006).

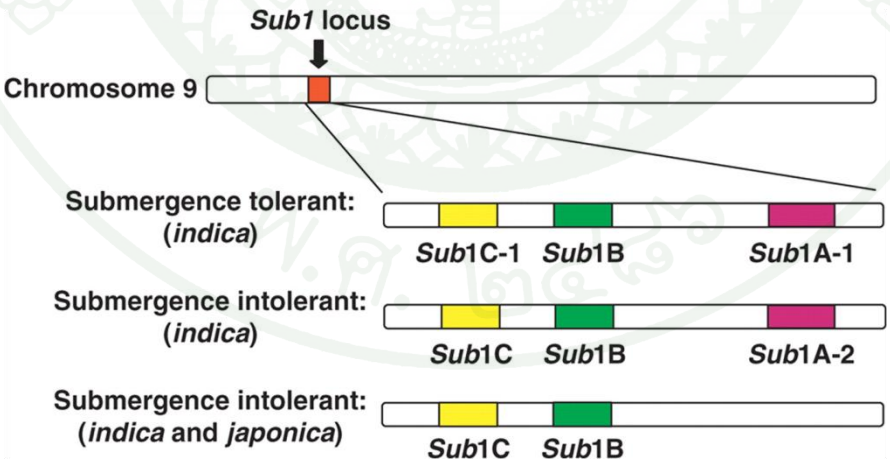

Figure 1 Graphical of *Sub1* haplotypes of *Oryza sativa* L.

Source: Fukao *et al.*(2009)

Rice plants carrying *Sub1A* show restricted growth under submerged conditions, and thus avoid energy consumption associated with plant elongation. After the water receded, rice plants can restart growth using the conserved energy. During submergence, higher induction of α-amylase genes (*RAmy3C*, *RAmy3D* and *RAmy3E*) and sucrose synthase genes (*Sus1*, *Sus2* and *Sus3*) were observed in submergence-intolerant plants; by contrast, there was lower expression of these genes under the same conditions in submergence tolerant plants (Fukao *et al.*, 2006). Rice α-amylases function during rice germination, breaking down starch into sugars, and accumulate in the seed embryo and aleurone cells, even under anoxia. These results suggest that *Sub1A* negatively regulates transcription of these genes, to reduce energy consumption during submergence. Additionally, ethanolic fermentation is necessary during low-oxygen and submergence stress, and pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) genes are increased under low-oxygen conditions. Both enzymes function in the ethanolic fermentation pathway. Expressions of two *PDC* genes and two *ADH* genes are induced during submergence in submergence-tolerant rice. This suggests that *Sub1A* positively regulates the expressions of *PDC* and *ADH* genes under hypoxia. The flash flood tolerance gene, *Sub1A*, would promote acclimation to low oxygen conditions by regulating expression of these genes. Plant growth is controlled by the accurate regulation of plant hormones, even during growth under flooded conditions. During flash floods, submergence-tolerant lines limit their growth through the gibberellic acid (GA) signal transduction pathway (Fukao and Bailey-Serres, 2008). Slender rice-1 (SLR1) and SLR1-Like-1 (SLRL1) are repressors of GA signaling. SLR1 and SLRL1 proteins accumulate during submergence in submergence-tolerant rice, but not in submergence intolerant rice. These results indicate that the restricted growth of submergence tolerant rice is caused by the accumulation of SLR1 and SLRL1, which repress the GA signal through *Sub1A* (Figure 2). The transcriptome analyses revealed that further to the above factors, some complex events may exist downstream of *Sub1A*, such as cytokinin response and detoxification of reactive oxygen species (Jung *et al.*, 2010).

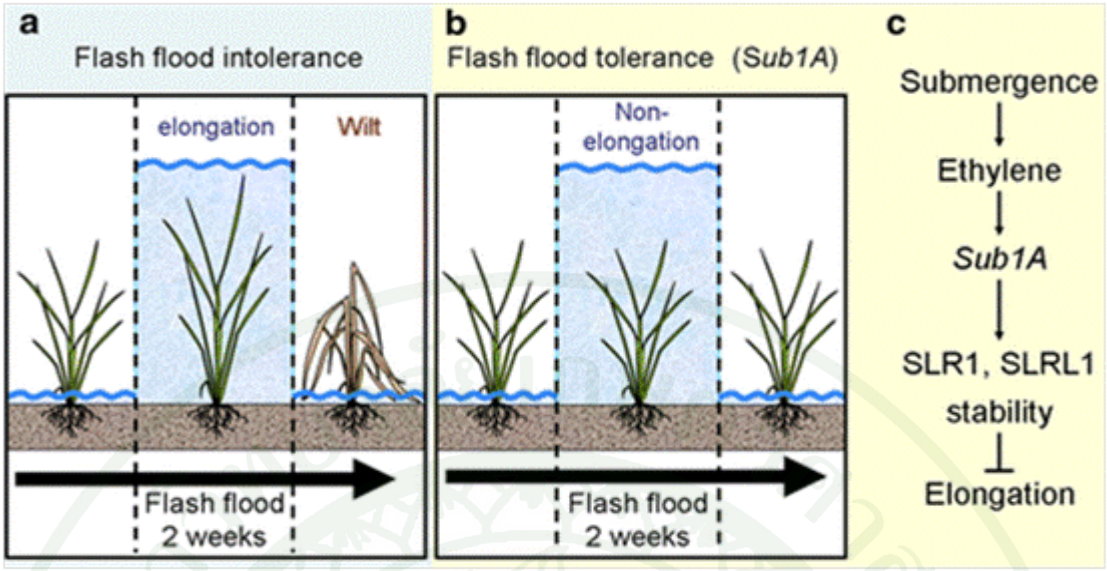

Figure 2 Model of flash flood tolerance and plant hormones in rice. a) The strategy of intolerant rice. b) The strategy of tolerance rice at the seedling stage. c) The scheme of flash flood tolerance. Flash flood tolerant rice carries *Sub1A*, which promotes the accumulation of SLR1 and SLRL1, negative regulators of GA signaling, and inhibits internodes elongation.

Source: Nagai *et al.* (2010)

Bacterial blight disease

Bacterial blight (BB) caused by the pathogen *Xanthomonas oryzae*. This disease is outbreaks occur in irrigated and rainfed lowland ecosystem when rainfall is heavy. The severity of infection is increasing when the high amount of fertilizer is applied. BB symptom can be infected in all stages of the rice plants and the disease can cause yield loss as high as 80% yield reduction, however, it depends on rice growth stage, geographic location or seasonal condition (Singh *et al.*, 1977). BB is caused by the short rod-shaped bacterium with round ends 1-2x0.8-1 um, monotrichous flagellum 6-8 µm, gram-negative, non-spore-forming and aerobic bacteria. Colonies of *Xoo* are circular, convex, whitish yellow to straw yellow, with smooth surface, entire margin, and opaque against transmitted light. *Xoo* can survive

on rice stubbles, straw and weed hosts. The BB is a vascular disease spreads through the xylem vessels. Lesions usually begin at the margin, a few centimeters (cm) from the tip, as water-soaked stripes. It can occur at all stages of the rice plants. At the seedling stage, the symptom first appeared as tiny water-soaked spots at the margin of the rice leaf blade. Then, it will enlarge and the rice plants turn yellow and wither. At the seedling stage, the lesions may start at anywhere on the leaf blade at the site of an injury. The lesions can occur on leaf sheath of susceptible cultivars. The affected leaves will turn yellow, roll up and wilt rapidly. At the tillering and reproductive stages, the symptom is known as leaf blight, a systemic infection that produces tannish-grey to white lesions along the vein. If plant produces panicles, the sterility percentage and number of immature grains will increase. Grains from diseased plants were easily broken during milling.

The identification and characterization of major genes for resistance and related factors have a great deal to the success in plant breeding programs. To date, more than 30 BB resistance genes have been identified from rice plants are currently reported and identified from cultivated rice and wild rice (Kameswara Rao *et al.*, 2002). Eleven are recessive genes including *xa5, xa5(t), xa8, xa13, xa15, xa19, xa20, xa24, xa28, xa31* and *xa32* (Kameswara Rao, 2003; Niño-Liu *et al.*, 2006). Three resistance genes including *xa15, xa19,* and *xa20* were induced by mutagenesis (Lee *et al.*, 2003). Six resistance genes including *Xa21, Xa1, xa5, xa13, Xa26* and *Xa27* had already been cloned (Song *et al.*, 1995; Yoshimura *et al.*, 1998; Iyer and McCouch, 2004; Sun *et al.*, 2004; Gu *et al.*, 2005; Chu *et al.*, 2006).

Most of these genes follow the classic gene-for-gene concept for the race-specific interaction between rice and *Xoo*. Avirulent gene in bacteria exhibits the specificity for resistance gene in the rice plant. Some resistance genes are effective only in adult plants, while other is effective at all stages of growth. Many BB resistance genes, i.e. *Xa3, Xa4, Xa6, xa9, Xa10, Xa21, Xa22, Xa23, Xa26* and *Xa30,* have been reported to locate on chromosome 11. Most of them are ultigene family and tightly link together. *Xa21* gene was introgressed from a wild species *O. longistaminata* into *O.sativa* background (Khush *et al.*, 1989). It confers resistance to

abroad range of *Xoo* strains. This R gene was first tagged with RAPD marker (Ronald *et al.*, 1992). RG103, the RFLP marker had found to be tightly linked to this gene at a distance of 1.2 cM. A PCR-base STS marker pTA248 was developed and can be used in marker-assisted selection and can be adopted a map-based cloning strategy. *Xa21* was the first BB resistance gene successful cloned. Currently, PB7/8 marker (Chunwongse *et al.*, 1993), derived from *Xa21* gene has been very effective using for selecting BB resistance *Xa21* gene in rice. Jantaboon *et al.* (2004) was used PB7/8 to select for homozygous at *Xa21* locus. This marker was shown present for the lines carry *Xa21* gene and was shown absent for not carry *Xa21* gene.

Cooking and eating quality

The cooking and eating quality of the rice grain is one of the most serious problems in many rice producing areas of the world. The grain quality of rice is a complex character composed of many components such as nutritional quality, appearance quality, cooking quality and eating quality. Grain quality is directly related to three attributes of the physical and chemical characteristics of the starch in the endosperm, amylose content (AC) (Juliano, 1985), gel consistency (GC) (Cagampang *et al.*, 1973) and gelatinization temperature (GT) (Little *et al.*, 1958).

Starch is a principle reserved carbohydrate in plants. Starch consists of two glucan polymers, namely amylose and amylopectin (Figure 3). Most cereal endosperm starch consists of 18-33% amylose and 72-82% amylopectin (Buléon *et al.*, 1998). Amylose and amylopectin have different structures and properties.

Amylose is a predominant linear polymer comprising α-1,4-glucosidic bonds with a very small amount of α(1-6) branches. The cluster is usually close to the reducing head of the molecule. It has a molecular weight approximately 1x105-1x106 (Tester *et al.*, 2004). The chain numbers in amylose molecules is different ranging from 2-11 with the individual chain lengths (CL) containing 250-670 glucose units. Rice starch amylose has degree of polymerization (DP_n) values of 920-1110, CL of 250-370 and slightly branched with average 2-5 chains (Vandeputte and Delcour,

2004). Amylose accounts for 18-33% of total starch. Moreover, amylose is also responsible for the characteristic binding of charged iodine molecules where each turn of helix holds about two iodine atoms and a blue color is produced.

Amylopectin is a very large molecule which spans from the hilum (center) to the periphery of starch granule and composes of small linear α-1,4-glucan chains linked together with branch points by α-1,6-glucosidic bonds. The average branches of amylopectin molecule are 5-6 %. It has a DP_n of 4700-12,800, CL values of 17-24. The individual chains may vary between 10-100 glucose units (Vandeputte and Delcour, 2004). The molecular weight of amylopectin is approximately 1x107-1x109 Rice starch amylopectin has a DP_n 8200-12,800 and CL of 19-23 (Buléon *et al.*, 1998). In the amylopectin molecules, branching points are arranged in clusters, which are not randomly distributed throughout the molecule. It contains three chains including, A 5 chains, B chains and only one C chain Based on the cluster model of amylopectin molecule, the most peripheral (exterior) chains which no other chains bonded are described as A chains. A group of B chains are those that carry one or more branches mostly of A chains. The C chain is the only one which has the reducing head calling backbone of the amylopectin molecules. Depending on the chain lengths and the number of clusters traversed within the granule, B chains are referred as B1-B4 (one to four clusters). The ratio of A- to B-chains is a parameter used for characterizing the different of amylopectin structure. The chain length distribution of amylopectin is an important characteristic which varies between plant species (Andersson, 2001).

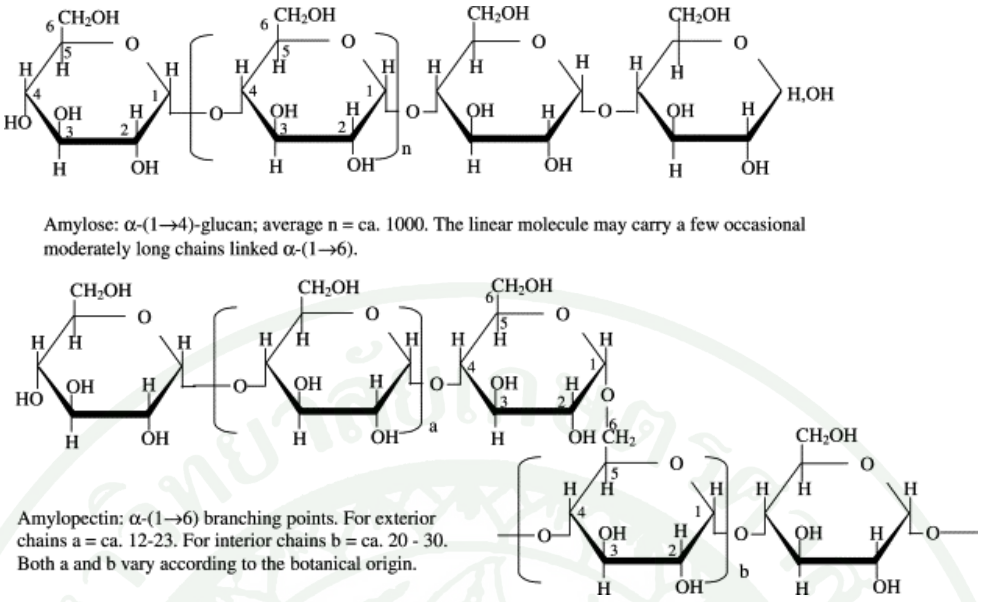

Figure 3 Structures of amylose and amylopectin.

Source: Tester *et al*.(2004)

Amylose content (AC)

Amylose content (AC) is associated with cooked rice tenderness. Varieties with low-amylose content such as japonica varieties typically have a soft, sticky cooked rice texture. In contrast, varieties with an intermediate- to high-amylose content, represent by most of the indica varities, typically produced cooked rice which separate and firm, drier texture (Juliano, 1971). In rice, amylose contents are classified as waxy (0-2 % amylose), very low (5-12% amylose), low (12-20 % amylose), intermediate (20-25 % amylose), or high (25-33 % amylose) (Vandeputte and Delcour, 2004).

Molecular marker technology has facilitated our understanding of the genetic basis of complex quantitative traits. Recent results from molecular marker based quantitative trait locus (QTL) analyses of AC have revealed that AC is mainly controlled by the *Waxy* gene locus (*Wx*) on chromosome 6 (He *et al.*, 1999). In rice, two functional alleles of the *Wx* gene have been found to exist that correspond to the

AC levels of indica and japonica rice varieties, respectively (Sano *et al.*, 1986). Wx^a is widely distributed in O. sativa spp. indica, a subspecies with higher AC, whereas Wx^b is mainly observed in japonica, a subspecies with intermediate AC. The rice *Waxy* gene (*Wx*) encodes a granule-bound starch synthase (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 exons with a 1.1 kb untranslated leader intron (Hirano and Sano, 1991). The amylose content of rice endosperm is regulated post-transcriptionally. It was found that sequence variation in the 5′ splice site of the leader intron affected the amylose content among the rice varieties (Wang *et al.*, 1995). Two alleles Wx^a and Wx^b were found to regulate the quantitative level of the gene product as well as the amylose content. The Wx^a allele contains an efficient intron 1 splice site (AGGTATA) for normal gene expression and plants expressing this allele contain intermediate to high grain amylose content. The Wx^b allele commonly carries a single nucleotide variation at the first intron splice site (AG(G/T)TATA). The G-to-T base substitution reduces the efficiency of the first ntron splicing, and subsequently depresses GBSS expression (Isshiki *et al.*, 1998). Recently, it has been learned that the amylose content is influenced by the temperature and that the splicing aberrant of the Wx^b allele is affected by extreme temperatures. Therefore, the amylose content can be enhanced at low temperatures but depressed at high temperatures (Hirano and Sano, 1998; Inukai *et al.*, 2000).

The waxy rice 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* mutations, by uses of EMS-induction and gamma ray induction, revealed base substitutions in several exons and introns, and base deletion in intron 4. In addition, a spontaneous *wx* mutation, in the japonica traditional cultivar ‘Kinoshita-mochi’, showed that exon 2 had a 23 bp duplication in the coding sequence (Inukai *et al.*, 2000). In Indochina, particularly in Thailand and Laos, glutinous rice is popularly grown for in-house consumption. In addition to direct consumption, its unique starch quality makes it to be in high demand by modified

food manufacturers for the production of such things as rice crackers. Glutinous rice is not only interesting for its origin but also for its adaptability to lowland rainfed fields, where growing conditions are harsh and diverse. In Thailand, 70% of the rice grown is on lowland rainfed fields. Here, glutinous rice production is constrained by salinity, low soil fertility, drought and submergence. Most of the glutinous rice varieties are photoperiod sensitive landraces. Being photoperiod sensitive landraces, most of these glutinous rice varieties have to be improved genetically emphasizing in a specific starch quality.

Gel consistency (GC)

Gel consistency, which is a measure of cold paste-viscosity of cooked milled rice flour, is a good index of cooked rice texture, especially among rices of high amylose content. Rice differs in gel consistency from-soft to hard (Cagampang *et al.*, 1973). Cooked rice with hard gel consistency harden faster than those with a soft one. Rice with soft gel consistency cook tender, and remain soft even upon cooling (Juliano, 1979). Rice with soft gel consistency is preferred by most rice consumers. Breeders are therefore trying to develop high-yielding varieties with soft gel consistency (Khush *et al.*, 1979).

Tremendous efforts have also been made to understand the genetic basis of GC. Because of its significant negative correlation with AC, it has long been believed that GC is also controlled by *Wx* or another gene tightly linked to the *Wx* locus (He *et al.*, 1999; Lanceras *et al.*, 2000). Two QTLs on chromosome 6 and one on chromosome 7 were detected for GC by using RIL population derived from the cross KDML105xCT9993 (Lanceras *et al.*, 2000). On the other hand, three QTLs, designated as qGC-1, qGC-2 and qGC-6, were identified using a population consisting of 190 DH lines derived from an F_1 hybrid between WYJ2 (japonica) and Zhenshan97B (indica) (Su *et al.*, 2011). Two QTLs for amylose content and gel consistency were detected and mapped on chromosomes 6, respectively, using 285 BC_2F_2 plants developed from an interspecific cross between cv IR64 and *Oryza rufipogon* (Septiningsih *et al.*, 2003). The QTL, qGC-6 with the largest effect on GC

was located in the interval between RM190 and RM510 on chromosome 6 (Tian *et al.*, 2005; Zheng *et al.*, 2008). He *et al.* (1999) identified two QTLs for GC on chromosomes 2 and 7 using a DH population consisting of 132 lines. Although these investigations largely contributed to the genetic basis of GC in rice, the basis for its molecular regulation remains unknown. Su *et al.* (2011) confirmed that the identified ORF for qGC-6 encodes granule-bound starch synthesis protein (*Wx* protein), which proves that *Wx* locus is responsible for gel consistency of japonica/indica cross in rice. Using 64 plants with extremely soft GC that were selected on recombinant break points between two SSR markers, RM540 and RM8200 in a BC_4F_2 population, qGC-6 was mapped to a 60-kb DNA region between two STS markers, S26 and S27.

Gelatinization temperature (GT)

Gelatinization temperature (GT), an important parameter for rice cooking quality, is the critical temperature at which the starch granules start to lose crystallinity and birefringence by irreversible expanding and to change the starch surface from polarized to soluble state called starch paste (Khush *et al.*, 1979). Estimated indirectly by digestion of the rice seeds in alkali solution (Juliano, 1979), the range of GT of different varieties of rice is classified as three groups: high (>74°C), intermediate (70°C-74°C), and low (<70°C) (Bhattacharya, 1979) GT rice. On the other hand, water and time required for rice cooking also show high correlation to GT, which means that rice varieties with high GT need more water and longer period of cooking than those with low GT.

The inheritance of GT has been widely studied since the 1990s (He *et al.*, 1999; Tian *et al.*, 2009). QTL mapping showed that GT may be controlled either by the alkali degeneration gene (*alk*) or by the *Wx* gene (Bao *et al.*, 2002; Fan *et al.*, 2005). Umemoto *et al*. (2002) reported that the starch synthase IIa gene (*SSIIa*) was located at the *alk* locus on chromosome 6 (Figure 4) in the rice genome.

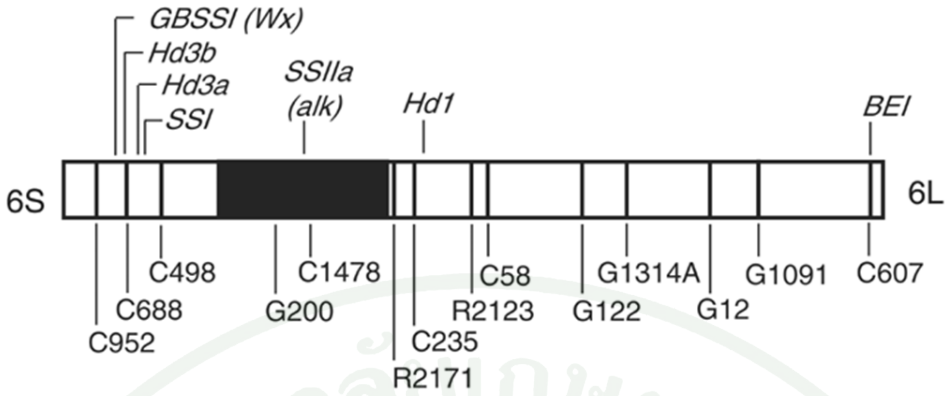

Figure 4 Position of gene involved in GBSSI (*Wx*);granule-bound starch synthase I and *SSIIa*(*alk*); starch synthase IIa.

Source: Umemoto *et al.* (2004)

Gao *et al* (2003) report the map-based cloning of the *alk* locus that encodes *SSIIa*, and found that nucleotide substitutions in the coding sequence of *SSIIa* may cause the alteration in GT. Jiang *et al.* (2004) cloned three isoforms of starch synthase II incluing *SSIIa* by cDNA library screening, finding that *SSIIa* was mainly expressed in endosperms. Three single nucleotide polymorphisms (SNPs) were reported to be associated with GT through haplotype analysis (Umemoto *et al.*, 2004). With shuffling experiments, Nakamura *et al*., (2005) further analyzed in detail the effect of amino acid replacement caused by these SNPs on enzyme activity of *SSIIa*, amylopectin structure and GT, and suggested two of the SNPs were essential for *SSIIa* activity, which proved to play a specific role in the synthesis of the long B1 chains by elongating short A and B1 chains. A study by Bao *et al.*, (2006) provided further support for the utilization of the GC/TT polymorphism via association mapping in marker assisted breeding for improvement of rice grain quality and developed a method including four primer in a single PCR to genotype the GC/TT SNP at 4329/4330 bp (Figure 5). The GC/TT polymorphism can differentiate rices with high or intermediated GT from low GT at a rate of about 90% correct prediction.

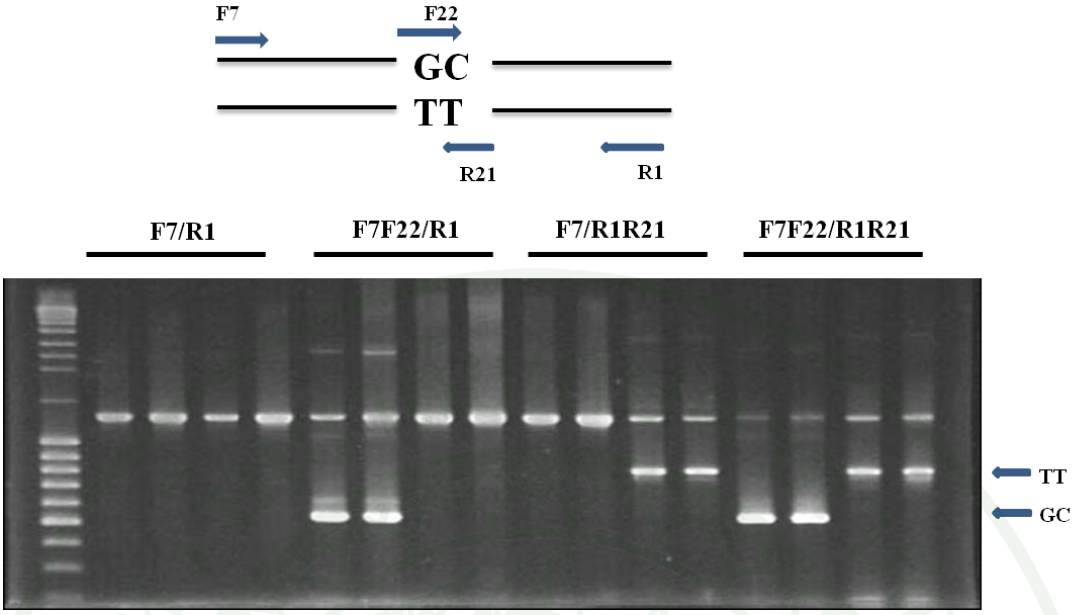

Figure 5 Schematic representation of PCR with two-pair confronting primer and gel image showing different PCR products amplified by different combinations of the primers.

Source: Bao *et al.* (2006)

Fragrance

Fragrant rice is the much valued quality factor because of this feature is given a premium price. In recent year, fragrant rice is highly favored and command higher prices than non-fragrant rice in international market due to its pleasant fragrance, soft texture, good cooking and eating quality. Over 100 volatile compounds have been detected in fragrant rice varieties, but the major compound responsible for the characteristic aroma is 2-acetyl-1-pyrroline (2AP) (Buttery *et al.*, 1983; Lorieux *et al.*, 1996). This compound, which is produced in all parts of the rice plant except the roots, has a very low odor threshold, allowing humans to detect it at minute concentrations in field-grown plants or crushed leaf tissue, as well as in the grain before, during, and after cooking (Buttery *et al.*, 1983).

Although several volatile flavour compounds have been identified that are responsible for fragrance (Yajima *et al.*, 1979; Mahatheeranont *et al.*, 2001), 2-acetyl-1-pyrroline (2AP) is the major active compound in fragrant rice. 2AP identified as a principal aroma compound and L-proline was identified as a precursor of aroma in rice (Yoshida *et al.*, 2002), the biochemical pathways of 2AP synthesis is presently unknown (Fitzgerald *et al.*, 2009). Moreover, some contradictions were reported about the origin of pyrroline, a precursor for 2AP. Vanavichit *et al.* (2005) attempted to elucidate the 2AP biosynthetic pathway and proposed that 2AP is synthesized via the polyamine pathway. The immediate precursor of 2AP was found to be 1-pyrroline (1P) which is formed from 4-aminobutyraldehyde (AB-ald; the immediate precursor of 4-aminobutyric acid (GABA)) Bradbury *et al*. (2008) suggested that γ-aminobutyraldehyde (GABald) is an effective substrate for BADH2 and that accumulation and spontaneous cyclisation of GABald to form Δ^1-pyrroline due to a non-functional BADH2 enzyme as the likely cause of 2AP accumulation in rice. However, in another study, increased expression of Δ^1- pyrroline-5-carboxylate synthetase in fragrant varieties compared with non-fragrant varieties, as well as concomitant elevated concentrations of its product, led to the conclusion that Δ1-pyrroline-5- carboxylate, usually the immediate precursor of proline synthesized from glutamate, reacts directly with methylglyoxal to form 2AP (Huang *et al.*, 2008), with no direct role proposed for BADH2.

Molecular study of grain fragrance was first discovered by Ahn *et al*. (1992). RFLP analysis showed that fragrance locus (*fgr*) is closely linked to a single-copy DNA clone, RG28, on chromosome 8 of rice at a distance of 4.5 cM. Bradbury *et al*. (2005a) has been identified fragrance is recessive traits due to the 8 base pair deletion and 3 single nucleotide polymorphisms (SNP) in exon 7 of the gene which encodes a putative betaine aldehyde dehydrogenase 2 (*badh2*) have led to the introduction of premature stop codon to produce a truncated protein which result in abrogation of the function of the enzyme BADH2 consequently accumulate substrate 2AP in fragrant varieties, while the functional *Badh2* gene codes for a 503 amino acid mature protein which consumes the substrate in non-fragrant varieties. Identification of the gene for fragrance and availability of large aromatic rice gene pool has created world-wide

interest to look for allelic variants at this locus. In addition to 8-bp deletion in exon 7, several variations including a 7-bp insertion in exon 8 (Amarawathi *et al.*, 2008); a 7-bp deletion in exon 2 (Shi *et al.*, 2008); absence of MITE (miniature interspersed transposable element) in promoter (Bourgis *et al.*, 2008) two new SNPs in the central section of intron 8 (Sun *et al.*, 2008); a TT deletion in intron 2 and a repeated $(AT)_n$ insert in intron 4 (Chen *et al.*, 2008) of *badh2* were reported in various fragrant varieties (Figure 6).

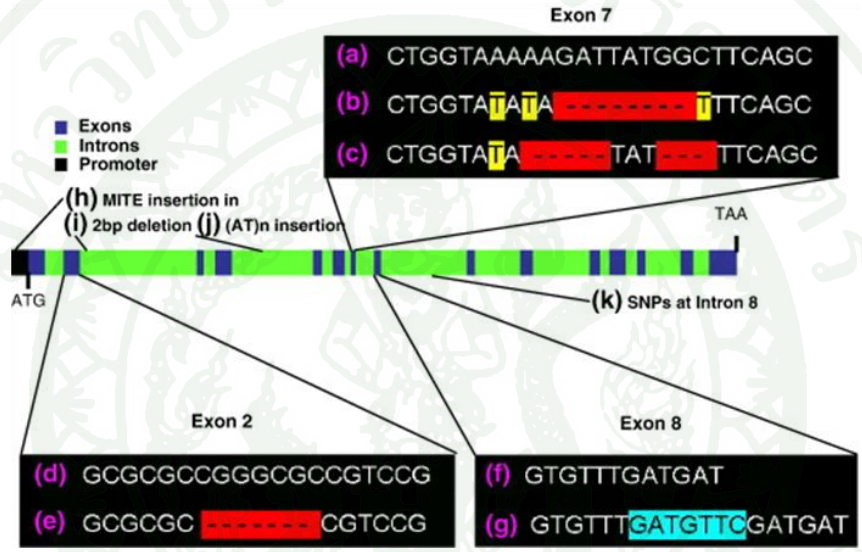

Figure 6 The structure of *Badh2* gene showing various mutations.

Source: Sakthivel *et al.* (2009)

Although many reports concluded that a single recessive gene governs fragrance, emerging evidences indicate there may be QTLs controlling the trait of fragrance. Lorieux *et al.* (1996) were the first to map QTLs for rice fragrance on chromosome 4 and 12. Amarawathi *et al.* (2008) identified 3 QTLs controlling fragrance each on chromosome 3, 4 and 8 in Basmati rice varieties. The QTL on chromosome 8 was found to co-locate on *badh2* locus while the QTL on chromosome 4 was the same as reported by Lorieux *et al.* (1996). Interestingly, betaine aldehyde dehydrogenase 1 (*badh1*) was found as one of the candidate gene in the QTL region in chromosome 4. Due to its similarity in the molecular function as that of *badh2* gene

on chromosome 8, *badh1* was reported as a likely candidate gene for fragrance.While elucidating the biochemical pathway of 2AP production in rice, Bradbury *et al*. (2005a) suggested that despite the low affinity of BADH1 to 4-aminobutyraldehyde (GABald), a presumed 2AP precursor, GABald may still be a substrate for BADH1 in vivo and that the presence of BADH1 may diminish the pool of GABald available to form 2AP. With their similar activities and with BADH1 being associated with a fragrance allele (Amarawathi *et al.*, 2008) they suggested that mutations that lead to a nonfunctional BADH1 or a reduction in expression levels of BADH1 may cause an increase in 2AP accumulation in rice. Considering the exceptions to *badh2* mutation from Fitzgerald *et al.* (2008), and involvement of more than one locus for the fragrance trait from Lorieux *et al.* (1996) and Amarawathi *et al.* (2008), it is increasingly becoming evident that the *badh2* gene alone may not be sufficient enough to explain the genetic and molecular basis of fragrance in rice. Further, involvement of several other compounds such as alkanals, alk-2-enals, alka-2,4-dienals, 2-pentylfuran and 2-phenylethanol in fragrance determination indicate that many other genes might play a role in effecting unique or modified fragrance.

Marker-assisted selection (MAS)

Conventional plant breeding that relies only on phenotypic selection has been historically effective. However, for some traits, phenotypic selection has made little progress due to challenges in measuring phenotypes or identifying individuals with the highest breeding value. The effects of environment, genotype by environment interaction, and measurement errors also contribute to observed differences. Evaluation of genotypes in multiple environments with replicated designs allows better estimation of breeding values, but requires additional time and expense. For some traits, it may be necessary to sacrifice the individual to measure phenotypes, or trait expression may depend on variable environmental conditions (e.g. disease pressure) and the stage of development (e.g. grain quality can only be assessed after flowering). Furthermore, plant breeders typically must simultaneously improve a suite of commercially valuable traits, which may limit gains from selection. Just as molecular plant breeding helps to expand genetic diversity, characterize genetic

architecture, and modify gene action, its methods can also be applied to increasing the efficiency of selection.

The potential benefits of using molecular markers linked to genes of interest in breeding programmes, thus moving from phenotype-based towards genotype-based selection, have been obvious for many decades. Molecular markers are typically derived from a small region of DNA, and should not be considered as normal genes as they usually do not have any biological effect. Instead, they can be thought of as constant landmarks in the genome with a known location on a chromosome and associated with a particular gene or trait. Molecular markers are tools for simultaneously advancing our understanding of plant genome and increasing the efficiency of plant breeding (Toojinda *et al.,* 1998). Marker-assisted selection (MAS) refers to the use of molecular marker that is tightly linked to target loci as a substitute for assist phenotypic screening. MAS is an approach that has been developed to avoid the problems connected with conventional plant breeding changing the selection criteria from selection of phenotypes towards selection of genes, either directly or indirectly. Molecular markers are clearly not environmentally regulated and are unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth. With the availability of an array of molecular markers and genetic maps, MAS has become possible both for traits governed by major genes as well as for quantitative trait loci (QTLs). Usefulness of a given molecular marker is dependent from its capability in revealing polymorphisms in the nucleotide sequence allowing discrimination between different molecular marker alleles. These polymorphisms are revealed by molecular techniques such as restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), microsatellite or simple sequence length polymorphisms (SSR), random amplified polymorphic sequences (RAPD), cleavable amplified polymorphic sequences (CAPS), single strand conformation polymorphisms (SSCP), single nucleotide polymorphisms (SNPs) and other (Mohan *et al.*, 1997; Rafalski, 2002).

Molecular markers for trait *selection* have numerous advantages over morphological markers used in conventional plant breeding. The main advantages of molecular markers include the following:

- Time saving: Genomic DNA can be isolated from any part of the plant tissue, and target trait information can be obtained with linked DNA markers before pollination, thus allowin g breeders to carry out more informed genetic crosses.

- Consistency: Phenotypic evaluation of genetic traits is often complicated by environmental factors. However, DNA markers are mostly neutral to environmental variation.

- Biosafety: Diagnostic tests for the presence or absence of traits for disease resistance can be conducted by DNA markers tightly linked to the target gene without resorting to pathogen inoculation in the field or greenhouse. Additionally, molecular markers facilitate introgression of genes into elite cultivars in advance of the occurrence of certain races of diseases or biotypes of insects.

- Efficiency: Evaluation of breeding lines in early generations of the breeding process with DNA markers can allow breeders to reject progenies from the program and improve the genetic quality of breeding materials.

- More accurate selection of complex traits: Polygenic traits are often difficult to select for using conventional breeding approaches. DNA markers linked to QTL allow them to be treated as single Mendelian factors.

Application of MAS in plant breeding

The advantages of molecular marker in plant breeding, with an emphasis on important MAS schemes. Collard and Mackill (2008) have classified breeding schemes into five broad areas: merker-assisted evaluation of breeding material; marker-assisted backcrossing; marker-assisted pyramiding; early generation selection;

and combined MAS, although there may be overlap between these categories. Generally, for line development, molecular markers have been integrated in conventional schemes or used to substitute for conventional phenotypic selection.

1. Marker-assisted evaluation of breeding material.

The first selection step in plant breeding is the choice of lines to mate as parents of new populations. Conventionally, the selection of such parents is based on a combination of phenotypic assessments, pedigree information, breeding records and chance. Now, the use of molecular markers enables a marker-assisted germplasm evaluation. This type of evaluation has the potential to make parental selection more efficient, to expand the gene pool of modern cultivars and to speed up the development of new varieties (Steele *et al.*, 2004).

2. Marker-assisted backcrossing

Backcrossing is a plant breeding method most commonly used to incorporate one or a few genes into an adapted or elite variety. In most cases, the parent used for backcrossing has a large number of desirable attributes but is deficient in only a few characteristics. The process, where a gene or a QTL from a population A is introduced to a population B by crossing A and B and then repeatedly backcrossing to B, is called introgression (Hospital, 2009). Here, molecular markers can be used to control the presence of the target gene or QTL and to accelerate the return of background genome to recipient type. Marker-assisted introgression is very effective for introgressing genes or QTLs from landraces and related wild species, because it reduces both the time needed to produce commercial cultivars and the risk of undesirable linkage drag with unwanted traits of the landrace or wild species.

The use of molecular markers in backcrossing greatly increases the efficiency of selection (Holland, 2004). Three general levels of marker-assisted backcrossing (MAB) can be described (Figure 7). In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is

referred to as ‘foreground selection’. This may be particularly useful for traits that have laborious or time-consuming phenotypic screening procedures. It can also be used to select for reproductive-stage traits in the seedling stage, allowing the best plants to be identified for backcrossing. Furthermore, recessive alleles can be selected, which is difficult to do using conventional methods.

The second level involves selecting backcross progeny with the target gene and recombination events between the target locus and linked flanking markers in order to minimize linkage drag. This is referred to as ‘recombinant selection’. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus. This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent.

The third level of MAB involves selecting backcross progeny with the greatest proportion of recurrent parent genome, using markers that are unlinked to the target locus. This is referred to as ‘background selection’. Background markers are markers that are unlinked to the target gene or QTL on all other chromosomes. 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. Data from simulation studies suggests that at least two but possibly three or even four backcross generations can be saved by using markers.

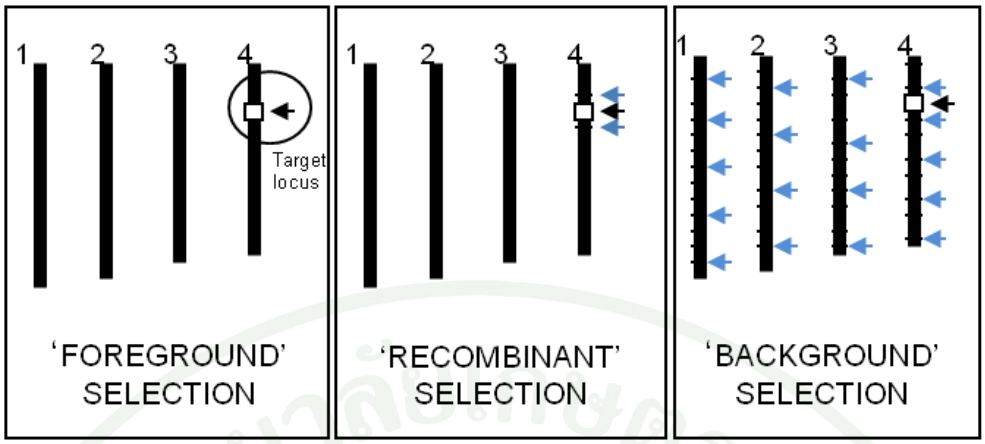

Figure 7 Three levels of selection during marker-assisted backcrossing.

Source: Collard and Mackill *et al.* (2008)

3. Marker-assisted pyramiding

Pyramiding is the process of combining several genes or QTLs together into a plant variety. Using phenotypic selection methods it is extremely difficult and sometimes impossible to pyramid the desired traits. A striking example is the breeding of durable disease resistance. When a variety is protected by one gene with a major effect against a disease, it is often not possible to introgress additional resistance genes to the same disease because they show the same phenotype. However, if resistance genes can be tagged with molecular markers, the number of resistance genes in any plant can be easily determined. The most widespread application for pyramiding has been for combining multiple disease resistance genes in order to develop durable disease resistance. (Collard and Mackill, 2008).

4. Early generation selection marker-assisted selection

Although markers can be used at any stage during a typical plant breeding programme, MAS is a great advantage in early generations because plants with undesirable gene combinations can be eliminated. This allows breeders to focus attention on a lesser number of high-priority lines in subsequent generations. When

the linkage between the marker and the selected QTL is not very tight, the greatest efficiency of MAS is in early generations due to the increasing probability of recombination between the marker and QTL. The major disadvantage of applying MAS at early generations is the cost of genotyping a larger number of plants. Ribaut and Betran (1999) proposed that a single MAS step could be performed on F_2 or F_3 populations derived from elite parents. The population sizes may soon become quite small due to the high selection pressure, thus providing an opportunity for genetic drift to occur at non-target loci, so it is recommended that large population sizes be used. This problem can also be minimized by using F_3 rather than F_2 populations, because the selected proportion of an F_3 population is larger compared with that of an F_2 population also proposed that, theoretically, linkage drag could be minimized by using additional flanking markers surrounding the target QTLs, much in the same way as in MAB

5. Combined marker-assisted selection

There are several instances when phenotypic screening can be strategically combined with MAS. In the first instance, 'combined MAS' may have advantages over phenotypic screening or MAS alone in order to maximize genetic gain. This approach could be adopted when additional QTLs controlling a trait remain unidentified or when a large number of QTLs need to be manipulated. Simulation studies indicate that this approach is more efficient than phenotypic screening alone, especially when large population sizes are used and trait heritability is low (Hospital *et al.*, 1997). In practice, all MAS schemes will be used in the context of the overall breeding programme, and this will involve phenotypic selection at various stages. This will be necessary to confirm the results of MAS as well as select for traits or genes for which the map location is unknown.

In some situations, there is a low level of recombination between a marker and QTL, unless markers flanking the QTL are used. In other words, a marker assay may not predict phenotype with 100% reliability. However, plant selection using such markers may still be useful for breeders in order to select a subset of plants

using the markers to reduce the number of plants that need to be phenotypically evaluated. This may be particularly advantageous when the cost of marker genotyping is cheaper than phenotypic screening, such as for quality traits. This was referred to as ‘stepwise selection’ by Langridge and Chalmers (2005). In addition to complementing conventional breeding methods, mapping QTLs for important traits may have an indirect benefit in a conventional breeding programme. In many cases, this occurs when traits which were thought to be under the complex genetic control are found to be under the influence of one or a few major QTLs. For example, submergence tolerance of rice was found to be under the control of the major QTL *Sub1*, which helped simplify the breeding for this trait (Xu *et al.*, 2006).

MATERIALS AND METHODS

Part I: Non-glutinous rice varieties

Plant materials

RILs (2,037 F_7 lines) were developed through an SSD method from F_2 individuals derived from a cross between IR57514-PMI-5-B-1-2 (IR57514) and KDML105. IR57514, developed by IRRI, was identified as widely adapted to the rainfed lowlands of the Mekong region (Naklang *et al.*, 1996; Romyen *et al.*, 1998) and has shown a high yield performance with good agronomic characteristics across several years and locations (Jearakongman *et al.*, 1995; Ouk *et al.*, 2006). Later, this line was identified as a submergence tolerant variety that carries a *Sub1* locus derived from the ancestor cultivar, FR13A. KDML105 is a fragrant, traditional Thai variety that has good culinary qualities. It adapts well to the rainfed lowlands of Thailand (Siangliw *et al.*, 2003) but is intolerant to flash flooding and has a low yield performance.

DNA extraction and PCR amplification

In each sample, leaf sample of one-month-old seedling were cut and stored in container at –20°C prior to DNA extraction. A total genomic DNA of two parents and individual plants of progeny were isolated from 0.5 g of leaf tissue according to the DNA trap method developed by DNA Technology Laboratory, Kasetsart University, Kamphaeng Saen, Thailand. The leaf sample was frozen with liquid nitrogen and ground into a fine powder with conical plastic grinder and the powder was then added to 1000 ul of extraction buffer and incubated at 65°C for one hour. The sample was placed in ice for 5 min and added 100 ul of neutralizer and mixed well using vortex genie. The content was spun in a centrifuge at 14000 rpm for 7 min and then aqueous solution was transferred to new 1.5 ml tube. DNA was precipitated in 500 ul of trapping buffer and gently mixed and spun at 2200 rpm for 1 min. The supernatant was removed and the pellet was washed twice with each 500 ul of washing buffer I and washing buffer II, spun at 2200 rpm and 14000 rpm for 1 min, receptively. The

sample was dried at 65°C for one hour, after that DNA was re-hydrated with 100 ul of elution buffer and incubated for 30 min at 65°C. After finished centrifugation of sample for 2 min at 14000 rpm, DNA was transferred into a new 0.2 ml tube to a final concentration of 50-100 ng per ul. The PCR reaction was performed in a 10ul reaction mixture containing 2 ul of template DNA (50 ng), 1 ul of 10xPCR buffer, 0.8 ul of 25 mM $MgCl_2$ (final concentration 2 mM), 2 ul of 1 mM dNTP (final concentration 0.2 uM), 0.4 ul of 5 uM forward and reverse primers (final concentration 0.2 uM), 0.5 ul of 1 unit of Taq DNA polymerase (final concentration 0.5 unit). The volume was completed to 10 ul with distilled water. Sample was covered with one drop of mineral oil. PCR reaction was initiated at 94°C denatured temperature for 3 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min and 30 sec and final 5 min incubation at 72°C was allowed for completion of primer extension. The amplified product was electrophoresed on a 4.5% denaturing silver-stained polyacrylamide gel.

DNA markers for MAS

Information on markers used in this study was obtained from the Gramene database (http://www.gramene.org). The R10783indel was used for selection of favored allele (submergence tolerant) of the *Sub1* locus located on chromosome 9 (Siangliw *et al.*, 2003; Toojinda *et al.*, 2003; Toojinda *et al.*, 2005). This marker was developed from RB0783, an expressed sequence tag marker from the Rice Genome Project, Japan. Three DNA markers, including Aromarker, Waxy and GT11 were used in selection of cooking quality. Aromarker was developed based on an 8-bps deletion in the seventh exon of the Os2AP (or *badh2*) gene on the chromosome 8 and can be used to distinguish fragrant from non-fragrant alleles. Waxy, a microsatellite marker developed by Cornell University, was used to amplify a fragment containing (CT)n repeat in the putative 5′ splice site leader intron of the *Wx* gene which is located on the short arm of chromosome 6 (Tian *et al.*, 2005). GT11, a closely linked microsatellite marker to the *SSIIa* gene developed by Rice Gene discovery Unit, Thailand, was used to distinguish high and low GT traits (Lanceras *et al.*, 2000).

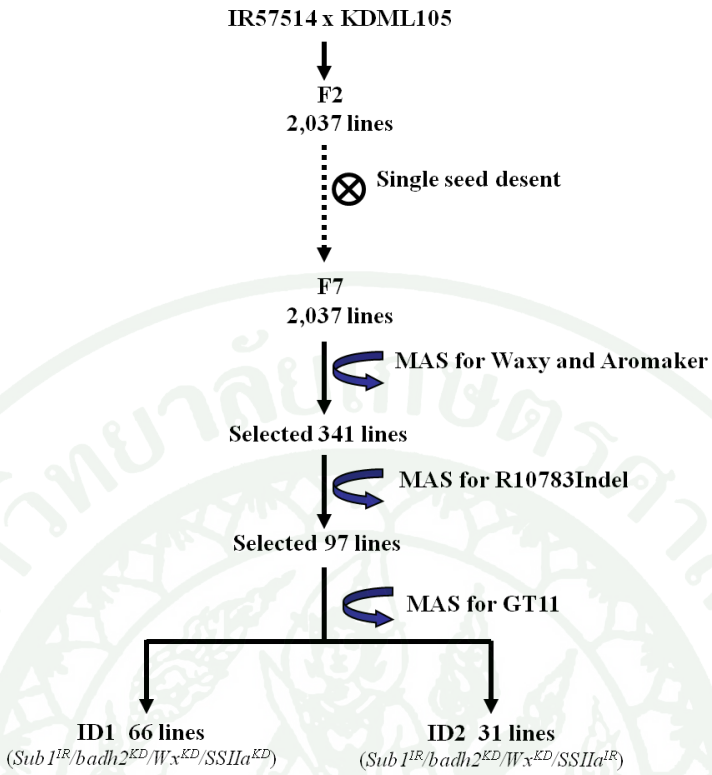

Figure 8 Breeding and selection scheme using step-wise marker-assisted selection non-glutinous rice.

Marker-assisted selection

MAS was applied using four molecular markers linked to the *Sub1*, *Wx*, *badh*2 and *SSIIa* genes as shown in Figure 8. Selective markers and their polymorphism information were shown in (Figure 9). Step-wise MAS was practiced for three rounds to increase genotyping efficiency. First, Waxy and Aromarker were used in the genotyping of 2,037 F_7 RILs. The RILs with two positive alleles were then genotyped with R10783indel. Lastly, the RILs with three positive alleles were screened with GT11. The RILs with positive alleles of all of the four loci were grouped as ID1 ($Sub1^{IR}$, $badh2^{KD}$, Wx^{KD} and $SSIIa^{KD}$), whereas the RILs carrying positive alleles of three of the loci and a negative allele at GT11 were grouped as ID2 ($Sub1^{IR}$, $badh2^{KD}$, Wx^{KD} and $SSIIa^{IR}$). The validation of specific alleles was performed using 66 ID1s and 31 ID2s to determine the submergence tolerance, amylose content (AC), fragrance

(FR) and alkali spreading value (ASV) and to evaluate the agronomic performance in paddy fields.

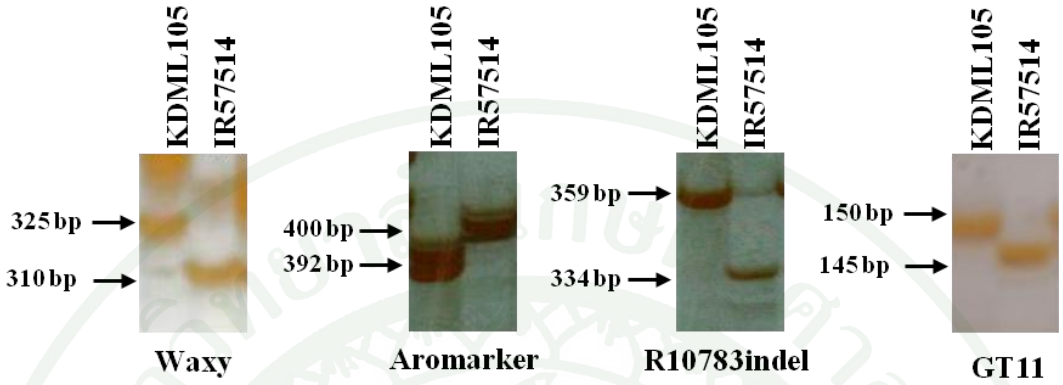

Figure 9 Selective markers and their polymorphism information between two parents for genotypic selection of individual plant in non-glutinous varieties.

Determination of the genetic constitution in ID populations

The 66 ID1 and 31 ID2 lines were assayed for their genetic background using 97 microsatellite markers that showed clear polymorphisms between the parental lines. The list of marker is shown in Appendix Table 1. These markers were well distributed throughout the 12 chromosomes of the rice genome and at least four markers spanning in each chromosome. Scoring the genotype of markers was calculated using the number of markers homozygous for KDML105 and IR57514 allele as estimated the percent genome contents. The ratios of segregation were tested for each marker using a chi-square (χ^2) test. The estimation of the genetic background was determined by counting the allele frequency of the markers.

Trait evaluation and data recordings

Submergence conditions (flash flooding for 15 days)

The lines, 66 ID1s and 31 ID2s, and three standard controls, including FR13A and IR57514 as controls for tolerance and KDML105 as an intolerance control, were

assessed for their submergence tolerance. The experiment was conducted under complete submergence in an outdoor lagoon located in Agronomy Field, Kasetsart University, Kamphangsean Campus, Thailand during the wet season of 2009. The experiment was arranged in a randomized complete block design with three replications. The IDs and controls were seeded in two-row plots of 1.25 m in length and 0.25 m between the rows. Four weeks after seedling, the number of seedlings was counted for each plot, and the lagoon was filled with water to a depth of 2 m. To impose severe submergence stress, seedlings were subjected to complete submergence for 15 days by keeping the water level at 1-1.2 m above the leaf tip throughout the experimental period. The lagoon was drained, and the seedlings were re-exposed to air for 10 days. The number of surviving plants and their height were recorded (Figure 10).

The percentage of surviving seedlings (PSS) was calculated as total number of surviving seedlings counted 10 days after the water was drained from the submerging ponds, divided by total number of seedlings counted before submergence. This value was then multiplied by 100.

$$PSS = \frac{\text{number of surviving seedling} \times 100}{\text{total number of seedling}}$$

The percentage of seedling elongation (PSE) was used as a measure of the increment in shoot height during submergence and calculated as the average difference in shoot height before and after desubmergence. To compare the impact of submergence on shoot elongation, the extension in height in each individual line was set to be 100%.

$$PSE = [\text{shoot height after submergence} - \text{shoot height before submergence}] \times 100$$

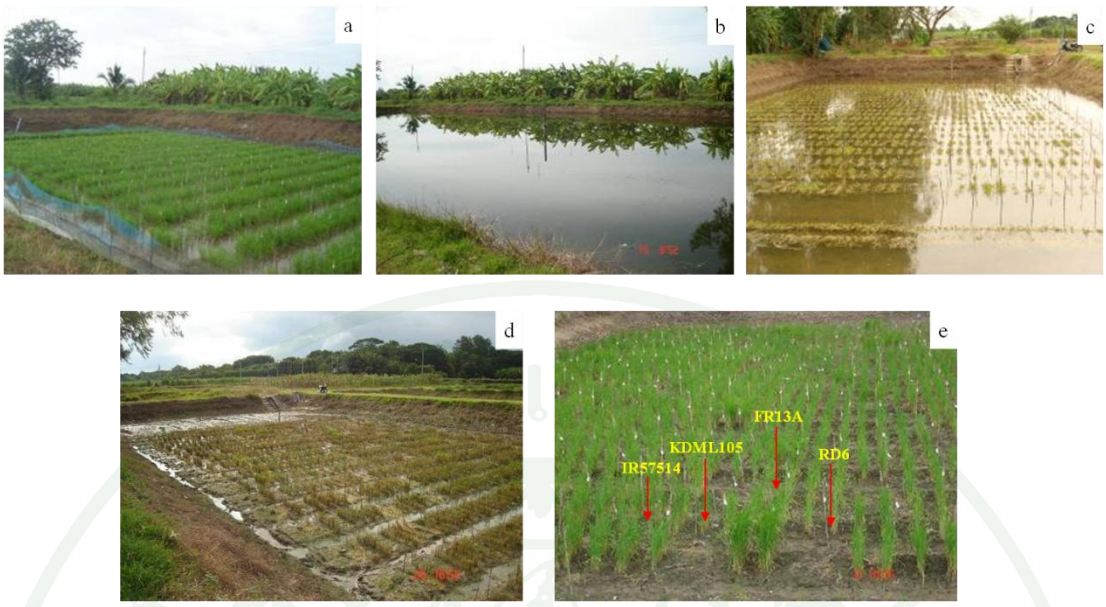

Figure 10 Evaluation of submergence tolerance in outdoor lagoon (a) Seedling at the age of 4 weeks (b) The lagoon was filled with water and keeping the water level at 1-1.2 m above the leaf tip throughout the experimental period (c) The lagoon was drained (d) The seedlings were re-exposed to air for 10 days (e) The number of surviving plants were recorded.

Normal irrigation

The parental lines and IDs were planted to evaluate their essential agronomic traits in an experimental field of the same area as the submergence experiment under irrigated conditions during the wet season of 2009. The planting method, field management and trait measurements, including days to flowering (DF), plant height (PH), numbers effective tiller per plant (NETP), numbers of filled grains per panicle (NFGP), numbers of grains per panicle (NGP), percent spikelet fertility (PSF) and weight of 1000 grains (TGW). DF was recorded at flowering time whenever 50% of individuals in each plot flowered. PH, NETP, NGP and NFGP were measured at maturity, based on three individuals randomly selected in each plot. PH was measured from soil surface to at the tip of the panicle (awn excluded), NGP and NFGP were counted manually, all measurements based on the main stem of those three individual randomly selected plants. Phenotypic acceptability and proneness to lodging were

recorded visually according to Standard Evaluation System (SES) of rice from IRRI. The measurement of GY per plot was used only the inner rows. Two border rows and border plants of each row were discarded. GY of each plot was adjusted to 14% moisture content. The data of GY per plot was used to calculate as tons per hectare. TGW was recorded after drying.

Evaluation of grain quality traits

Seeds of the IDs and their parents were harvested from the border rows at the physiological maturity stage and dried naturally in a greenhouse. Seed samples (100g) were taken from each replication and mixed together. Only 50g of the mixed samples was mechanically dehulled and polished by a minipolisher; these milled samples were used in the grain quality tests.

Evaluation for presence or absence of fragrance IDs and their parents was determined by both sensory and chemical methods. Grain fragrance was determined by a sensory test developed from Rice Gene Discovery Unit, Kasetsart University, Thailand (Wanchana *et al.*, 2005), five seeds of brown rice were placed into 1.5 ml centrifuge tube and 200 ul of distilled water was added and incubated at 65ºC for three hours with the lids on. The samples were allowed to cool and the lids were then opened one by one and the samples were smelled and scored for fragrance by three panelists. Presence of rice fragrance compound, detection of 2-acetyl-1-pyrroline (2AP) concentration in ten ID2 was conducted by chemical methods using the seeds harvested from Thailand. Ten grams sample of each lines and two parents were sent to identify volatile components using Gas-chromatography/mass-spectrometry (GC-MS) at Department of Chemistry, Faculty of Science, Chiang Mai University, Thailand (Wongpornchai *et al.*, 2004; Yoshihashi *et al.*, 2004).

Amylose content (AC) was measured using the procedure of Juliano (1985) with minor modification. 100 mg of rice powder was incubated at room temperature for overnight in a solution of 1 ml of 95% ethanol and 9 ml of 1N sodium hydroxide to gelatinize the starch. After making up volume of the content to 100 ml with

distilled water, 5 ml was taken into new conical flask. The sample was added with 1ml of 1N acetic acid solution and 2 ml of iodine reagent (0.2 gm iodine and 2 gm Potassium iodide in 100 ml water) and volume is made up to 100 ml with distilled water and mixed well. The absorbance was recorded at 620 nm using a spectrophotometer. The AC was estimated using a standard curve developed from known quantities of purified potato amylose from Fluka Thailand. Rice varieties may be classified as high-, intermediate-, low-, very low and no- (glutinous or waxy) amylose classes with >25%, 20-25%, 12-20%, 5-12% and 0-5% of the apparent amylose (Table 1) (Juliano, 1971).

Table 1 Classification of rice varieties based on proportion of amylose content and its related to texture of cooked rice.

Varietal class	%Amylose	Texture
Waxy	0-5%	sticky,glossy, firm
Very Low	5-12%	moist, sticky, split
Low	12-20%	moist, sticky, split
Intermediate	20-25%	moist, tender, do not hard
High	25-33%	dry, fluffy and hard

Gel consistency (GC) was measured by the length in a culture tube of cold gel according to the method of Cagampang *et al.* (1973). One hundred milligrams of rice powder was put in a 10 mm x 110 mm culture tube and wetted with 0.2 ml of 95% ethanol containing 0.025% Bromothymol blue was added. Two milliliters of 0.2N KOH was added. The sample was mixed using vortex Genie mixer. The test tube was covered with glass marble. The sample was cooked in a boiling water bath for 8 min, making sure that the tube content reach 2/3 the height of the tube. The test tube was removed from the water bath and let stand at room temperature for 5 min. The tube was cooled in an ice-water bath for 20 min and laid horizontally on a laboratory table lined with millimeter graphing paper. The total length of the gel was measured in millimeter one hour later as distance from the bottom of the tube to the front of the gel migration. The gel length thus obtained provides a measurement of the GC: the longer

the distance, the softer the gel. The gel consistency value was evaluated by hard (26-40mm), medium (41-60mm) and soft (61-100mm), short gel indicates hard GC and long gel represents soft GC.

Gelatinization temperature (GT) was indirectly measured by evaluating the Alkali Spreading Value (ASV) using the method of Little *et al.* (1958). Each sample was tested three times. Each time, six intact milled grains were put in a petridish, to which 10 ml of 1.7% KOH was added. The grains were carefully separated from each other using a forceps and incubated at 30ºC for 23 hours to allow spreading of the grains. The spreading value of the grains was scored on a numerical scale of 1 to 7 by visual assessment (Figure 11). Alkali spreading value (ASV) corresponds to GT (Table 2). A larger ASV represents more spreading in alkali, indicating a lower GT and a smaller ASV indicates a higher GT.

Table 2 Alkali spreading scores for measuring gelatinization temperature (GT).

Scores	Alkali Spreading Behavior	Range	GT
1	Kernel not affected	High	74-79ºC
2	Kernel swollen	High	74-79ºC
3	Kernel swollen; collar complete or narrow	Intermediate	70-74ºC
4	Kernel swollen; collar complete and wide	Intermediate	70-74ºC
5	Kernel split or segregated; collar complete and wide	Low	55-69ºC
6	Kernel dispersed; merging with collar	Low	55-69ºC
7	Kernel completely dispersed and intermingled	Low	55-69ºC

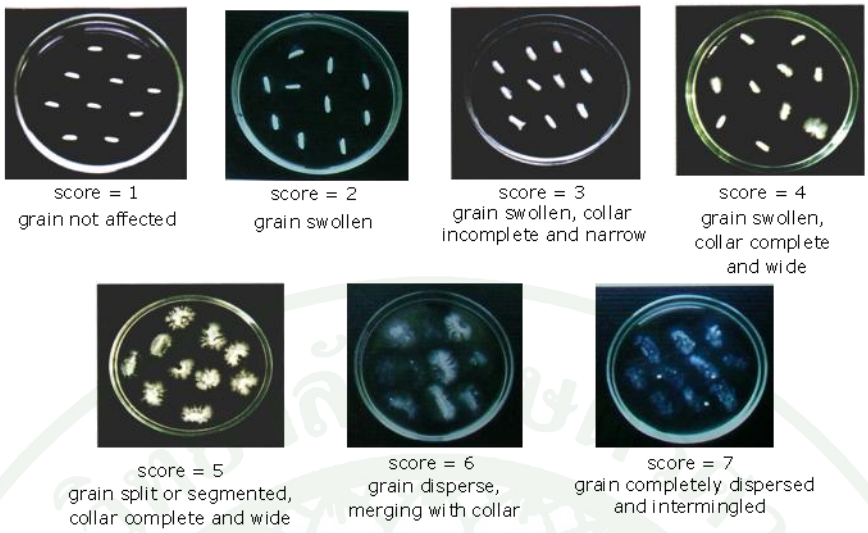

Figure 11 Alkali spreading scores for measuring gelatinization temperature (GT) at 1.7%KOH.

The grain morphology, including the size and shape, were taken using the milled kernels: 10 milled kernels were used for the measurements of grain length (GL) and grain breadth (GB) using Vernier calipers, and the length/breadth ratios (L/B) were calculated.

Statistical and QTL analysis

The locations of the QTLs affecting the measured quantitative traits were determined using the interval-mapping module of the MapQTL computer package (version 4.0) (Van Ooijen *et al.*, 2002). QTL-likelihood plots were produced using the markers that flank the LOD score peak of identified QTLs in the interval-mapping method, as cofactors for QTL mapping on the MapQTL computer program. For each trait and chromosome the LOD score thresholds were calculated by permutation analysis in order to achieve the chromosome-wide significance levels of 5%.

The statistical analysis was performed for each parameter based on a randomized complete block design model using STATGRAPHICS Plus 3.0 software. The means were compared by the LSD test if the F value was significant.

Associations among different traits were examined by correlation analysis. The fragrance, AC, GC and ASV of the IDs were compared with those of KDML105, whereas their PSS and PSE were compared with those of IR57514. The effect of the genetic background was determined by one-way analysis of variance (ANOVA) using the phenotype as the dependent parameter and the markers as the independent parameters. Simple linear regression analysis was used to estimate the effect of the markers on the trait values.

Part II: Glutinous rice varieties

Plant materials

One hundred ninety F_6 recombinant inbred lines were developed from a cross between RD6 and KDIII (KDML105-*Sub1*-*Xa21*-*Bph*) including RGD03039-432-22, RGD03040-721-1 and RGD03040-721-11. RGD03039-432-22, RGD03040-721-1 and RGD03040-721-11 are elite lines developed by marker assisted QTL pyramiding (MAP) at Rice Gene Discovery Unit (RGDU). These lines carry *Sub1*, *Xa21* and qBph12 conferring submergence tolerance (SUB) bacterial blight resistance (BB) and brown planthopper resistance respectively. RD6 is a glutinous jasmine rice cultivar that has been popularly grown in the rainfed lowland areas of the North and Northeast of Thailand. It has a good cooking and eating qualities with desirable fragrance but very susceptible to various biotic and abiotic constraints including flash flooding, and bacterial blight disease.

DNA markers for MAS

DNA marker information used in this study was obtained from the Gramene database (http://www.gramene.org). The R10783indel marker was used for the selection the *Sub1* locus located on chromosome 9 (Siangliw *et al.*, 2003; Toojinda *et al.*, 2003; Toojinda *et al.*, 2005; Ruanjaichon *et al.*, 2008).This marker was developed from RB0783, an expressed sequence tag marker from the Rice Genome Project, Japan. PB7/8 marker was used for the selection of *Xa21* locus located on chromosome 11 (Chunwongse *et al.*, 1993). *Xa21* gene has been very effective against broad spectrum of BB isolates worldwide. Glu-23 marker was used for the selection of Wx gene determined the glutinous or non-glutinous grain types (Wanchana *et al.*, 2003). This marker designed based upon the 23-bp duplicated sequence in the *Wx* gene. It is very effective to distinguish glutinous and non-glutinous grain types. By using the Glu-23F/R primers, 196 bp and 173 bp DNA fragments were amplified from the glutinous and non-glutinous varieties, respectively.

Marker-assisted selection (MAS)

Stepwise MAS was applied using three DNA markers linked to *Wx*, *Sub1* and *Xa21* genes as shown in Figure 12. Selective markers and their polymorphism information were shown in (Figure 13). First, R10783indel was genotyped 106 F_1 plants from the cross between RD6 and KDIII (RGD03039-432-22, RGD03040-721-1, RGD03040-721-11). Fifteen F_1 plants were selected and allowed for self-pollinated to produce 1,000 F_2 plants. Then Glu-23 and R10783indel markers were used to identify the F_2 plants carrying the positive alleles at these two loci. One hundred and forty eight F_2 plants were selected (100 F_2 plants had Wx^{RD6}, $Sub1^{KDIII/RD6}$ and 48 F_2 plants had Wx^{RD6}, $Sub1^{KDIII}$). In F_3 generation, 946 F_3 plants were planted in the field and plant type selection was applied. One hundred ninety two F_3 plants were selected and planted in the field (F_4 generation). R10783indel and PB7/8 markers were used to identified the F_4 families carrying the positive alleles at these two loci. Finally, we selected 46 F_4 families including 17 F_4 families carrying Wx^{RD6}, $Sub1^{KDIII}$ *and* $Xa21^{KDIII}$, 21 F_4 families carrying Wx^{RD6}, $Sub1^{KDIII}$ *and* $Xa21^{KDIII/RD6}$ and 8 F_4 families carrying Wx^{RD6}, $Sub1^{KDIII/RD6}$ *and* $Xa21^{KDIII/RD6}$. In F_5 generation, 540 plants were planted in the field and R10783indel and PB7/8 markers were used to identify the F_5 plants carrying the homozygouse positive allele at two loci. Two groups of genotype (ID3 and ID4) were chosen for this study. The ID3 consisted of 98 plants that have the Wx^{RD6}, $Sub1^{KDIII}$ *and* $Xa21^{KDIII}$ profile and ID4 consisted of ninety two plants that have the Wx^{RD6}, $Sub1^{KDIII}$ *and* $Xa21^{RD6}$ profile. ID3 and ID4 were planted in the field for plant type selection and advanced the generation into the F_6 generation. Finally, 52 lines of ID3 and 43 lines of ID4 were selected based on their agronomic performance and grain quality appearance in the F_6 generation (planted as observation nursery in 2010). These lines were used to evaluate for submergence tolerance, and bacterial blight resistance.

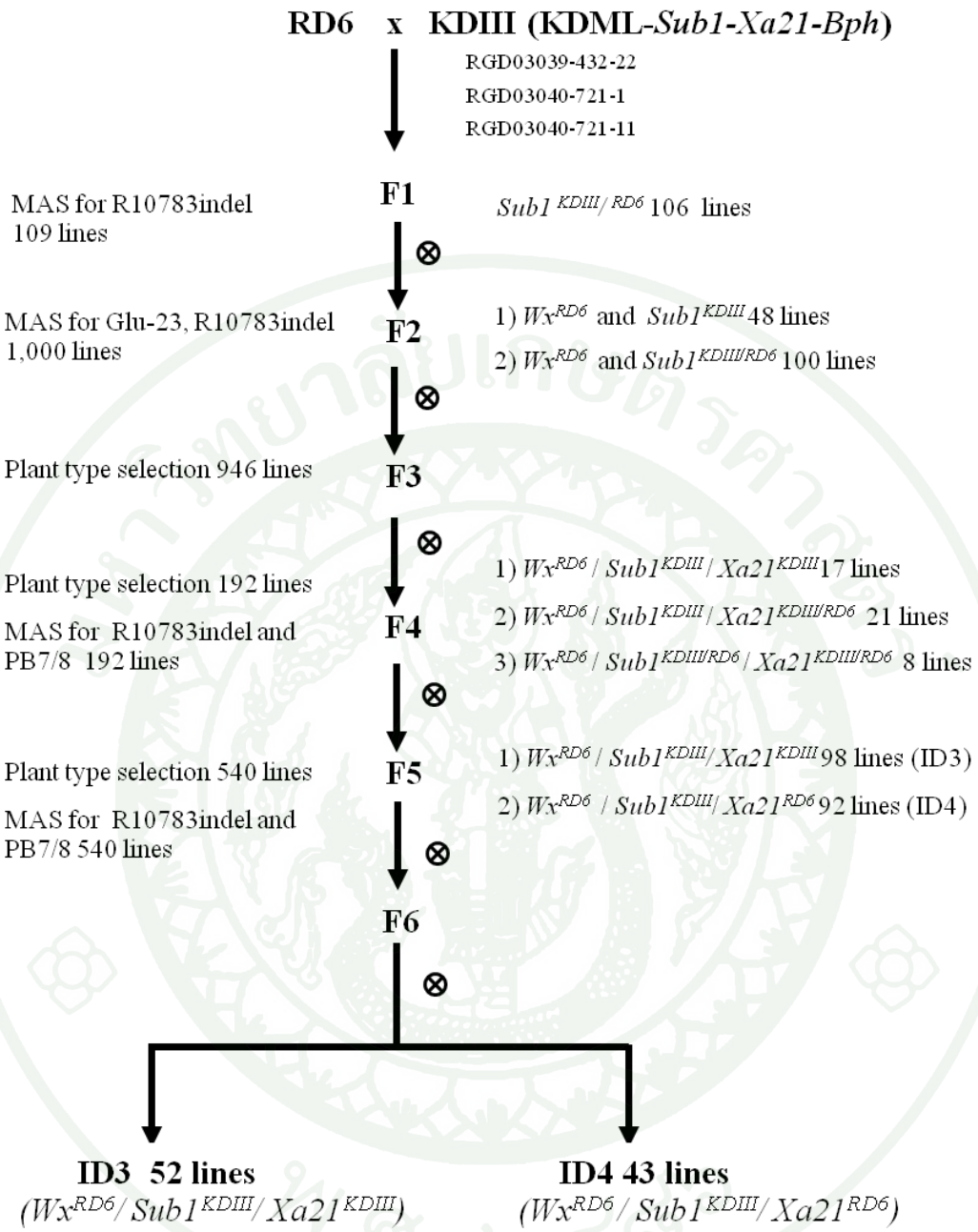

Figure 12 Breeding and selection schemes using marker-assisted selection glutinous rice.

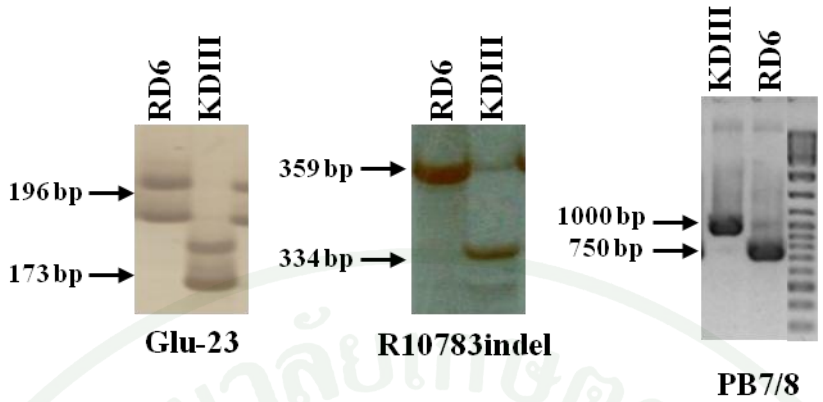

Figure 13 DNA markers and their polymorphism between two parents were used for the genotypic selection in the RILs (RD6 x KDIII).

Trait evaluations and data recordings

Submergence tolerance

ID3 and ID4 were evaluated for submergence tolerance using FR13A as positive (tolerance) and RD6 as negative (intolerance) controls. The evaluation protocol and the data recordings were followed the experiment mentioned in the Part I. The experiment was conducted at Agronomy Field, Kasetsart University, Kamphangsean Campus, Thailand in July to September 2010.

Resistance to bacterial blight disease (BB)

ID3 and ID4 were evaluated for BB resistance against three *Xoo* isolates TXO85, TXO95 and TXO153. TXO85 and TXO95 isolates were collected from Surin province located in Northeast of Thailand while TXO153 isolate was collected from Nakorn-Pathom province located in the Central part of Thailand. IR1188 and RD6 were used as positive (resistance) and negative (susceptible) checks. The experiment was arranged in randomize complete block design with two replications. The two groups of IDs and control were seeded in tray (4 seeds per plot).

All *Xoo* isolates were grown in peptone sucrose agar medium (5g peptone, 20g sucrose, 3g beef extract and 15g agar, adjusted to 1 liter with dH_2O) for 72 hours at 28ºC. The bacterial cells were suspended in sterile water adjusted to 109 CFU/ml. The artificial inoculation using the leaf-clipping method (Kauffman *et al.*, 1973) was performed at a tillering stage (60 days after sowing) in the greenhouse at Kasetsart University, Kampaeng Saen Campus. Bacterial cells were inoculated to two fully expanded leaves of each plant. The inoculated plants were kept in a greenhouse without water over night and then water was applied regularly (five times per day) until the inoculated plants and the susceptible control clearly showed the symptoms. After 12-18 days inoculation, lesion length (LL) from the cut leaf tips were measured in centimeter. BB resistance was classified based on the LL. The LL of < 0-3 cm, 3.1-6.0 cm, 6.1-9.0 cm and > 9.0 cm were classified as resistance (R), moderate resistance (MR), moderate susceptible (MS), and highly susceptible (S) respectively (Yang *et al.*, 2003).

Determination of the genetic constitution in ID populations

The 52 (ID3) and 43 (ID4) recombinant inbred lines were assayed for their genetic constitution using 49 microsatellite markers. These markers had clearly shown polymorphism between the parental lines. The list of marker is shown in Appendix Table 2. These markers are well distributed throughout the 12 chromosomes of the rice genome (at least three markers per each chromosome).

Data analysis

The QTL analysis was followed the method mentioned in the Part I. The statistical analysis was performed for each parameter based on a randomized completed block design model using STATGRAPHICS Plus 3.0 software. The means of tested genotypes were compared to each other and standard check by the LSD test if the F value was significant.

RESULTS

Part I: Non-glutinous rice varieties

The proportion of superior genotype (ID1)

Single seed descent produced 2,037 F_7 RILs derived from the F_2 plants. Only 341 RILs were identified as carrying positive homozygous alleles of KDML105 at the Waxy and Aromarker markers. The observed heterozygosity at 2 loci after 7 generations was 3.1% or 63 lines. Of the 341 lines, 97 RILs were identified as carrying a positive homozygous allele of IR57514 at R10783indel. GT11 was used to classify the 97 RILs into two groups, high (IR57514 allele) and low (KDML105 allele) gelatinization temperature. Lastly, 66 RILs, accounting for 3.24 % of the RIL population, were identified as having submergence tolerance and jasmine-like cooking qualities based on the foreground markers. This proportion was less than the expected value of four independent-genes segregation ($[1/2]^4$ = 1/16 or 6.25%); hence, there must have been some heterozygous target alleles in the population, which reduced the ratio of the expected homozygosity.

The genetic structure of the IDs

A total of 97 polymorphic SSR marker distributed throughout 12 rice chromosomes were used for background analysis in 97 lines from the IDs population. The proportion of KDML105 and IR57514 alleles was calculated (Figure 14). Without any selective pressure, the segregation ratio of the marker at each locus should fit to 1:1. A skewing of the alleles caused by the MAS was found for the markers linked to Waxy and Aromarker on chromosomes 6 and 8, respectively, favoring the KDML105 allele and for the markers linked to R10783indel on chromosome 9, favoring the IR57514 allele. Skewing was also observed on non-targeted chromosomes, such as on chromosomes 1 and 5, favoring KDML105 and on chromosomes 3, 4, 7 and 10, favoring IR57514. The genetic background of individual

IDs contained 36 to 70% of the KDML105 genomic background, whereas the percentage average of KDML105 was 53.3%.

Submergence tolerance performance of IDs and parents

To investigate the submergence tolerance performance at seedling stage, a rice population consisted of 66 ID1 and 31 ID2 total 97 individuals derived from MAS which showed $Sub1^{IR}$ region. The means of the PSS in the ID1 (63.7) and ID2 (64.4) groups were slightly lower than that of the tolerant parent, IR57514 (PSS = 77.3), but significantly higher than that of the intolerant parent, KDML105 (PSS = 4.6). All of the IDs exhibited lower levels of PSE than KDML105. The PSS of individuals ranged from 31.3 to 82.6 and 31.3 to 82.7 for ID1 and ID2, respectively. The means of the PSE of the ID1 and ID2 groups were not significantly different from that of IR57514 (PSE = 42.4) but significantly lower than that of the intolerant parent, KDML105 (PSE = 74.9). The PSE of individuals ranged from 36.0 to 60.9 and 29.2 to 66.4 for ID1 and ID2, respectively. These results indicated that the genotypes with a positive allele of the Sub1 locus increased the PSS by decreasing the PSE under submergence stress. The PSS showed highly significant negative correlations with the PSE ($r = -0.64$, $P < 0.01$). The phenotypic distribution of the PSS of the ID1and ID2 lines after submergence was illustrated in Figure 15 and Figure 16 shown distribution of all ID population for the PSS after submergence.

We investigated the selection efficiency based on R10783indel according to the results from the genetic analysis and the phenotypic analysis by submergence tolerance. Analysis using R10783indel as the marker was performed on all IDs individuals (Figure 17). Based on the phenotype data of submergence tolerance, the selection accuracy of the marker was calculated. The results showed that submergence tolerance had high selection accuracy of 100% survival but different on degree. Thus R10783indel could be applied to the MAS of the trait of submergence tolerance.

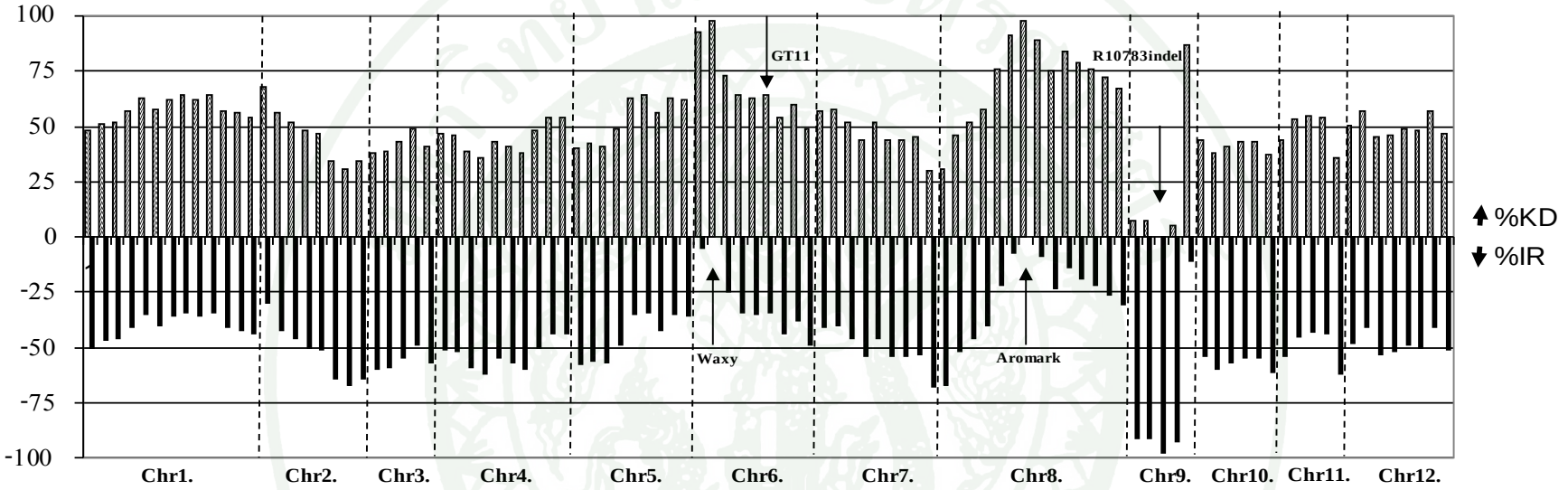

Figure 14 Frequency distribution of the genetic constitution of selected lines on 12 chromosomes.

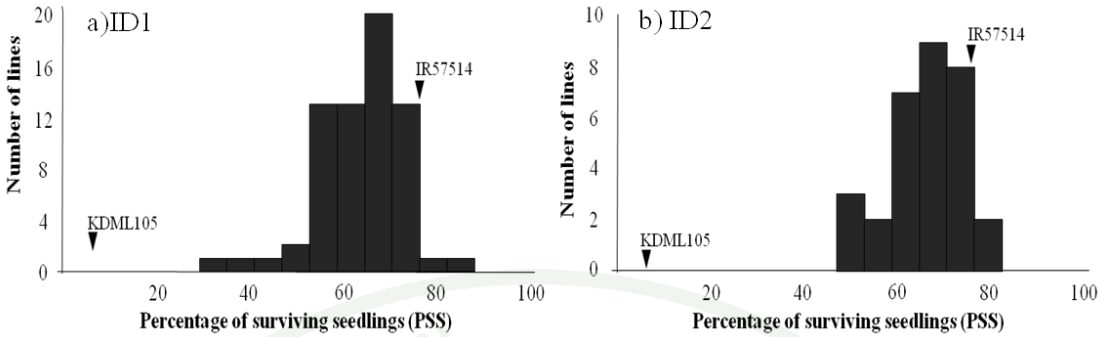

Figure 15 Distribution of percentage of surviving seedlings (PSS) after submergence a) ID1 b) ID2, the average PSS of KDML105 and IR57514 were 4.6±4.0 and 77.3±9.8, respectively.

Figure 16 Distribution of ID population for percentage of surviving seedlings (PSS) after submergence.

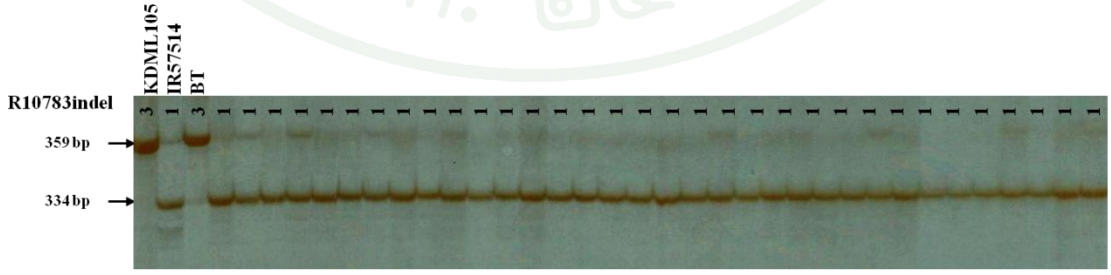

Figure 17 Genotype performance of IDs using R10783indel carried homozygous IR57514 allele linked to submergence tolerance.

Performance of IDs and parents for grain quality

There were distinct differences between the parents for the AC, FR, 2AP, ASV, GB and L/B, and no differences for the GC or GL were observed (the results of grain quality performance were presented in Appendix Table 3). The means of the AC, FR and ASV were not significantly different between the ID1 and ID2 groups and KDML105, except for the ASV of ID2 with an ASV value similar to IR57514. These results indicated that the use of Waxy, Aromarker and GT11 markers effectively selected for the AC, FR, 2AP and ASV traits in this MAS program. All of the IDs exhibited a lower AC than IR57514; the AC of individuals varied between 13 to 20% for ID1 and 14 to 20% for ID2. These values were similar to those of KDML105 (AC = 14.7%). The ASV was positively correlated with the AC ($r = 0.38$, $P < 0.01$) but negatively correlated with the GC ($r = -0.40$, $P < 0.01$). FR was evaluated with numerical score 0 to 1 (0 = non-fragrance, 1 = fragrance). Two parents were different in aroma rating, referring aroma score of 1 in KDML105 and 0 in IR57514. The FR of all of the IDs was as strong as that of KDML105 when determined by a sensory test. However, the 2AP concentration varied between 0.976 and 2.233 ppm among the 10 ID1 lines tested: it was found at 2.761 ppm in KDML105 and was undetectable in IR57514 (Table 3). The KDML105 and IR57514 lines demonstrated similar GC values of 83 and 85 mm, respectively. Transgressive segregations in both directions (lower and higher than that of the parents) were observed for the GC in both of the ID populations, and the GC of individuals ranged from 61.0 to 134.0 mm and 77.5 to 125.0 mm for ID1 and ID2, respectively. These results indicated that a quantitative inheritance of the GC trait is independent of the *Wx* locus in this experiment. For physical grain quality, IR57514 had a shorter GL, a wider GB and a lesser L/B ratio than did KDML105. The mean GL of ID1 was 7.24 mm, in which individuals ranged from 6.65 to 8.25 mm. All of the individuals in ID1 had a wider GB (ranging between 1.73 to 2.25 mm) and a lesser L/B ratio (ranging from 3.10 to 4.18) than KDML105 (Table 4).

Table 3 Fragance sensory score and amount of 2AP in 10 ID1 and two parents.

Entries	Pedigree	Fragance sensory score	2APconc (ppm)
1	IR57514	0	0.000
2	KDML105	1	2.761
3	PSL99093-47-2-5R	1	1.504
4	PSL99093-49-1-5R	1	2.189
5	PSL99093-134-3-5R	1	1.424
6	PSL99094-37-6-5R	1	1.322
7	PSL99094-38-1-5R	1	0.976
8	PSL99094-167-7-5R	1	2.233
9	PSL99094-167-8-5R	1	2.013
10	PSL99094-189-6-5R	1	2.153
11	PSL99094-202-3-5R	1	1.196
12	PSL99094-274-5-5R	1	1.807

The agronomic performance of the IDs and parental lines

The results of agronomic traits were presented in Appendix Table 3. The seven measured agronomic traits of the parents, ID1 and ID2, are shown in Table 5. Significant differences between the parents were observed for the DF, PH and NFGP, with KDML105 showing greater trait values for DF (119 d) and PH (135 cm) but smaller trait values for NFGP (127 grains). Transgressive segregations in both directions were observed in the ID1 and ID2 groups for all of the measured traits. The trait values of ID1 individuals ranged from 86 to 113 d for the DF, 76.7 to 175.0 cm for PH, 8 to 23 for NPP, 41 to 152 for NFGP, 54.0 to 97.7 % for PSF and 19.5 to 30.4 g for TGW. The trait values of ID2 individuals ranged from 86 to 110 d for the DF, 71.0 to 183.0 cm for PH, 7 to 19 for NPP, 38 to 160 for NFGP, 42.6 to 96.9 % for PSF and 20.2 to 31.6 g for TGW.

Table 4 Comparison of the grain-quality performance of ID1, ID2 and the two parental lines calculated by the combined analysis of variance data in all of the traits.

Traits	Parents		ID1		ID2	
	IR57514	KDML105	mean±SD	range	mean±SD	range
PSS	77.3[a]	4.6[b]	63.7±9.6[a]	31.3-82.6	64.4±10.5[a]	31.3-82.7
PSE	42.4[a]	74.9[b]	47.3±6.5[a]	36.0-60.9	46.3±7.6[a]	29.2-66.4
AC	33.6[a]	14.7[b]	16.8±1.6[b]	13.0-20.0	16.5±1.5[b]	14.4-19.9
ASV(GT)	1.0[a]	4.0[b]	5.0±0.7[b]	3.9-6.1	1±0.1[a]	1.0-1.2
Fragance	0	1	1	-	1	-
2AP*	0.000[a]	2.761[b]	1.682±0.456[b]	0.976-2.233	-	-
GC(mm)	83.0[a]	85.0[a]	84.6±14.7[a]	61.0-134.0	97.8±13.3[a]	77.5-125.0
length	6.87[a]	7.30[b]	7.24±0.29[b]	6.65-8.27	7.28±0.31[b]	6.63-8.02
breadth	1.94[a]	1.69[b]	2.03±0.11[b]	1.73-2.25	2.10±0.10[b]	1.90-2.25
L/B	3.54[a]	4.32[b]	3.58±0.21[a]	3.10-4.18	3.48±0.20[a]	3.16-3.87

PSS= percentage of surviving seedlings; PSE=percentage of seedling elongation; AC=amylose content; ASV=alkali spreading values; 2AP=2-acetyl-1-pyrroline; GC = gel consistencies; GL=grain length; GB=grain breadth; L/B=length/breadth ratio

* Ten lines of the ID1 were analyzed for the presence of 2AP

[a–b]Indicates a significant difference of trait mean values among groups by the LSD test ($P < 0.05$)

Table 5 Comparison of the agronomic traits of ID1, ID2 and the two parental lines calculated by the combined analysis of variance data in all of the traits.

Traits	Parents		ID1		ID2	
	IR57514	KDML105	mean±SD	range	mean±SD	range
DF	110[a]	119[b]	96.9±8.1[a]	86-113	97.3±6.1[a]	86-110
PH	109.0[a]	135.0[b]	126.3±28.7[b]	76.7-175.0	131.1±31.3[b]	81.0-183.0
NETP	12[a]	11[a]	12.3±3.4[a]	8-23	12.9±3.3[a]	7-19
NFGP	139[a]	127[b]	111.9±23.5[b]	41-152	111.4±29.3[b]	38-160
NGP	160[a]	146[b]	136.3±25.8[b]	59.5-205.0	145.3±30.3[b]	79.0-224.0
PSF	86.9[a]	87.3[a]	82.1±10.1[a]	54.0-97.7	76.6±14.1[a]	42.6-96.9
TGW	26.8[a]	25.1[a]	24.6±2.3[a]	19.5-30.4	25.5±3.0[a]	20.2-31.6

DF=days to flowering; PH, plant height; NETP=numbers effective tiller per plant; NFGP=numbers of filled grains per panicle; NGP=numbers of grains per panicle; PSF=percent spikelet fertility and TGW=weight of 1000 grains

[a–b]Indicates a significant difference of trait mean values among groups by the LSD test ($P < 0.05$)

Genetic modifiers

Although all of the IDs carrying *Sub1* are submergence tolerant, they presented significant variations of the PSS and PSE. Four QTL for the PSS and PSE values were identified and mapped to rice chromosomes 1, 2, 6 and 9 (Table 6). QTL associated with PSS (qPSS2 and qPSS6) and PSE (qPSE1 and qPSE9) mapped to different rice chromosomes. The IR57514 alleles at qPSS2 and qPSS6 contributed to higher PSS while KDML105 alleles at qPSE1 and qPSE9 contributed to higher PSE. This indicates that the PSS was not correlated with the PSE in the ID population in which all lines carry the *Sub1* locus.

All of the IDs presented a low AC class (AC < 20) but a slightly variation of the AC was found among individuals. This indicated that *Wx* locus selected by marker Waxy played a decisive role for AC. Two additional QTL, qAC5 and qAC8 were detected for AC trait, accounting for 9.7 and 13.5% of the total phenotypic variance (Table 6). The allele from IR57514 at qAC5 increased AC by 0.7% while that from KDML105 at qAC8 increased AC by 0.99%. All the ID1 have high ASV while all the ID2 have low ASV. Four QTL including qASV5, qASV6, qASV7 and qASV8 were detected for ASV. The largest effect QTL, qASV6, flanked by markers RM314 and GT11 on chromosome 6 explained 90.2% of the variation, indicating that this QTL played a decisive role for ASV. The allele from KDML105 at this locus increased ASV by 2 scores. GT11 is used to classify the ID population into the ID1 and ID2. No QTL for FR was detected since all the IDs were fragrance genotype. Two QTL, qGC6 and qGC7 for GC were identified to be flanked by markers RM314-RM564 and RM5711-RM182, respectively. The allele from IR57514 at qGC6 and qGC7 increased GC by 7.7 and 5.9 mm and explained 23.8 and 14.4% of the variance, respectively.

Four QTL, qGL2, qGL4.1, qGL4.2 and qGL4.3, for GL were found to be located on chromosomes 2 and 4 (Table 6). The alleles from KDML105 at qGL4.1, qGL4.2 and qGL4.3 increased GL by 0.14, 0.10 and 0.13 mm, respectively while that from IR57514 at qGL2 increased GL by 0.14 mm. Four QTL, qGB2, qGB5, qGB6

and qGL11, for GL were mapped on chromosomes 2, 5, 6 and 11. The allele from IR57514 at all loci except qGL11 had positive effect, which could increase GB from 0.03 to 0.05 mm. Coincidental QTLs for GL and GB on chromosome 2 (qGL2 and qGB2) with an opposite of the positive allele indicated that these QTL might belong to the same locus functioning to determine both GL and GB. Three QTL mapped to RM168-RM3202, RM7187-RM317 and RM219-RM7306 on chromosomes 3, 4 and 9 were detected for L/B. Individual QTL accounted for 26.3, 14.6 and 34.5% of total phenotypic variation respectively. IR57514 contributed a positive allele at qL/B3 and qL/B9 and negative allele at qL/B4.

Two QTL, qDF5 and qDF7, were detected for DF and explained 10.9 and 14.2 % of variance. At qDF5, the allele from KDML105 increased DF by 2.5 d while the allele from IR57514 at qDF7 increased DF by 2.8 d. The effect of photoperiod was not observed because the experiment was conducted under short days of wet season. KDML105 is photosensitive but IR57514 is non-photosensitive. Two QTL for PH were detected on chromosomes 1 and 12. The major QTL, qPH1, accounted for 76.8% of the variation with a LOD score of 24.1. The allele from KDML105 increased PH by 26.3 cm. The minor QTL, qPH12, accounted for 26.4% of the variation and a positive allele came from IR57514 that increased PH by 15.2 cm.

Single QTL, qNPP1, was detected on chromosome 1 for NPP, explaining 12.7% of the total variance in the IDs. Two QTL, qNFGP5 and qNFGP9, were identified for NFGP, explaining 14.4 and 10% of the total variance in the IDs. The alleles from IR57514 increased NFGP by 9.7 and 15.4 grains respectively. Two QTL for PSF were detected on chromosomes 5 and 6. The qPSF5 and qPDF6 explained 12.4 and 16.8% of the total variance in the IDs and the positive alleles were derived from IR57514 at both loci. Three QTL, qTGW2, qTGW5 and qTGW6, were identified for TGW, explaining 15.5, 37.1 and 16.8% of the total variance in the IDs. The alleles from IR57514 at qTGW2 and qTGW6 increased TGW by 1.07 and 1.05g respectively, while the allele from KDML105 at qTGW5 increased TGW by 1.57g.

Table 6 Putative QTL identified for submergence tolerance, cooking quality and agronomic characters.

Trait	Marker interval	QTL names	Chromosome	LOD	Additive	R^2
PSS	RM263-RM6	qPSS2	2	2.15	-3.85	15.7
	GT11-RM564	qPSS6	6	2.24	-2.95	10.0
PSE	RM3285-RM5781	qPSE1	1	5.29	3.74	24.2
	RM316-RM5799	qPSE9	9	2.24	4.62	10.0
AC	RM153-RM159	qAC5	5	2.18	0.77	9.7
	RM72-RM44	qAC8	8	2.60	-0.99	13.5
GC	GT11-RM564	qGC6	6	4.52	-7.73	23.8
	RM5711-RM182	qGC7	7	2.15	-5.97	14.4
ASV	RM3838-RM164	qASV5	5	2.02	0.75	13.2
	GT11-RM564	qASV6	6	37.38	2.06	90.2
	RM182-RM11	qASV7	7	2.79	0.81	16.4
	RM72-RM44	qASV8	8	4.65	-1.05	25.1
GL	RM263-RM6	qGL2	2	4.82	-0.14	20.9
	RM335-RM119	qGL4.1	4	4.47	0.14	24.6
	RM7187-RM317	qGL4.2	4	2.63	0.1	11.6
	RM3534-RM280	qGL4.3	4	2.90	0.13	22.4
GB	RM263-RM6	qGB2	2	4.58	-0.05	20.3
	RM26-RM334	qGB5	5	2.04	-0.03	9.2
	GT11-RM564	qGB6	6	4.48	-0.04	19.0
	RM209-RM224	qGB11	11	2.30	0.04	16.6
L/B	RM168-RM3202	qL/B3	3	2.49	-0.11	26.3
	RM7187-RM317	qL/B4	4	3.36	0.08	14.6
	RM219-RM7306	qL/B9	9	2.42	-0.14	34.5
DF	RM169-RM516	qDF5	5	2.00	2.5	10.9
	RM5711-RM182	qDF7	7	3.27	-2.84	14.2
PH	RM3285-RM5781	qPH1	1	24.10	26.3	76.8
	RM463-RM235	qPH12	12	3.34	-15.21	26.4

Table 6 (Continued)

Trait	Marker interval	QTL names	Chromosome	LOD	Additive	R^2
NPP	RM3285-RM5781	qNPP1	1	2.46	-1.24	12.7
NFGP	RM153-RM159	qNFGP5	5	3.32	-9.65	14.4
	RM316-RM5799	qNFGP9	9	2.24	-15.37	10.0
PSF	RM26-RM334	qPSF5	5	2.81	-4.24	12.4
	GT11-RM564	qTGW6	6	3.92	-1.05	16.8
TGW	RM263-RM6	qTGW2	2	3.44	-1.07	15.5
	RM159-RM169	qTGW5	5	2.28	1.57	37.1
	GT11-RM564	qTGW6	6	3.92	-1.05	16.8

PSS= percentage of surviving seedlings; PSE=percentage of seedling elongation; AC=amylose content; ASV=alkali spreading values; GC = gel consistencies

R^2, the fitted model explains the variability of the trait.

Part II: Glutinous rice varieties

Stepwise selection to develop superior genotype (ID3)

Table 7 and 8 showed the summary of stepwise selection. Fifteen F1 plants (5 plants from each cross) were self-pollinated to produce 1000 F_2 plants in which they were assayed with two markers, Glu-23 and R10783indel. Only forty eight F_2 plants were identified as carrying positive homozygous alleles at the Glu-23 and R10783indel markers (the $Wx^{RD6}/Sub1^{KDIII}$ genotype). This observed homozygosity at two loci was lower than the expectation in the F_2 generation (250 plants). PTS was applied in the F_3, F_4 and F_6 generations to reduce the number of plants for MAS. Finally, 95 F_6 plants were identified as carrying positive homozygous alleles at Glu-23, R10783indel and PB7/8 (52 as $WX^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$ and 43 as $Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$). These F_6 plants were selected from two crosses (RD6 x RGD03040-721-11 and RD6 x RGD03039-432-22).

Table 7 Summary of stepwise selection in each generation (F_1-F_6) of the single crosses derived from RD6 x KDIII. MAS and PTS stand for marker assisted selection and plant type selection respectively.

Gene rations	No. of plants	Selection (1)	No. of selected plants (1)	Selection (2)	No. of selected plants (2)	Selection (3)	No. of selected plants (3)
F_1	109	MAS (R10783indel)	106	PTS	15		
F_2	1,000	MAS (Glu-23, R10783indel)	148 (48 *Wx*^{RD6}/*Sub1*^{KDIII} and 100 *Wx*^{RD6}/*Sub1*$^{KDIII/RD6}$)				
F_3	8,880	PTS	946				
F_4	946	PTS	192	MAS (Glu-23, R10783indel)	192 (95 *Wx*^{RD6}/*Sub1*^{KDIII} and 97 *Wx*^{RD6}/*Sub1*$^{KDIII/RD6}$)	MAS (PB7/8)	46 (17 *Wx*^{RD6}/*Sub1*^{KDIII}/*Xa21*^{KDIII}, 21 *Wx*^{RD6}/*Sub1*^{KDIII}/*Xa21*$^{KDIII/RD6}$, 8 *Wx*^{RD6}/*Sub1*$^{KDIII/RD6}$/*Xa21*$^{KDIII/RD6}$)
F_5	540	MAS (Glu-23, R10783indel)	423 (*Wx*^{RD6}/*Sub1*^{KDIII})	MAS (PB7/8)	190 (98 *Wx*^{RD6}/*Sub1*^{KDIII}/*Xa21*^{KDIII} and 92 *Wx*^{RD6}/*Sub1*^{KDIII}/*Xa21*^{RD6})		
F_6	190	PTS	95 (52 *Wx*^{RD6}/*Sub1*^{KDIII}/*Xa21*^{KDIII} and 43 *Wx*^{RD6}/*Sub1*^{KDIII}/*Xa21*^{RD6})				

Table 8 Genotypes and numbers of plants in the F_1-F_6 generations. RD6 was used as female parent and KDIII (RGD03040-721-1, RGD03040-721-11, RGD03039-432-22) were used as male parent.

Generation	Genotypes	Male parents			Total plants
		RGD03040-721-1	RGD03040-721-11	RGD03039-432-22	
F_1	$Wx^{KDIII/RD6}/Sub1^{KDIII/RD6}$	5	5	5	15
F_2	$Wx^{RD6}/Sub1^{KDIII}$	16	18	14	48
	$Wx^{RD6}/Sub1^{KDIII/RD6}$	42	38	20	100
		345	**405**	**250**	**1000**
F_3		**423**	**346**	**177**	**946**
F_4	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	2	11	4	17
	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII/RD6}$	1	18	2	21
	$Wx^{RD6}/Sub1^{KDIII/RD6}/Xa21^{KDIII/RD6}$	0	8	0	8
		88	**88**	**16**	**192**
F_5	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	1	66	31	98
	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	0	78	14	92
		60	**420**	**60**	**540**
F_6	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	0	30	22	52
	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	0	6	37	43
		0	**36**	**59**	**95**

The genetic structure of the IDs

Figure 18 displays the genetic structure of the ID population based 49 SSR markers distributed throughout 12 rice chromosomes. With selection pressure (MAS and PTS) in each generation, the segregation ratio of each marker will not fall into a 1:1 ratio. A skewing of the alleles caused by MAS was found for the markers linked to Glu-23, Aromarker and R10783indel on chromosomes 6, 8 and 9 respectively. Skewing was also observed on non-targeted chromosomes, such as on chromosomes 1, 2, 5, 7 and 10, favoring RD6 allele. The genetic background of individual IDs contained 6.3 to 94.7.% of the RD6 genomic background, whereas the percentage average of RD6 was 51.6%.

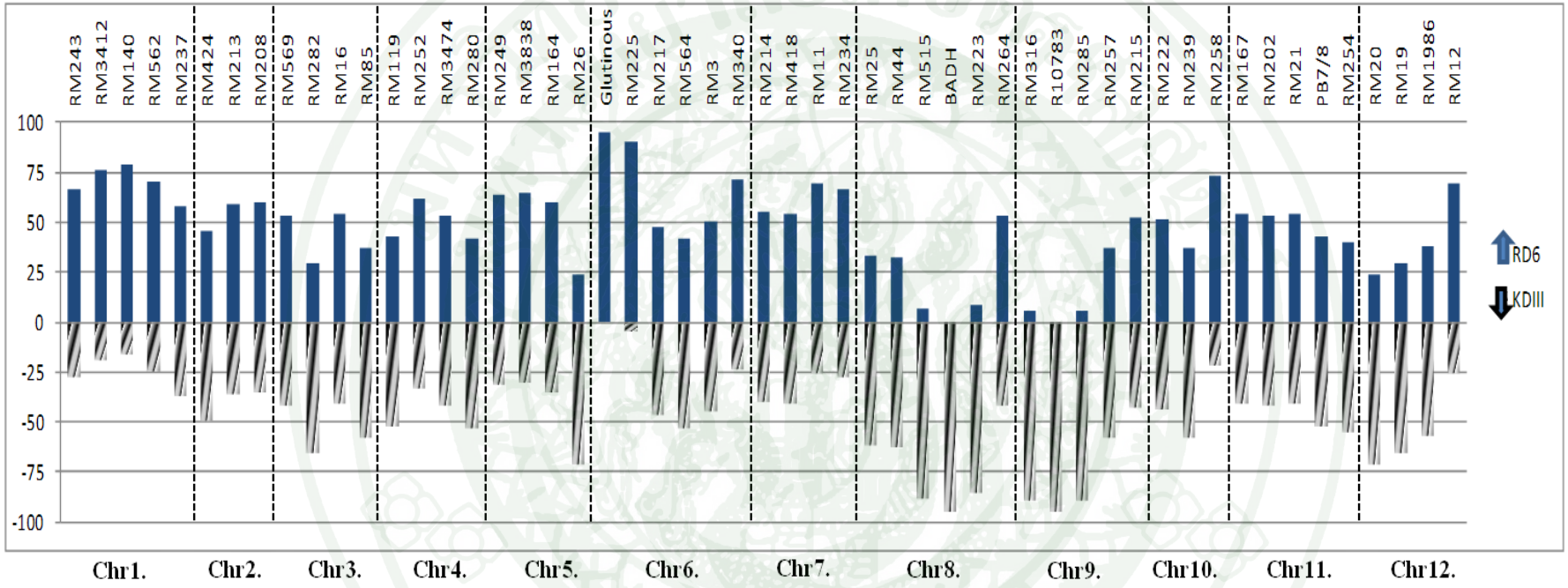

Figure 18 Frequency distribution of the genetic constitution of selected lines on 12 chromosomes (ID3 and ID4).

Submergence tolerance performance of IDs and parents

The phenotypic distribution of the PSS of the ID3and ID4 was illustrated in Figure 19 (a) and (b) and Figure 20. PPS of individual ID3, ID4 lines and parents was presented in Appendix Table 4. A significant negative correlation was found between PSS and PSE ($r = -0.18$, at $P < 0.05$). PSS of the ID3 (28.7 %) and ID4 (28.6 %) were lower than that of tolerant check, FR13A (PSS = 97.7) but significantly higher than that of the intolerant parent RD6 (PSS = 0.0 %). The PSS of individual ID3 and ID4 ranged from 9.6 to 63.2 % and 10.8 to 66.3 % respectively (Table 9). The PSE of the ID3 (13.3 %) and ID4 (11.6 %) were not significantly different than that of the RD6 (12.2 %) and FR13A (PSE = 11.2 %). The PSE of individuals ranged from 1.7 to 21.6 % and 5.4 to 19.9 % for ID3 and ID4 respectively.

Table 9 Comparisons of PSS and PSE of the ID3, ID4 and two controls based on combined analysis of variance.

Group	PSS (%)		PSE (%)	
	mean±SD	range	mean±SD	range
ID3	28.7±13.0[b]	9.6-63.2	13.3±4.4[a]	1.7-21.6
ID4	28.6±11.4[b]	10.8-66.3	11.8±3.4[bc]	5.4-19.9
RD6	0±0[c]	0-0	11.2±2.2[bc]	7.1-15.5
FR13A	97.7±0.3[a]	96.9-97.9	12.2±2.6[ab]	8.4-19.3

PSS= percentage of surviving seedlings; PSE=percentage of seedling elongation

[a,b,c] Indicate significant difference of trait mean values among groups ($P<0.05$)

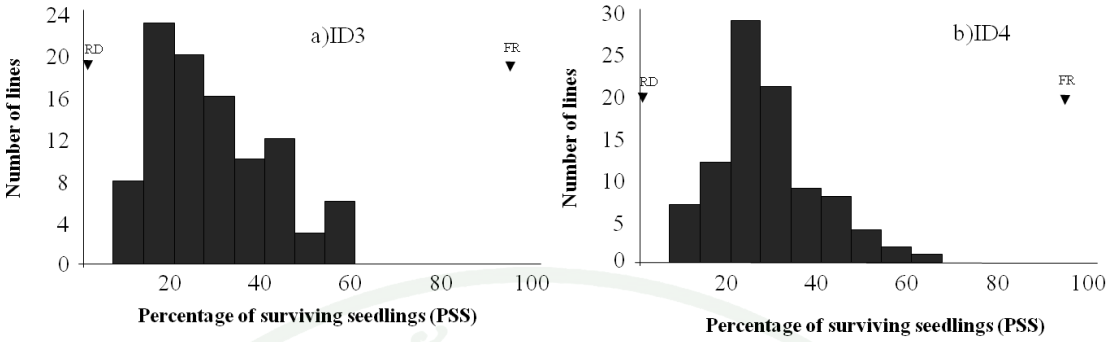

Figure 19 Frequency distributions of the PSS of (a) ID3 and (b) ID4. PSS of RD6 and FR13Awere averaged 0±0 and 97.7±0.3 %, respectively.

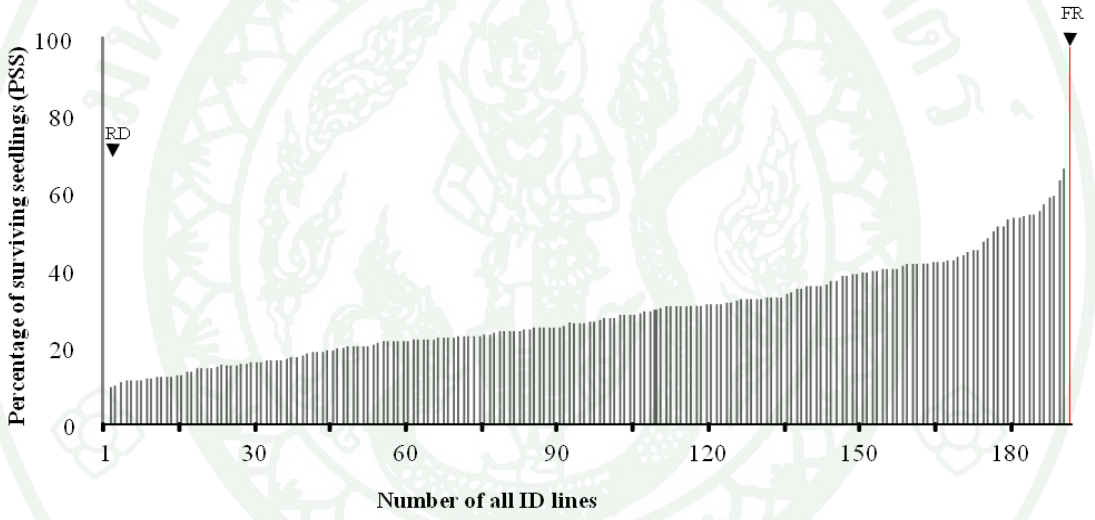

Figure 20 PSS of individuals (ID3 and ID4), FR13A (tolerance check) and RD6 (intolerance parent).

Bacterial blight resistance performance of IDs and parents

We challenged ID3, ID4, IR1188 (resistance check) and RD6 (susceptible parent) with three *Xoo* isolates. TXO85 and TXO95 are the isolate collected from Surin province in Northeast of Thailand and TXO153 is the isolate collected from Nakorn-pathom province in Central of Thailand. IR1188 carrying *Xa21* showed a resistant reaction (R) whereas RD6 showed a susceptible reaction (S) to all tested *Xoo*

isolates. Lesion length (LL) of the ID3, ID4, IR1188 and RD6 is presented in Table 10.

For TXO85 inoculation, the averaged LL was 1.2±1.0 and 11.4±2.5 cm for IR1188 and RD6 respectively. The LL of the ID3 (2.4±0.9 %) was not significantly different from that of the IR1188 (resistance check) but significantly different from that of the RD6 (susceptible parent). The LL of the ID4 (9.2±1.8 %) was significantly higher than that of the IR1188 but significantly lower than that of the RD6 (susceptible parent). The LL of individuals ranged from 0.6 to 5.6 % and 5.2 to 16.3 % for ID3 and ID4, respectively. The LL of ID3 showed a skewness toward the moderate resistance (MR), and ID4 showed a skewness toward the moderate susceptible (MS) and susceptible (S) (Figure 21).

For TXO95 inoculation, the averaged LL was 1.3±1.0 and 16.4±3.0 cm for IR1188 and RD6 respectively. The LL of the ID3 (3.9±1.8 %) was not significantly different from that of the IR1188 (resistance check) but significantly different from that of the RD6 (susceptible parent). The LL of the ID4 (16.2±2.8 %) was significantly higher than that of the IR1188 but not significantly difference from that of the RD6 (susceptible parent). The LL of individuals ranged from 0.6 to 7.8 % and 10.3 to 27.5 % for ID3 and ID4, respectively. The LL of ID3 showed a skewness toward the resistance (R) and the moderate resistance (MR), and ID4 showed a skewness toward the susceptible (S) (Figure 22).

For TXO153 inoculation, the averaged LL was 2.5±1.3 and 18.1±3.9 cm for IR1188 and RD6 respectively. The LL of the ID3 (4.9±1.7 %) was higher than that of the IR1188 (resistance check) but lower than that of the RD6 (susceptible parent). The LL of the ID4 (16.2±2.4 %) was significantly higher than that of the IR1188 but not significantly difference from that of the RD6 (susceptible parent). The LL of individuals ranged from 1.3 to 8.4 % and 9.4 to 23.8 % for ID3 and ID4, respectively. The LL of ID3 showed a skewness toward the moderate resistance (MR), and ID4 showed a skewness toward the susceptible (S) (Figure 23). The phenotypic

distribution of the ID3 and ID4 lines against all three isolates was illustrated in Appendix Table 4.

Table 10 Comparison of the LL of the ID3, ID4, IR1188 and RD6 based on the combined analysis of variance.

Isolates	Control		ID3		ID4	
	IR1188	RD6	mean±SD	range	mean±SD	range
TXO85	1.2±1.0[a]	11.4±2.5[c]	2.4±0.9[a]	0.6-5.6	9.2±1.8[b]	5.2-16.3
TXO95	1.3±1.0[a]	16.4±3.0[b]	3.9±1.8[a]	0.6-7.8	16.2±2.8[b]	10.3-27.5
TXO153	2.5±1.3[a]	18.1±3.9[c]	4.9±1.7[a]	1.3-8.4	16.2±2.4[b]	9.4-23.2

[a–b]Indicates a significant difference of trait mean values among groups by the LSD test (P <0.05)

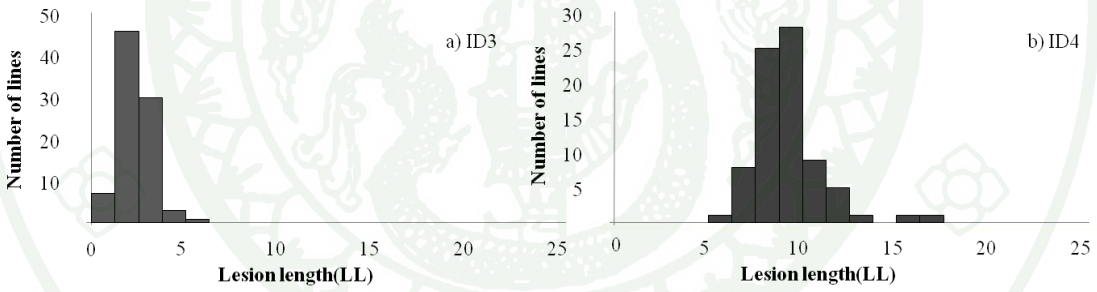

Figure 21 Frequency distribution of the LL of the ID3 and ID4 after inoculated with TXO85. The arrows showed the mean of LL for IR1188 (1.2±1.0 %) and RD6 (11.4±2.5 %).

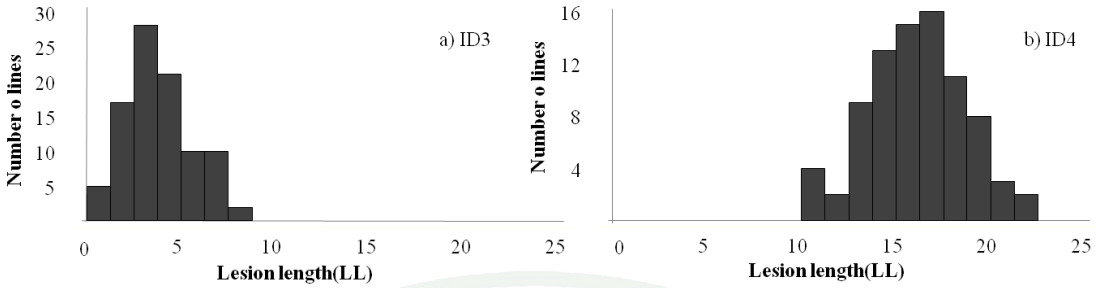

Figure 22 Frequency distribution of the LL of the ID3 and ID4 after inoculated with TXO95. The arrows showed the mean of LL for IR1188 (1.3±1.0 %) and RD6 (16.4±3.0 %).

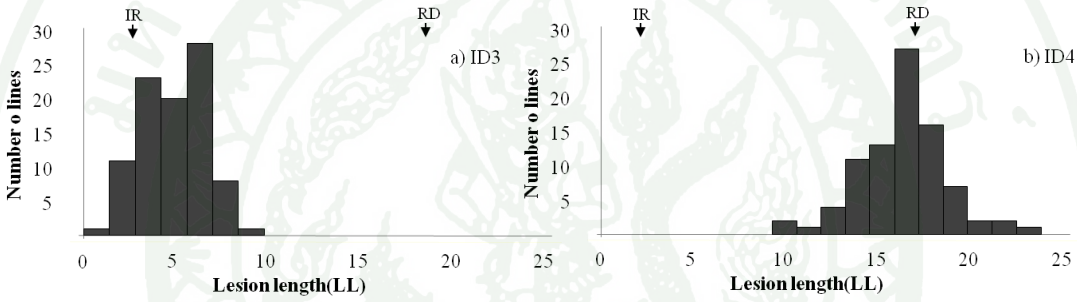

Figure 23 Frequency distribution of the LL of the ID3 and ID4 after inoculated with TXO153. The arrows showed the mean of LL for IR1188 (2.5±1.3 %) and RD6 (18.1±3.9 %).

The agronomic performance of the IDs and parental lines

Table 11 and Appendix Table 5 presented the agronomic trait performance of the IDs. Significant differences for the DF and NETP were observed between the parents, The DF of the RD6 was 94 d but greater trait values for NETP (26.22g). Transgressive segregations in both directions were observed in the ID3 and ID4 for all of the measured traits. The trait values of ID3 individuals ranged from 92 to 106 d for the DF, 128 to 171 cm for PH, 9 to 25 for NETP, and 21.8 to 29.6 g for TGW. The trait values of ID4 individuals ranged from 97 to 104 d for the DF, 124 to 171 cm for PH, 8 to 18 for NETP, and 21.4 to 28.5 g for TGW.

Table 11 Comparisons of the agronomic traits of ID3, ID4 and the two parental lines calculated by the combined analysis

Traits	Parents		ID3		ID4	
	RD6	RGD03040-721-11	mean±SD	range	mean±SD	range
DF	94±0.0[a]	102±0.0[b]	99.9±3.4[b]	92-106	102±0.0[b]	97-104
PH	146.4±1.9[a]	142.6±1.4[a]	143.4±7.8[a]	128-171	142.6±1.4[a]	124-171
NETP	13.5±2.2[ab]	12.3±1.6[ab]	13.7±3.3[a]	9-25	12.3±1.6[ab]	8-18
TGW	26.22±0.58[a]	24.40±0.58[bc]	25.63±1.61[ab]	21.8-29.6	24.40±0.58[bc]	21.4-28.5

DF=days to flowering ; PH=plant height; NETP=numbers effective tiller per pant and TGW=weight of 1,000 grains

[a-c] Indicates a significant difference of trait mean values among group by the LSD test ($P<0.05$)

Genetic modifiers

Genome scan was performed to monitor the genotype of 95 F_6 lines (ID3 and ID4). Of 249 markers being screened, Only 49 (19.6%) markers showed a polymorphism. We constructed genetic linkage map of the ID population. The linkage map was 1,618 cM, with average distance of 33 cM between adjacent markers. Several QTLs associated with PSS, PSE, TXO85, TXO95, TXO153, DF, PH, NETP and TGW were detected as showed in Table 12

Although all of the IDs (ID3 and ID4) carrying *Sub1* are submergence tolerant, they presented significant variations of the PSS and PSE. Six QTL for the PSS and PSE were identified and mapped to rice chromosomes 2, 3, 4, 6 and 12 (Table 12). QTL associated with PSS (qPSS3, qPSS4 and qPSS12) and PSE (qPSE2, qPSE3 and qPSE6) differ in their locations. The RGD03040-721-11 alleles at qPSS3 (RM282-RM16), qPSS4 (RM252-RM3474), qPSS12 (RM19-RM1986) and qPSE2 (RM424-RM213) contributed to higher PSS and PSE while RD6 alleles at qPSE3 (RM569-RM282) and qPSE6 (RM217-RM564) contributed to higher PSE. This

indicates that the PSS was not correlated with the PSE in this ID population in which all lines carry the *Sub1* locus.

When ID3 and ID4 were challenged with three Xoo isolates, TXO85, TXO95 and TXO153, the same QTLs were detected on chromosomes 11 (RM21-PB7/8). The RGD03040-721-11 allele was contributed QTL chromosome 11 for the less of LL. The large effect QTL on chromosome 11, $qBB_{TXO85}11$, $qBB_{TXO95}11$ and $qBB_{TXO153}11$, flanked by markers RM21 and PB7/8 explained 82.0, 88.4 and 86.2% of the variation in the IDs, respectively.

Two QTL, qDF2 and qDF12, were detected for DF and explained 18.1 and 18.3 % of phenotypic variance respectively. The allele inherited from RGD03040-721-11 increased the DF by 1.19 and 1.25 d respectively. Two QTL for PH were detected on chromosomes 1 and 2 and a positive allele inherited from RGD03040-721-11 increased the PH by 3.65 and 4.86 cm. Seven QTL, qNETP2, qNETP5, qNETP6, qNETP8, qNETP9, qNETP11 and qNETP12, located on chromosomes 2, 5, 6, 8, 9, 11 and 12 respectively were associated with NETP. These QTL individually explained 13.8, 23.1, 14.6, 21.1, 55.7, 17.9 and 22.3 % of the phenotypic variance explained respectively. Seven QTL, qTGW1, qTGW3, qTGW6, qTGW7, qTGW8, qTGW11 and qTGW12, were identified for TGW, explaining 19.4, 47.7, 47.6, 33.7, 16.8, 19.2 and 20.1 % respectively. Allele inherited from RD6 at qTGW1, qTGW3, qTGW6, qTGW7, qTGW8 and qTGW12 increased TGW by 1.18, 1.18, 1.07, 0.73 and 0.84 g respectively, while allele inherited from RGD03040-721-11 at qTGW11 increased by 0.75g.

Table 12 Putative QTL identified for submergence tolerance, bacterial blight resistance and agronomic characters.

Trait	Marker interval	QTL names	Chromosome	LOD	Additive	R^2
PSS	RM282-RM16	qPSS3	3	3.36	-6.02	24.6
	RM252-RM3474	qPSS4	4	3.95	-5.29	17.4
	RM19-RM1986	qPSS12	12	5.48	-6.72	28.0
PSE	RM424-RM213	qPSE2	2	2.17	-1.26	25.3
	RN569-RM282	qPSE3	3	2.04	1.38	29.9
	RM217-RM564	qPSE6	6	3.16	1.59	40.7
TXO85	RM21-PB7/8	$qBB_{TXO85}11$	11	32.44	3.18	82.0
TXO95	RM21-PB7/8	$qBB_{TXO95}11$	11	41.60	6.37	88.4
TXO153	RM21-PB7/8	$qBB_{TXO153}11$	11	38.28	5.46	86.2
DF	RM424-RM213	qDF2	2	4.11	-1.19	18.1
	RM19-RM1986	qDF12	12	3.32	-1.25	18.3
PH	RM562-RM237	qPH1	1	3.20	-3.65	14.4
	RM424-RM213	qPH2	2	5.72	-4.86	32.3
NETP	RM213-RM208	qNETP2	2	3.07	-1.60	13.8
	RM164-RM26	qNETP5	5	5.41	2.29	23.1
	RM564-RM3	qNETP6	6	3.26	1.59	14.6
	RM515-RM223	qNETP8	8	4.89	3.25	21.1
	RM285-RM257	qNETP9	9	3.38	4.71	55.7
	RM202-RM21	qNETP11	11	4.07	-1.77	17.9
	RM19-RM1986	qNETP12	12	3.96	2.10	22.3
TGW	RM140-RM562	qTGW1	1	3.60	-0.90	19.4
	RM282-RM16	qTGW3	3	6.38	1.18	47.7
	RM3-RM340	qTGW6	6	5.41	1.18	47.6
	RM11-RM234	qTGW7	7	8.30	1.07	33.7
	RM25-RM44	qTGW8	8	3.70	0.73	16.8
	RM21-RM254	qTGW11	11	4.29	-0.75	19.2
	RM1986-RM12	qTGW12	12	3.84	0.84	20.1

DISCUSSIONS

Ideotype breeding for superior rice varieties can be achieved through the precision of MAS. Gene-specific (R10783indel, Aromarker, Glu-23 and PB7/8) and tightly linked (Waxy and GT11) markers were employed to select ideal rice genotype. In IR57514 x KDML105 cross, 66 ideal rice genotypes (ID1) that combined favorable alleles at the *Sub1, Wx, badh2* and *SSIIa* loci were selected. In RD6 x KDIII crosses, 52 ideal rice genotype (ID3) that combined favorable alleles at the *Sub1*, *Wx* (glutinous) and *Xa21* loci were selected. The evaluation for submergence tolerance indicated that the PSS and PSE of the ID1 group were not significantly different from those of IR57514 and the PSS of the ID3 and ID4 were significantly different from those of RD6. The evaluation for cooking quality indicated that FR, AC and ASV values of the ID1 group were not significantly different from that of KDML105 and all ID3 and ID4 are glutinous. The evaluation for bacterial blight resistance indicated that LL of the ID3 was less than that of the ID4. Recombinants derived from a recombination event between the markers and the target genes were not observed in both populations. These results indicated a high accuracy of MAS. In the current study, QTLs for submergence tolerance, the *Sub1* locus (Nandi *et al.*, 1997; Sripongpangkul *et al.*, 2000; Kamolsukyunyong *et al.*, 2001; Siangliw *et al.*, 2003; Toojinda *et al.*, 2003), cooking quality, *Wx*, *badh2* and *SSIIa* loci (Pinson, 1994; Lorieux *et al.*, 1996; Lanceras *et al.*, 2000; Wanchana *et al.*, 2003; Aluko *et al.*, 2004; Amarawathi *et al.*, 2008) and *Xa21* (Wang *et al.*, 1996; Williams *et al.*, 1996; Sanchez *et al.*, 2000; Joseph *et al.*, 2004) were revalidated, and the potential MAS for submergence, cooking quality and bacterial blight resistance was confirmed (Zhou *et al.*, 2003; Wan *et al.*, 2005; Neeraja *et al.*, 2007). The results indicated the following: 1) the *Sub1* locus is the major gene contributing to a high plant survival through the reduction of plant growth to store carbohydrates and energy when rice plants experience a complete submergence (Xu and Mackill, 1996); 2) the *badh2* locus is the major gene determining the presence of 2AP in rice grains (Bradbury *et al.*, 2005b); 3) the *Wx* locus is the major gene determining the amount of rice grain amylose (Sano, 1984); and 4) the *SSIIa* locus is the major gene determining the ASV trait that is associated with the GT trait (Umemoto *et al.*, 2002). MAS can be integrated into an ongoing rice breeding program, which can accelerate the development of ideal

genotypes (submergence tolerance and jasmine-like cooking quality) within five years.

MAS causes a distorted segregation of markers favoring the responsive alleles along various segments of chromosomes where the selective markers are located (Xu *et al.*, 1997). These selections affect the frequencies of alleles at linked marker loci, causing the distorted segregation of markers to extend over some distance along carrier chromosomes (Xu *et al.*, 1997; Septiningsih *et al.*, 2003; Thomson *et al.*, 2003). In the IR57514 x KDML105 cross, we observed selective distortions on chromosomes 6, 8 and 9, with the peak near the selective markers Waxy, Aromarker and R10783indel and extend over 89.2, 116.9 and 93.0 cM, respectively. And in the RD6 x KDIII populations, we observed selective distortions on chromosomes 6, 8 and 9, with the peak near the selective markers Glu-23, Aromarker and R10783indel and extend over 231.1, 233.1 and 96.3 cM, respectively. In cases where the target markers are linked to genes controlling agronomic traits, these linkage drags could lead to a negative association with the traits (Jairin *et al.*, 2009; Liu *et al.*, 2009). With this in mind, we observed in the IR57514 x KDML105 population that the resistance allele of the *Sub1* was associated with qL/B9 and qNFGP9; the positive allele (Wx^{KD}) of Waxy was associated with qASV6. Our observations are agreed with (He *et al.*, 1999; Lanceras *et al.*, 2000; Bao *et al.*, 2002; Aluko *et al.*, 2004; Fan *et al.*, 2005). Linkage drag could bring difficulties in utilizing IR57514 and KDML105 in the breeding program. Distorted segregation of markers was observed on non-carrier chromosomes such as chromosomes 1, 3, 4, 5, 7 and 10 in the IR57514 x KDML105 population and chromosomes 1, 2, 5, 7, 10 and 12 in the RD6 x KDIII populations. This could be caused by a small population size of the IDs (97 individuals in the IR57514 x KDML105 population and 95 individuals in the RD6 x KDIII populations) (Septiningsih *et al.*, 2003; Aluko *et al.*, 2004) and an unintentional selection occurring during the development of RIL through the SSD in the IR57514 x KDML105 population. For example, the tall PH plants tend to be kept in each generation of the SSD, leading to a majority of tall PH plants found in the RIL. This evidence led to a distortion of markers found on the long arm of the chromosome 1 where the QTL for PH (qPH1) is located. In the present study, distortion of non-carrier chromosomes

leads to a lower productivity of some individual ID such as lower NPP, NFGP and PSF caused by KDML alleles on markers on chromosomes 1 and 5 (Table 5).

In this study, the significant variations among the individual IDs were observed for traits related to submergence tolerance, PSS and PSE. These indicated a quantitative nature of such traits. To date, there have been a number of reports on QTLs associated with submergence tolerance (Xu and Mackill, 1996; Nandi *et al.*, 1997; Sripongpangkul *et al.*, 2000; Siangliw *et al.*, 2003; Toojinda *et al.*, 2003; Angaji, 2008). The *Sub1* tolerance gene inherited from FR13A has been known to control ethylene- and gibberellin-mediated changes in gene expression that include a regulation of genes controlling carbohydrate consumption and cell elongation (Jackson and Ram, 2003; Fukao *et al.*, 2006; Xu *et al.*, 2006). The effect of *Sub1* on carbohydrate consumption and cell elongation was confirmed by this study in the IR57514 x KDML105 population. A significantly improved submergence tolerance (a high PSS) through the reduction of the PSE was observed (the PSE of most ID1 and ID2 was significantly different from KDML105), indicating that the presence of *Sub1* inherited from IR57514 clearly reduced the elongation of the plant under the submergence event. The PSS and PSE of the ID1 and ID2 showed a significant negative correlation, and the different genomic locations of the QTLs for the PSS and PSE were detected. These results indicated that there might be other mechanisms, in addition to the reduction of the PSE that leads to a high PSS when rice plants are subjected to complete submergence. The results also clearly showed that the genetic background played a critical role in determining the level of submergence tolerance in rice plants that carry the Sub1 locus, a major QTL for submergence tolerance.

The mean values of the AC, FR and ASV of the ID1 group were not significantly different from those of KDML105. According to the profile of markers Waxy, Aromarker and GT11, all ID1 individuals were expected to carry Wx^{KD}, $badh2^{KD}$ and $SSIIa^{KD}$, but the AC, FR and ASV among individuals were slightly different from each other (Table 6), suggesting that they are quantitatively inherited traits. Genetic studies have indicated that the AC, FR and ASV are controlled by the major genes, *Wx* (Sano, 1984), *badh2* (Bradbury *et al.*, 2005b) and *SSIIa* (Umemoto

et al., 2002), respectively, with one or more modifiers (He *et al.*, 1999; Wang *et al.*, 2007; Lou *et al.*, 2009) and might be affected by quantitative genes of the triploid endosperm, cytoplasm and maternal genomes (Shi *et al.*, 1997; Bao and Xia, 1999) In this study, two QTL, qAC5 and qAC8, acting as modifiers were responsible for the variations in AC in the ID1 (13.0 to 20.0 %) and ID2 (14.4 to 20.0 %) (He *et al.*, 1999; Aluko *et al.*, 2004; Fan *et al.*, 2005). For the FR, no QTL was detected in this study because no variation for FR was observed in the ID population (all IDs had a scored as 1, fragrance). However, we analyzed the amount of 2AP in the grains of 10 individuals of the ID1 group. We found that the amount of 2AP varied greatly from 0.976 to 2.233 ppm, which is less than that of KDML105 (2.761). Therefore, this variation indicated that there are some modifiers determining the amount of 2AP in the ID population (Lorieux *et al.*, 1996; Bradbury *et al.*, 2005b). The variation of ASV was observed only in the ID1 group, varying from 4 to 6 scores. Because all of the ID1 individuals carried the $SSIIa^{KD}$ allele, qASV5, qASV7 and qASV8 associated with the ASV were considered as modifiers in this study. The allele from IR57514 at the qASV8 locus increased the ASV value, whereas the allele from KDML105 at the qASV5 and qASV7 loci increased the ASV value. These combinations of three loci were responsible for the variation of the ASV presenting in the ID1 population (Lanceras *et al.*, 2000; Aluko *et al.*, 2004; Fan *et al.*, 2005).

The gel consistency of cooked rice helps in the determination of the texture of cooked rice, and the genetic determinant of the GC has been extensively studied in rice (He *et al.*, 1999; Fan *et al.*, 2005). There have been many reports that the *Wx* gene, encoding granule bound starch synthase (GBSS), or a major gene linked to the *Wx* locus determines the GC (Tan *et al.*, 1999; Fan *et al.*, 2005; Tian *et al.*, 2005; Zheng *et al.*, 2008). In this study, the parental varieties, IR57514 and KDML105, have high values of GC (83 to 85 mm), indicating that their cooked rice is more tender and sticky. The GC of the ID1 and ID2 groups were not significantly different from those of IR57514 and KDML105, but some of the ID lines, as a result of transgressive segregation, presented high (hard) and low (soft) GC. This result indicated that neither Wx^{KD} nor $SSIIa^{KD}$ was responsible for the variation of the GC in these populations. Through the QTL mapping, qGC6 and qGC7 were identified for

the GC and mapped to the vicinity of the qASV6 and qASV7 respectively. This is in agreement with previous results reported by He *et al*. (1999), Aluko *et al*. (2004) and Fan *et al*. (2005). Co-localization of the QTL affecting cooking quality, as a result of either pleiotropic effect or close linkage, have been reported as genetic basis of correlation among the quality traits (Yoshida *et al.*, 2002). In this study, the positive alleles of the coincidental QTL for GC and ASV came from different parents. We did not observe any recombinant in the ID population. This evidence suggested pleiotropic effect rather than close linkage of these QTL determining GC and ASV. Alleles from IR57514 at both QTL loci increased the GC while decreased the ASV. Further studies using near isogenic lines are needed to elucidate the pleiotropic QTL at these position. Co-localization of the QTL for AC and ASV resulted from pleiotropic effect or close linkage was found on chromosome 8. The qAC8 and qASV8 were mapped to RM72-RM44 interval. Allele from IR57514 increased the AC and ASV (Aluko *et al.*, 2004; Wan *et al.*, 2004).

In addition to the development of the ideal genotypes, we identified QTL associated with physical grain quality and some important agronomic characteristics in the IR57514 x KDML105 population. A total of 23 QTLs for nine traits such as GL, GW, L/B, DF, PH, NPP, NFGP, PSF and TGW were identified (Table 6). We found co-localization of the QTL on five genomic regions located on chromosomes 1, 2, 4, 5 and 6. The first genomic region on the long arm of chromosome 1 covered by RM3285 and RM5781 contained qPH1 and qNPP1. Allele from KDML105 increased PH but decreased NPP. The qPH1 was mapped very closely to the sd1 (Hemamalini *et al.*, 2000; Li *et al.*, 2003b; Septiningsih *et al.*, 2003). There are previous reports of the QTL for NPP mapped to this region (Septiningsih *et al.*, 2003). The sd1 has been widely studied and epistatic effect of sd1 on other traits was extensively reported (Hemamalini *et al.*, 2000; Sripongpangkul *et al.*, 2000; Spielmeyer *et al.*, 2002; Li *et al.*, 2003b). Genetic relationships between qPH1 and sd1 and qNPP1 and sd1 need further studies. The second region flanked by RM263 and RM6 contained qGL2, qGB2 and qTGW2. Pleiotropic effect has been demonstrated as genetic basis for grain quality (Fan *et al.*, 2006; Zhang *et al.*, 2006; Song *et al.*, 2007; Bai *et al.*, 2010). In this region, allele from IR57514 increased GL, GB and TGW (Septiningsih *et al.*,

2003; Yan *et al.*, 2003; Wan *et al.*, 2005). The third region carrying qGL4.2 and qL/B4 was located within RM7187-RM317 interval. Allele from KDML105 at these two loci increased GL and L/B (Wan *et al.*, 2005). The fourth region was located between RM26 and RM334 on chromosome 5. Two QTL including qGB5 and qPSF5 in which the allele from IR57514 increase GB and PSF were co-located (Tan *et al.*, 1999; Wan *et al.*, 2005; Wan *et al.*, 2008). The final region flanked by GT11 and RM564 contained qGB6, qPSF6 and qTGW6. This is the same region where *SSIIa* is located. Allele from IR57514 increased GB, PSF and TGW. The relationships among *SSIIa*, qGB6 and qTGW6 need further studies. According to the QTL mapping, both parents contributed positive alleles of the QTL for GL, GB, L/B, DF, PH and TGW. This suggests that we can obtain superior genotypes for GL, GB, L/B, DF, PH and TGW by phenotypic selection. As mentioned earlier, the numbers of lines developed through SSD should be high (5,000-10,000 lines) in order to increase probability of obtaining IDs with all desired characteristics.

In this study, it is clearly demonstrated that breeding for glutinous rice with submergence tolerance and BB resistance can be deployed by MAS and PTS. In the RD6 x KDIII populations, our validation experiments indicated that the ID3 and ID4 are glutinous rice and tolerant to flash flooding. The ID3 are resistant to BB disease. Utilizing gene specific markers (within *Sub1* and *Xa21* genes) through MAS can overcome those limiting in the conventional breeding method by enhancing the efficiency of selection and increasing the probability of obtaining the desired genotype (IRRI, 2002; Collard *et al.*, 2005; Jena and Mackill, 2008).

In the RD6 x KDIII populations, we identified QTL associated with some agronomic characteristics. A total of 18 QTLs for four traits such as DF, PH, NETP and TGW were identified. We found co-localization of the QTL on two genomic regions on chromosome 2 and 12. The first region on chromosome 2 was flanked by RM424 and RM213. The qDF2 and qPH2 were co-located and allele inherited from the RGD03040-721-11 increased both DF and PH. And the second region on chromosome 12 was flanked by RM19 and RM1986. The qDF12, qNETP12 and

qTGW12, were co-located and allele inherited from RD6 increased the NETP and TGW but decreased the DF.

In conventional breeding, breeders select plant based on the phenotypic performance in the field or nursery. In this study, we integrated MAS and PTS to develop the ideal genotypes. In the IR57514 x KDML105 population, SSD was deployed to develop the large F_7 recombinant population. Stepwise MAS was then deployed to identify the ideal genotype. The stepwise MAS reduce the number of plants for MAS. However, plant type selection may still be useful in order to selection subset of plants to reduce the number of plants that need to be phenotypically evaluated. There are optimizations of marker methods in terms of integration between MAS and conventional breeding may have advantages over phenotypic screening or MAS alone in order to challenges for the greater adoption and impact of MAS on rice breeding.

CONCLUSIONS

Ideotypes breeding for superior rice varieties were successfully developed through the integration of MAS and a conventional method in a single-cross breeding. MAS have also been successfully applied to improve submergence tolerance, bacterial blight resistance and cooking quality in the ideal rice genotype. In IR57514 x KDML105 cross, ID1 that combined favorable alleles at the *Sub1*, *Wx*, *badh2* and *SSIIa* loci were selected. The IDs with $Sub1^{IR}$ were tolerant to complete submergence and the IDs with Wx^{KD}, $badh2^{KD}$ and $SSIIa^{KD}$ showed a KDML105 grain quality. In RD6 x KDIII crosses, ID3 that combined favorable alleles at the *Sub1*, *Wx* (glutinous) and *Xa21* loci were selected. The IDs with $Sub1^{KDIII}$ were tolerant to complete submergence and the IDs with $Xa21^{KDIII}$ were resistant to bacterial blight and the IDs with Wx^{RD6} showed a RD6 quality In self-pollinating crops, SSD has been proven as an effective breeding method which is characterized with less production cost and it is more rapid compared to pedigree, modified pedigree and bulk methods (Fahim *et al.*, 1998) in developing superior genotypes. In this study, we identified the superior genotype of Hom Cholasit, an elite line derived from the ID2 group, with desirable traits of submergence tolerance, a low amylose content, fragrance, lodging resistance (short plant height), a nonphotoperiod sensitivity and a high yield (7.2 t/ha in the farmer's field). Since 2008, this variety has been widely planted by farmers in the irrigated flood-prone areas of Thailand. Currently, the SSD method, combined with MAS, is routinely used to develop new rice genotypes in Thailand. The improved ID lines should be immediately useful for flood-prone areas and will help farmers to increase their production and income.

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APPENDIX

Appendix Table 1 Molecular markers used for background determination in non-glutinous variety. All SSR markers were obtained from www.grameme.org.

Chromosome	Marker names	Number of marker
1	RM495, RM84, RM220, RM243, RM23, RM3412, RM562, RM5, RM246, RM8097, RM212, RM5781, RM3285	13
2	RM154, RM279, RM174, RM424, RM341, RM263, RM6, RM208	8
3	RM3202, RM569, RM5761, RM16, RM168	5
4	RM335, RM119, RM273, RM252, RM7187, RM317, RM3474, RM3534, RM280, RM559	10
5	RM153, RM122, RM159, RM169, RM516, RM3838, RM164, RM26, RM334	9
6	RM170, RM204, RM314, RM121, RM564, RM541, RM7309, RM30	8
7	RM5711, RM11, RM182, RM3826, RM1132, RM18, RM234, RM248, RM172	9
8	RM152, RM38, RM72, RM44, RM515, RM3459, RM210, RM256, RM149, RM447, RM458, RM264	12
9	RM316, RM5799, RM219, RM7306	4
10	RM222, RM216, RM258, RM228, RM333, RM591	6
11	RM116, RM202, RM287, RM209, RM224	5
12	RM20, RM19, RM101, RM1047, RM309, RM463, RM235, RM12	8

Appendix Table 2 Molecular markers used for background determination in glutinous variety. All SSR markers were obtained from www.grameme.org.

Chromosome	Marker names	Number of marker
1	RM140, RM3412, RM562, RM243, RM237	5
2	RM208, RM213, RM424	3
3	RM16, RM569, RM85, RM282	4
4	RM252, RM3474, RM119, RM280	4
5	RM3838, RM249, RM164, RM26	4
6	RM225, RM340, RM3, RM217, RM564	5
7	RM11, RM234, RM214, RM418	4
8	RM264, RM25, RM44, RM223, RM515	5
9	RM215, RM257, RM316, RM285	4
10	RM258, RM222, RM239	3
11	RM167, RM21, RM202, RM254	4
12	RM12, RM1986, RM19, RM20	4

Appendix Table 3 Data performance of target and agronomic traits of all ideotypes non-glutinous rice varieties.

Entries	Pedigree	PSS	PSE	AC	GC	ASV	GL	GB	L/B	DF	PH	NETP	NFGP	NGP	PSF	TGW
	IR57514	77.3	42.4	33.6	83.0	1	6.87	1.94	3.54	110	109	12	139	160	86.9	26.8
	KDML105	4.6	74.9	14.7	85.0	4	7.30	1.69	4.32	119	135	11	127	146	87.3	25.1
1	PSL99093-1-1-5R	53.4	63.9	18.1	104.5	6	7.50	1.99	3.77	98	148	14	128	137	93.4	23.1
2	PSL99093-31-7-5R	66.0	45.8	17.9	73.0	6	7.39	2.10	3.52	90	77	16	120	133	90.2	27.1
3	PSL99093-31-8-5R	61.2	43.7	14.7	85.0	5	6.89	2.03	3.40	90	86	19	101	112	90.6	30.4
4	PSL99093-31-10-5R	61.3	48.0	15.2	101.0	1	7.69	2.21	3.48	87	94	17	110	122	90.2	29.5
5	PSL99093-32-7-5R	58.0	41.2	15.4	85.5	4	7.22	1.90	3.80	89	92	9	143	152	94.1	21.4
6	PSL99093-33-6-5R	75.3	44.1	15.9	80.5	4	7.39	2.00	3.69	89	133	14	125	144	87.1	24.6
7	PSL99093-33-14-5R	33.6	60.9	17.7	82.5	4	7.30	1.99	3.67	87	133	12	111	128	86.3	26.0
8	PSL99093-47-2-5R	66.4	49.6	17.2	83.0	4	7.10	2.08	3.41	99	140	9	137	168	81.3	26.8
9	PSL99093-47-4-5R	65.0	45.4	17.1	95.0	5	7.44	1.99	3.74	99	142	10	104	123	84.1	24.3
10	PSL99093-48-5-5R	74.3	42.7	16.4	83.0	5	7.14	1.88	3.80	100	144	9	142	170	83.5	26.1
11	PSL99093-49-1-5R	57.0	43.8	15.9	80.0	5	7.23	1.73	4.18	93	138	13	105	139	75.5	20.8
12	PSL99093-60-12-5R	59.1	58.1	13.0	73.0	4	6.65	2.07	3.21	95	149	11	117	135	86.3	24.2
13	PSL99093-111-7-5R	67.6	47.9	17.1	85.0	5	7.31	2.00	3.65	107	149	9	119	174	68.4	24.8
14	PSL99093-120-7-5R	66.0	55.7	15.4	72.5	5	6.87	2.22	3.10	94	138	10	120	153	78.4	24.1
15	PSL99093-133-4-5R	57.4	50.7	15.7	89.5	1	6.82	1.99	3.43	99	147	19	98	114	86.3	21.6
16	PSL99093-134-3-5R	54.0	51.6	15.6	85.5	4	7.00	1.83	3.83	97	136	23	152	173	87.9	19.5
17	PSL99093-134-6-5R	51.9	49.8	15.2	85.0	1	6.98	2.10	3.32	96	148	17	152	173	87.9	22.7
18	PSL99093-135-3-5R	51.0	58.6	16.6	77.5	1	6.82	1.94	3.52	99	146	7	134	149	89.6	20.2
19	PSL99093-150-4-5R	66.5	50.9	17.3	125.0	1	7.23	2.10	3.44	93	164	14	107	125	85.9	26.1
20	PSL99093-152-3-5R	75.2	42.9	17.4	78.5	1	7.36	2.20	3.35	99	162	9	160	174	91.9	26.2
21	PSL99093-152-5-5R	77.3	48.1	14.5	91.0	1	7.46	2.08	3.59	90	150	13	128	142	90.1	24.1
22	PSL99094-27-3-5R	75.3	47.1	15.6	65.5	5	7.43	2.06	3.61	90	143	11	139	156	89.4	23.9
23	PSL99094-27-5-5R	65.1	44.4	15.4	69.5	5	7.03	2.03	3.46	90	93	15	111	126	88.4	23.8

Appendix Table 3 (Continued)

Entries	Pedigree	PSS	PSE	AC	GC	ASV	GL	GB	L/B	DF	PH	NETP	NFGP	NGP	PSF	TGW
24	PSL99094-27-5-5R	71.1	41.9	15.1	76.5	4	7.05	2.00	3.52	90	78	18	117	132	88.6	23.6
25	PSL99094-37-4-5R	71.1	46.1	14.9	67.0	4	6.97	1.87	3.73	89	78	11	114	127	89.8	22.6
26	PSL99094-37-5-5R	75.9	38.9	14.6	61.0	5	6.67	1.99	3.35	96	84	15	117	137	85.0	22.6
27	PSL99094-37-6-5R	61.5	43.2	16.7	66.0	6	6.88	1.94	3.55	96	80	9	104	129	80.5	23.7
28	PSL99094-37-7-5R	64.7	51.8	15.3	71.5	4	6.86	1.83	3.75	91	82	11	101	124	81.0	21.9
29	PSL99094-38-1-5R	72.8	43.6	17.0	61.5	6	7.18	1.81	3.97	90	87	15	130	145	89.6	22.3
30	PSL99094-38-2-5R	56.9	39.6	17.1	68.0	6	7.17	1.90	3.77	90	84	18	125	137	90.9	23.0
31	PSL99094-42-3-5R	71.4	36.5	16.4	92.5	1	7.19	2.25	3.20	93	145	12	110	114	96.9	28.8
32	PSL99094-46-5-5R	56.5	47.3	16.0	94.0	1	6.63	2.08	3.19	99	164	14	133	162	82.4	22.5
33	PSL99094-52-1-5R	66.5	36.2	18.7	74.0	6	7.25	2.00	3.63	102	161	15	100	112	89.7	19.7
34	PSL99094-53-1-5R	66.6	46.8	17.3	67.5	5	7.40	2.03	3.65	101	103	15	123	150	81.7	25.5
35	PSL99094-53-2-5R	67.0	46.8	17.0	76.0	5	7.53	2.00	3.77	104	105	20	117	151	77.4	24.8
36	PSL99094-63-5-5R	58.3	56.1	18.2	88.0	5	7.12	2.04	3.49	107	167	11	125	128	97.7	27.1
37	PSL99094-78-7-5R	66.1	37.6	15.9	82.5	1	7.79	2.06	3.78	101	109	11	141	161	87.5	26.9
38	PSL99094-78-8-5R	75.5	31.5	15.2	97.0	1	7.66	1.99	3.85	101	106	18	156	195	80.0	29.4
39	PSL99094-79-2-5R	71.6	36.6	16.1	82.5	5	7.34	2.07	3.55	91	103	18	116	136	84.9	23.2
40	PSL99094-80-5-5R	67.5	41.2	14.6	87.0	4	6.99	1.97	3.55	90	148	12	79	108	73.0	22.0
41	PSL99094-80-7-5R	68.2	38.5	15.8	92.0	5	7.18	2.08	3.45	87	113	14	124	131	95.0	26.4
42	PSL99094-81-1-5R	63.7	42.5	14.4	98.5	5	6.97	1.97	3.54	100	104	13	103	121	85.5	23.6
43	PSL99094-88-1-5R	58.6	50.5	16.2	89.5	5	7.12	2.06	3.46	91	134	10	109	133	81.9	25.1
44	PSL99094-97-7-5R	57.5	41.9	14.0	94.0	5	6.77	1.96	3.46	86	90	13	121	137	88.3	22.7
45	PSL99094-99-1-5R	82.3	45.3	18.6	71.0	5	7.17	2.04	3.52	87	90	10	103	111	92.8	21.8
46	PSL99094-126-2-5R	50.6	50.4	15.3	119.0	1	6.96	1.93	3.61	110	159	12	90	158	56.8	21.2
47	PSL99094-135-5-5R	73.5	41.4	19.5	96.5	1	7.52	2.14	3.51	91	99	13	125	145	86.5	23.5
48	PSL99094-135-6-5R	64.2	35.3	16.4	101.0	1	7.20	2.18	3.30	96	95	14	111	117	94.8	24.7

Appendix Table 3 (Continued)

Entries	Pedigree	PSS	PSE	AC	GC	ASV	GL	GB	L/B	DF	PH	NETP	NFGP	NGP	PSF	TGW
49	PSL99094-135-9-5R	73.6	38.2	16.5	120.0	1	7.04	2.23	3.16	97	99	12	126	145	87.2	28.2
50	PSL99094-135-11-5R	66.7	29.2	16.6	77.5	1	7.29	2.22	3.28	91	100	13	115	175	65.9	28.5
51	PSL99094-136-2-5R	67.7	45.7	16.4	85.5	1	7.22	2.24	3.22	104	167	11	119	160	74.4	24.6
52	PSL99094-155-8-5R	53.3	49.9	15.4	80.5	4	7.48	2.21	3.38	104	109	18	106	122	86.5	28.8
53	PSL99094-157-3-5R	64.7	46.3	14.2	66.0	5	7.50	2.06	3.64	89	135	13	100	120	83.3	23.3
54	PSL99094-157-4-5R	58.8	49.4	18.3	70.5	6	7.50	2.06	3.64	86	135	9	109	128	85.2	22.1
55	PSL99094-157-5-5R	65.0	55.0	17.1	88.0	6	7.12	1.88	3.79	89	134	11	100	128	78.4	24.0
56	PSL99094-167-7-5R	69.8	39.9	18.3	87.0	6	7.81	2.21	3.53	109	163	10	125	145	86.2	29.3
57	PSL99094-167-8-5R	59.8	47.1	19.6	91.0	6	7.26	2.12	3.42	108	148	10	124	141	87.9	26.8
58	PSL99094-171-4-5R	63.1	55.2	16.7	109.0	5	7.10	2.13	3.33	90	137	11	105	113	93.3	23.5
59	PSL99094-186-4-5R	70.7	48.9	18.6	105.0	6	7.91	2.15	3.68	106	166	8	148	181	81.7	27.5
60	PSL99094-186-4-5R	68.9	44.1	18.7	86.0	5	8.27	1.98	4.18	106	105	12	113	146	77.4	29.2
61	PSL99094-187-3-5R	62.5	48.5	16.9	99.5	4	7.28	1.83	3.98	113	104	8	132	155	85.2	23.2
62	PSL99094-187-4-5R	66.6	43.8	14.9	134.0	4	7.34	1.99	3.69	113	114	12	142	167	85.0	26.1
63	PSL99094-187-8-5R	61.7	49.7	15.6	81.0	4	7.63	1.97	3.87	89	128	8	110	130	84.2	24.2
64	PSL99094-189-2-5R	66.8	42.4	16.7	85.5	5	7.05	2.25	3.13	108	114	13	146	154	94.5	24.7
65	PSL99094-189-5-5R	69.0	45.4	20.0	92.0	6	7.27	2.19	3.32	106	175	10	120	144	83.3	27.3
66	PSL99094-189-6-5R	52.5	49.3	19.0	115.5	6	7.17	2.09	3.43	106	164	9	103	130	78.8	23.5
67	PSL99094-195-6-5R	59.3	43.3	16.3	119.0	1	7.50	2.23	3.36	100	100	10	127	147	86.7	28.8
68	PSL99094-199-4-5R	68.5	44.0	19.9	99.5	1	7.33	2.13	3.44	91	161	7	148	173	85.3	26.3
69	PSL99094-201-2-5R	74.7	36.0	18.1	83.5	5	7.36	1.96	3.76	89	95	15	100	115	86.5	24.6
70	PSL99094-202-3-5R	54.5	55.5	17.0	112.5	5	7.13	2.02	3.53	105	146	10	138	157	87.9	25.2
71	PSL99094-202-4-5R	54.1	52.0	16.6	104.5	4	6.90	2.00	3.45	95	151	14	146	166	87.7	25.8
72	PSL99094-206-1-5R	68.3	43.4	17.6	108.5	6	7.33	2.14	3.43	96	168	9	152	187	81.5	28.0
73	PSL99094-206-2-5R	71.3	41.9	20.0	81.0	6	7.08	2.15	3.29	97	156	9	126	162	77.8	25.0

Appendix Table 3 (Continued)

Entries	Pedigree	PSS	PSE	AC	GC	ASV	GL	GB	L/B	DF	PH	NETP	NFGP	NGP	PSF	TGW
74	PSL99094-206-8-5R	82.7	43.9	18.7	92.5	6	7.43	2.17	3.42	104	161	9	116	193	60.3	27.8
75	PSL99094-207-10-5R	63.7	50.3	19.6	65.0	5	6.86	2.02	3.40	90	95	10	76	88	86.3	23.4
76	PSL99094-212-1-5R	71.9	54.8	15.3	76.5	5	7.70	2.06	3.74	99	173	13	95	128	74.1	25.5
77	PSL99094-212-2-5R	73.7	40.2	19.4	80.5	5	7.50	2.09	3.59	99	171	11	81	88	91.5	26.1
78	PSL99094-212-5-5R	71.1	51.6	16.6	115.0	1	8.02	2.17	3.70	102	183	13	94	146	64.3	31.6
79	PSL99094-217-3-5R	31.3	66.4	16.2	87.0	5	7.08	2.02	3.50	109	144	16	107	141	75.9	26.4
80	PSL99094-224-1-5R	71.8	34.8	14.9	104.5	1	7.47	1.93	3.87	97	166	12	70	114	61.7	30.7
81	PSL99094-238-5-5R	68.4	48.8	17.0	106.5	1	7.61	2.05	3.71	98	152	11	120	165	72.9	28.3
82	PSL99094-238-6-5R	68.6	45.5	15.6	100.5	1	7.32	2.11	3.47	105	164	13	127	183	69.6	23.1
83	PSL99094-238-12-5R	36.7	56.1	16.3	87.0	6	7.58	2.09	3.63	111	135	9	58	108	54.0	26.4
84	PSL99094-238-12-5R	50.1	51.8	18.2	61.0	6	7.68	2.21	3.48	111	138	11	115	205	56.1	24.4
85	PSL99094-239-5-5R	68.3	45.9	17.9	107.0	1	7.41	2.10	3.53	100	148	8	131	224	58.5	23.6
86	PSL99094-239-7-5R	61.0	48.6	19.2	94.5	1	7.56	2.04	3.71	104	92	16	58	135	42.6	25.5
87	PSL99094-257-8-5R	68.1	44.5	14.6	113.0	5	7.10	2.11	3.36	102	97	20	69	118	58.1	25.9
88	PSL99094-257-9-5R	60.3	51.2	14.4	113.5	1	7.17	2.06	3.48	105	87	14	38	79	47.5	25.7
89	PSL99094-257-10-5R	82.3	35.1	19.7	101.5	1	7.01	2.16	3.25	105	87	17	80	116	68.5	27.1
90	PSL99094-258-4-5R	76.0	44.9	14.4	97.0	1	7.13	1.90	3.75	99	130	11	93	131	70.9	22.5
91	PSL99094-274-5-5R	55.6	50.1	18.2	89.5	6	7.17	2.00	3.59	106	136	9	41	60	68.1	21.0
92	PSL99094-313-3-5R	61.0	56.7	18.1	92.5	6	7.04	2.06	3.42	89	124	11	74	112	66.1	24.2
93	PSL99094-313-4-5R	61.4	41.5	15.7	82.5	1	7.20	2.04	3.53	86	81	19	68	102	66.5	23.3
94	PSL99094-319-2-5R	68.2	47.5	16.0	85.0	1	7.20	2.02	3.56	91	143	8	99	148	66.9	22.1
95	PSL99094-326-1-5R	63.2	38.7	17.2	79.0	5	7.10	2.03	3.50	88	145	15	69	122	56.1	25.9
96	PSL99094-326-2-5R	69.2	47.8	17.6	83.0	5	7.56	1.98	3.82	86	134	9	54	85	63.3	19.9
97	PSL99094-328-6-5R	62.8	51.9	17.6	92.0	1	6.97	2.12	3.29	86	115	14	92	119	77.2	24.6

Appendix Table 4 Data validation of the phenotype and genotype of 190 F_6 individuals from cross of RD6 and KDIII containing combinations of submergence tolerance and BB resistance genes.

Entries	Pedigree	PPS (%)	Lesion length(cm)					
			TXO85		TXO95		TXO153	
	FR13A	97.7						
	RD6	0.0	11.4	S	16.4	S	18.1	S
	IR1188		1.2	R	1.3	R	2.5	R
1	RGD05002-13-72-B-3-1-B	25.1	3.3	MR	5.4	MR	3.5	MR
2	RGD05010-30-17-B-2-1-B	53.5	2.0	R	3.9	MR	5.5	MR
3	RGD05010-30-17-B-5-1-B	26.6	2.4	R	-		4.6	MR
4	RGD05010-30-17-B-10-1-B	32.6	2.0	R	2.5	R	2.3	R
5	RGD05010-30-49-B-7-1-B	59.2	5.6	MR	2.1	R	-	
6	RGD05010-13-42-6-6-1-1	22.5	3.8	MR	7.3	MS	4.1	MR
7	RGD05010-13-42-6-6-2-1	43.4	-		3.0	R	5.4	MR
8	RGD05010-13-42-6-6-3-1	19.9	3.7	MR	4.9	MR	2.9	R
9	RGD05010-13-42-6-6-4-1	14.4	1.2	R	3.1	MR	4.0	MR
10	RGD05010-13-42-6-6-5-1	30.6	1.9	R	2.7	R	2.8	R
11	RGD05010-13-42-6-6-5-2	29.4	2.5	R	3.0	R	1.3	R
12	RGD05010-13-42-6-6-6-1	24.2	1.9	R	3.1	MR	2.6	R
13	RGD05010-13-42-6-6-7-2	30.4	1.4	R	2.0	R	2.1	R
14	RGD05010-13-42-6-6-8-1	41.0	3.1	MR	2.8	R	3.8	MR
15	RGD05010-13-42-6-6-9-2	35.8	1.3	R	0.6	R	2.5	R
16	RGD05010-13-42-6-6-10-2	11.3	1.5	R	1.1	R	2.1	R
17	RGD05010-13-42-6-10-1-1	39.9	0.8	R	1.1	R	2.8	R
18	RGD05010-13-42-6-10-1-2	41.7	-		2.6	R	1.8	R
19	RGD05010-13-42-6-10-3-1	20.3	-		3.2	MR	3.0	R
20	RGD05010-13-42-6-10-4-1	41.4	3.2	MR	3.1	MR	3.9	MR
21	RGD05010-13-42-6-10-6-1	36.0	1.5	R	5.0	MR	2.6	R
22	RGD05010-13-42-6-10-7-1	32.1	2.4	R	2.8	R	3.9	MR
23	RGD05010-13-42-6-10-8-1	36.9	1.8	R	1.1	R	3.4	MR
24	RGD05010-13-42-6-10-9-1	54.1	1.5	R	1.2	R	1.6	R
25	RGD05010-13-42-6-11-1-1	27.6	2.4	R	3.6	MR	3.3	MR
26	RGD05010-13-42-6-11-1-2	57.0	0.7	R	-		-	
27	RGD05010-13-42-6-11-7-2	22.5	1.8	R	1.7	R	6.2	MS
28	RGD05010-13-42-6-11-8-1	21.2	1.3	R	1.7	R	2.3	R
29	RGD05010-13-61-12-2-7-1	25.9	1.0	R	1.6	R	2.4	R
30	RGD05010-13-61-12-2-10-1	34.2	1.3	R	2.2	R	3.0	R
31	RGD05010-15-58-3-1-1-1	53.9	3.2	MR	1.9	R	7.3	MS
32	RGD05010-15-58-3-1-2-1	29.9	1.6	R	4.9	MR	4.1	MR
33	RGD05010-15-58-3-1-3-1	48.1	3.5	MR	5.2	MR	5.3	MR
34	RGD05010-15-58-3-1-4-1	55.0	1.6	R	2.5	R	4.5	MR
35	RGD05010-15-58-3-1-6-1	63.2	3.4	MR	1.5	R	6.2	MS

Appendix Table 4 (Continued)

Entries	Pedigree	PPS (%)	Lesion length(cm)					
			TXO85		TXO95		TXO153	
36	RGD05010-15-58-3-1-10-1	44.9	3.3	MR	2.6	R	4.7	MR
37	RGD05010-15-58-3-10-5-1	41.6	2.8	R	1.7	R	5.8	MR
38	RGD05010-15-58-3-10-6-1	53.0	2.8	R	2.7	R	3.7	MR
39	RGD05010-15-58-3-10-8-1	26.0	2.3	R	1.8	R	5.7	MR
40	RGD05010-15-58-3-10-9-1	50.1	2.4	R	2.5	R	3.2	MR
41	RGD05010-15-58-3-10-10-1	40.4	2.7	R	1.8	R	-	
42	RGD05010-29-7-4-1-5-1	16.8	2.9	R	4.1	MR	-	
43	RGD05010-29-7-4-5-2-1	32.1	-		2.9	R	6.5	MS
44	RGD05010-29-7-4-5-6-1	23.7	-		-		7.8	MS
45	RGD05010-29-7-6-3-1-1	30.8	2.0	R	-		4.8	MR
46	RGD05010-29-7-6-3-2-1	40.1	2.8	R	3.3	MR	6.0	MR
47	RGD05010-29-7-6-3-3-1	38.8	2.1	R	2.5	R	6.2	MS
48	RGD05010-29-7-6-3-4-1	37.2	3.6	MR	3.6	MR	6.2	MS
49	RGD05010-29-7-6-3-5-1	30.9	3.7	MR	2.9	R	5.0	MR
50	RGD05010-29-7-6-3-7-1	42.0	3.9	MR	3.0	R	5.2	MR
51	RGD05010-29-7-6-5-1-1	15.6	3.3	MR	-		-	
52	RGD05010-29-7-6-5-4-1	44.5	3.5	MR	2.2	R	4.4	MR
53	RGD05010-29-7-6-5-10-1	42.3	2.3	R	7.8	MS	6.6	MS
54	RGD05011-18-25-B-1-1-B	19.6	2.5	R	4.9	MR	5.3	MR
55	RGD05011-23-20-B-1-1-B	10.1	2.4	R	2.4	R	3.8	MR
56	RGD05011-23-20-B-3-1-B	9.6	1.8	R	2.7	R	2.0	R
57	RGD05011-23-20-B-4-1-B	17.1	2.4	R	3.5	MR	5.0	MR
58	RGD05011-23-20-B-5-1-B	14.1	2.3	R	2.8	R	6.4	MS
59	RGD05011-23-20-B-5-2-B	16.3	2.2	R	3.1	MR	3.1	MR
60	RGD05011-23-20-B-6-1-B	11.5	3.5	MR	4.1	MR	7.9	MS
61	RGD05011-23-20-B-9-1-B	11.6	2.1	R	4.6	MR	5.6	MR
62	RGD05011-23-20-B-10-1-B	13.5	2.3	R	2.7	R	5.7	MR
63	RGD05011-28-27-B-4-1-B	15.0	2.1	R	4.1	MR	5.4	MR
64	RGD05011-28-27-B-5-1-B	15.8	1.9	R	3.9	MR	5.5	MR
65	RGD05011-28-27-B-8-1-B	13.4	2.7	R	4.8	MR	7.0	MS
66	RGD05011-28-27-B-10-1-B	11.3	3.0	R	5.0	MR	4.7	MR
67	RGD05010-13-50-B-3-1-B	32.4	2.0	R	6.6	MS	4.6	MR
68	RGD05011-28-27-3-B-1-1	16.0	2.6	R	6.0	MR	6.8	MS
69	RGD05011-28-27-3-B-2-1	11.9	2.6	R	7.0	MS	6.9	MS
70	RGD05011-28-27-3-B-3-1	18.8	3.8	MR	6.9	MS	5.6	MR
71	RGD05011-28-27-3-B-4-1	19.2	2.5	R	7.0	MS	4.1	MR
72	RGD05011-28-27-3-B-5-1	16.7	1.9	R	6.6	MS	8.4	MS
73	RGD05011-28-27-3-B-6-1	21.7	-		6.2	MS	6.2	MS
74	RGD05011-28-27-3-B-7-1	27.1	-		5.5	MR	6.9	MS
75	RGD05011-28-27-3-B-8-1	21.1	-		6.4	MS	6.0	MR
76	RGD05011-28-27-3-B-9-1	34.7	2.7	R	7.8	MS	3.7	MR
77	RGD05011-28-27-3-B-10-1	22.9	2.8	R	7.5	MS	6.3	MS

Appendix Table 4 (Continued)

Entries	Pedigree	PPS (%)	Lesion length(cm)					
			TXO85		TXO95		TXO153	
78	RGD05011-28-27-4-B-1-1	14.9	2.1	R	5.9	MR	5.8	MR
79	RGD05011-28-27-4-B-2-1	24.9	2.3	R	6.1	MS	4.8	MR
80	RGD05011-28-27-4-B-3-1	17.5	1.3	R	4.7	MR	3.8	MR
81	RGD05011-28-27-4-B-4-1	28.2	2.3	R	4.9	MR	5.0	MR
82	RGD05011-28-27-4-B-5-1	28.4	3.7	MR	3.7	MR	-	
83	RGD05011-28-27-4-B-6-1	18.6	0.6	R	2.9	MR	4.0	MR
84	RGD05011-28-27-4-B-8-1	16.5	1.0	R	4.2	MR	5.9	MR
85	RGD05011-28-27-4-B-9-1	21.9	1.7	R	4.4	MR	6.2	MS
86	RGD05010-15-72-4-3-2-1	30.6	-		4.8	MR	5.5	MR
87	RGD05010-15-72-4-3-4-1	38.8	-		4.2	MR	6.6	MS
88	RGD05010-15-72-4-3-6-1	26.2	3.3	MR	7.1	MS	6.7	MS
89	RGD05010-15-72-4-3-7-1	16.3	2.9	R	6.9	MS	7.2	MS
90	RGD05010-15-72-4-3-8-1	15.2	2.5	R	5.7	MR	6.5	MS
91	RGD05010-15-72-4-6-1-1	24.7	2.2	R	5.7	MR	6.9	MS
92	RGD05010-15-72-4-6-2-1	35.1	2.4	R	5.0	MR	6.2	MS
93	RGD05010-15-72-4-6-3-1	28.8	3.1	MR	5.6	MR	6.2	MS
94	RGD05010-15-72-4-6-4-1	42.4	3.4	MR	3.2	MR	7.6	MS
95	RGD05010-15-72-4-6-5-1	24.1	2.9	R	4.6	MR	5.3	MR
96	RGD05010-15-72-4-6-7-1	20.2	1.8	R	3.3	MR	4.0	MR
97	RGD05010-15-72-4-6-9-1	12.1	1.1	R	3.9	MR	7.5	MS
98	RGD05010-15-72-4-6-10-1	17.6	-		3.1	MR	7.2	MS
99	RGD05011-18-24-B-1-2-B	26.4	-				10.3	S
100	RGD05011-18-24-B-2-1-B	23.4	9.2	S	17.2	S	13.5	S
101	RGD05011-18-24-B-2-2-B	35.6	8.2	MS	20.9	S	22.1	S
102	RGD05011-18-24-B-3-1-B	38.6	-	MR	13.3	S	10.8	S
103	RGD05011-18-24-B-3-2-B	30.3	-	MR	10.3	S	9.4	S
104	RGD05011-18-24-B-4-2-B	43.6	7.1	MS	11.2	S	16.5	S
105	RGD05011-18-24-B-5-1-B	21.1	8.8	MS	15.3	S	19.4	S
106	RGD05011-18-24-B-5-2-B	22.7	8.2	MS	18.7	S	17.2	S
107	RGD05011-18-24-B-7-1-B	12.2	9.4	S	13.7	S	16.9	S
108	RGD05011-18-24-B-7-2-B	14.3	8.8	MS	17.2	S	17.2	S
109	RGD05011-18-24-B-9-1-B	18.8	10.8	S	19.9	S	15.4	S
110	RGD05011-18-24-B-9-2-B	28.4	9.8	S	19.8	S	14.2	S
111	RGD05011-18-24-B-10-1-B	42.0	10.6	S	18.0	S	15.8	S
112	RGD05011-18-24-B-10-2-B	32.0	9.8	S	17.9	S	18.1	S
113	RGD05010-15-7-4-6-2-1	40.2	9.0	S	18.3	S	16.6	S
114	RGD05010-15-7-4-6-3-1	22.0	5.2	MR	13.2	S	14.5	S
115	RGD05010-15-7-4-6-4-2	29.3	-		-		-	
116	RGD05010-15-7-4-6-6-1	23.8	-		-		-	
117	RGD05010-15-7-4-6-6-2	36.0	7.8	MS	17.1	S	17.6	S
118	RGD05010-15-7-4-6-8-1	30.4	9.4	S	11.5	S	23.2	S
119	RGD05010-15-7-4-6-8-2	25.0	8.0	MS	10.9	S	16.6	S

Appendix Table 4 (Continued)

Entries	Pedigree	PPS (%)	Lesion length(cm)					
			TXO85		TXO95		TXO153	
120	RGD05010-15-7-4-6-9-2	21.2	-		14.9	S	19.1	S
121	RGD05010-15-7-4-6-10-1	21.1	12.2	S	14.4	S	12.0	S
122	RGD05010-15-7-4-6-10-2	38.3	9.0	MS	19.6	S		
123	RGD05010-15-7-8-1-3-1	26.3	8.2	MS	14.4	S	18.3	S
124	RGD05010-15-7-8-1-3-2	24.7	8.4	MS	13.1	S	16.6	S
125	RGD05010-15-7-8-1-5-1	12.3	-		15.1	S	12.5	S
126	RGD05010-15-7-8-1-5-2	23.9	8.6	MS	14.4	S	13.4	S
127	RGD05010-15-7-8-1-6-1	11.2	7.2	MS	10.9	S	12.9	S
128	RGD05010-15-7-8-1-6-2	10.8	7.9	MS	14.1	S	16.0	S
129	RGD05010-15-7-8-1-7-1	12.2	-		15.6	S	19.1	S
130	RGD05010-15-7-8-1-7-2	15.3	8.5	MS	17.6	S	16.4	S
131	RGD05010-15-7-8-1-8-1	15.2	9.1	S	16.4	S	17.3	S
132	RGD05010-15-7-8-1-8-2	29.4	12.3	S	14.1	S	16.1	S
133	RGD05010-15-7-8-1-10-1	27.5	7.3	MS	14.9	S	15.8	S
134	RGD05010-15-7-8-1-10-2	41.9	8.1	MS	13.7	S	12.9	S
135	RGD05010-15-58-1-2-1-1	31.1	10.7	S	16.2	S	14.1	S
136	RGD05010-15-58-1-2-1-2	41.3	9.6	S	17.8	S	17.1	S
137	RGD05010-15-58-1-2-2-1	39.9	9.8	S	16.6	S	17.1	S
138	RGD05010-15-58-1-2-3-1	47.0	7.1	MS	18.7	S	17.9	S
139	RGD05010-15-58-1-2-3-2	54.1	10.8	S	16.4	S	17.3	S
140	RGD05010-15-58-1-2-4-1	40.4	9.2	S	18.8	S	15.9	S
141	RGD05010-15-58-1-2-5-1	51.4	10.8	S	16.1	S	18.9	S
142	RGD05010-15-58-1-2-5-2	58.7	7.8	MS	18.8	S	17.3	S
143	RGD05010-15-58-1-2-7-1	39.2	10.0	S	16.3	S	15.4	S
144	RGD05010-15-58-1-2-7-2	51.0	15.5	S	21.5	S	16.4	S
145	RGD05010-29-7-1-3-8-1	21.3	9.5	S	19.8	S	19.3	S
146	RGD05010-29-7-1-3-8-2	22.2	8.0	MS	19.2	S	21.8	S
147	RGD05010-29-7-1-3-9-1	32.7	9.8	S	17.7	S	16.2	S
148	RGD05010-29-7-1-3-9-2	32.5	11.1	S	18.2	S	16.8	S
149	RGD05010-29-7-1-3-10-1	16.1	6.6	MS	17.4	S	16.2	S
150	RGD05010-29-7-1-4-3-1	31.2	8.9	MS	20.2	S	16.2	S
151	RGD05010-29-7-1-4-6-1	30.6	-		-		-	
152	RGD05010-29-7-1-4-9-1	22.2	-		19.4	S	16.4	S
153	RGD05010-29-7-1-4-9-2	32.4	8.2	MS	16.6	S	15.1	S
154	RGD05010-29-7-1-4-10-1	28.5	9.8	S	13.1	S	16.0	S
155	RGD05010-29-7-1-4-10-2	39.2	-		-		-	
156	RGD05010-13-42-4-5-6-1	66.3	9.3	S	12.3	S	15.8	S
157	RGD05010-13-42-4-5-6-2	22.4	8.0	MS	20.6	S	18.7	S
158	RGD05010-13-42-4-5-6-3	19.6	7.5	MS	14.2	S	14.0	S
159	RGD05010-29-7-2-2-1-1	30.6	7.5	MS	14.0	S	17.0	S
160	RGD05010-29-7-2-2-1-2	25.0	9.2	S	17.6	S	18.4	S
161	RGD05010-29-7-2-2-1-3	23.2	9.5	S	15.2	S	17.3	S

Appendix Table 4 (Continued)

Entries	Pedigree	PPS (%)	Lesion length(cm)					
			TXO85		TXO95		TXO153	
162	RGD05010-29-7-2-2-1-4	25.0	8.1	MS	15.3	S	18.4	S
163	RGD05010-29-7-2-2-1-5	19.8	16.3	S	16.4	S	16.9	S
164	RGD05010-29-7-2-2-1-6	20.0	7.9	MS	15.1	S	16.9	S
165	RGD05010-29-7-2-2-3-1	22.3	8.8	MS	16.7	S	16.3	S
166	RGD05010-29-7-2-2-3-2	30.3	8.2	MS	16.2	S	15.7	S
167	RGD05010-29-7-2-2-3-3	22.5	7.8	MS	16.3	S	20.0	S
168	RGD05010-29-7-2-2-3-4	27.6	9.0	MS	17.5	S	17.5	S
169	RGD05010-13-43-B-4-1-B	12.7	7.9	MS	14.8	S	14.5	S
170	RGD05010-13-43-B-4-2-B	11.3	7.7	MS	16.4	S	19.3	S
171	RGD05010-13-50-B-2-1-B	19.9	10.7	S	16.0	S	17.4	S
172	RGD05010-13-50-B-2-2-B	21.4	9.8	S	22.3	S	15.3	S
173	RGD05010-13-50-B-10-1-B	18.3	11.2	S	18.1	S	17.2	S
174	RGD05010-13-50-B-10-2-B	31.3	10.3	S	-		15.7	S
175	RGD05010-15-7-8-2-1-1	36.1	-		14.5	S	-	
176	RGD05010-15-7-8-2-1-2	31.0	8.2	MS	13.4	S	20.5	S
177	RGD05010-15-7-8-2-3-1	53.3	8.6	MS	14.3	S	16.5	S
178	RGD05010-15-7-8-2-3-2	32.6	7.4	MS	15.9	S	13.7	S
179	RGD05010-15-7-8-2-4-1	21.7	-		-		14.4	S
180	RGD05010-15-7-8-2-4-2	21.5	8.9	MS	15.6	S	13.9	S
181	RGD05010-15-7-8-2-5-1	21.5	10.0	S	27.5	S	14.9	S
182	RGD05010-15-7-8-2-5-2	41.4	8.5	MS	16.8	S	13.3	S
183	RGD05010-15-7-8-2-6-2	19.2	11.6	S	13.1	S	15.7	S
184	RGD05010-15-7-8-2-7-1	45.0	11.6	S	15.7	S	14.0	S
185	RGD05010-15-7-8-2-7-2	33.5	9.2	S	14.6	S	15.7	S
186	RGD05010-15-7-8-2-9-1	23.8	11.6	S	17.0	S	18.1	S
187	RGD05010-15-7-8-2-9-2	24.4	13.4	S	13.2	S	13.3	S
188	RGD05010-15-7-11-4-8-2	32.4	8.1	MS	15.1	S	15.8	S
189	RGD05010-15-7-11-4-10-1	14.1	7.9	MS	-		14.9	S
190	RGD05010-15-7-11-4-10-2	15.6	9.7	S	16.0	S	15.0	S

Appendix Table 5 Data performance of target and agronomic traits of all ideotypes glutinous rice varieties.

Entries	Pedigree	Gene(s)	PPS	Lesion length (cm)						DF	PH	NETP	TGW
			(%)	TXO85		TXO95		TXO153		(d)	(cm)		(g)
	FR13A		97.7										
	RD6		0.0	11.4	S	16.4	S	18.1	S				
	IR1188			1.2	R	1.3	R	2.5	R				
	RGD03040-721-11									102	143	12	26.22
	RD6									94	146	13	24.40
1	RGD05010-30-17-B-2-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	53.5	2.0	R	3.9	MR	5.5	MR	102	151	12	24.69
2	RGD05010-30-49-B-7-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	59.2	5.6	MR	2.1	R	-		102	145	12	21.87
3	RGD05010-13-42-6-6-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	43.4	-		3.0	R	5.4	MR	102	149	14	23.56
4	RGD05010-13-42-6-6-7-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	30.4	1.4	R	2.0	R	2.1	R	102	149	12	23.42
5	RGD05010-13-42-6-6-8-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	41.0	3.1	MR	2.8	R	3.8	MR	102	149	16	-
6	RGD05010-13-42-6-10-6-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	36.0	1.5	R	5.0	MR	2.6	R	102	140	25	23.99
7	RGD05010-13-42-6-10-7-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	32.1	2.4	R	2.8	R	3.9	MR	102	151	12	24.02
8	RGD05010-13-42-6-10-9-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	54.1	1.5	R	1.2	R	1.6	R	100	140	18	23.69
9	RGD05010-13-42-6-11-1-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	57.0	0.7	R	-		-		102	155	10	23.20
10	RGD05010-13-61-12-2-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	34.2	1.3	R	2.2	R	3.0	R	94	136	12	27.67
11	RGD05010-15-58-3-1-1-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	53.9	3.2	MR	1.9	R	7.3	MS	102	143	9	26.40
12	RGD05010-15-58-3-1-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	29.9	1.6	R	4.9	MR	4.1	MR	100	146	10	26.17
13	RGD05010-15-58-3-1-3-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	48.1	3.5	MR	5.2	MR	5.3	MR	100	143	15	25.64
14	RGD05010-15-58-3-1-4-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	55.0	1.6	R	2.5	R	4.5	MR	100	148	13	25.82

Appendix Table 5 (Continued)

Entries	Pedigree	Gene(s)	PPS	Lesion length (cm)						DF	PH	NETP	TGW
			(%)	TXO85		TXO95		TXO153		(d)	(cm)		(g)
15	RGD05010-15-58-3-1-6-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	63.2	3.4	MR	1.5	R	6.2	MS	102	145	15	26.46
16	RGD05010-15-58-3-1-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	44.9	3.3	MR	2.6	R	4.7	MR	100	144	11	25.41
17	RGD05010-15-58-3-10-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	41.6	2.8	R	1.7	R	5.8	MR	100	129	10	24.77
18	RGD05010-15-58-3-10-6-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	53.0	2.8	R	2.7	R	3.7	MR	100	128	14	25.20
19	RGD05010-15-58-3-10-8-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	26.0	2.3	R	1.8	R	5.7	MR	100	136	12	24.02
20	RGD05010-15-58-3-10-9-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	50.1	2.4	R	2.5	R	3.2	MR	97	143	11	23.62
21	RGD05010-15-58-3-10-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	40.4	2.7	R	1.8	R	-		100	150	9	24.78
22	RGD05010-29-7-4-1-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	16.8	2.9	R	4.1	MR	-		104	140	11	25.02
23	RGD05010-29-7-6-3-1-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	30.8	2.0	R	-		4.8	MR	102	145	11	26.71
24	RGD05010-29-7-6-3-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	40.1	2.8	R	3.3	MR	6.0	MR	102	144	11	26.38
25	RGD05010-29-7-6-3-3-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	38.8	2.1	R	2.5	R	6.2	MS	102	141	11	28.98
26	RGD05010-29-7-6-3-4-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	37.2	3.6	MR	3.6	MR	6.2	MS	102	142	11	26.17
27	RGD05010-29-7-6-3-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	30.9	3.7	MR	2.9	R	5.0	MR	102	149	11	27.73
28	RGD05010-29-7-6-3-7-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	42.0	3.9	MR	3.0	R	5.2	MR	102	136	19	26.55
29	RGD05010-29-7-6-5-4-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	44.5	3.5	MR	2.2	R	4.4	MR	102	139	11	26.61
30	RGD05010-29-7-6-5-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	42.3	2.3	R	7.8	MS	6.6	MS	100	142	16	26.11
31	RGD05011-18-25-B-1-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	19.6	2.5	R	4.9	MR	5.3	MR	104	135	25	23.67
32	RGD05011-23-20-B-3-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	9.6	1.8	R	2.7	R	2.0	R	104	139	22	28.62
33	RGD05011-23-20-B-4-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	17.1	2.4	R	3.5	MR	5.0	MR	102	148	18	28.39

Appendix Table 5 (Continued)

Entries	Pedigree	Gene(s)	PPS	Lesion length (cm)						DF	PH	NETP	TGW
			(%)	TXO85		TXO95		TXO153		(d)	(cm)		(g)
34	RGD05011-23-20-B-5-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	14.1	2.3	R	2.8	R	6.4	MS	102	150	16	28.13
35	RGD05011-23-20-B-9-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	11.6	2.1	R	4.6	MR	5.6	MR	104	164	19	29.59
36	RGD05011-23-20-B-10-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	13.5	2.3	R	2.7	R	5.7	MR	106	171	20	26.59
37	RGD05010-13-50-B-3-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	32.4	2.0	R	6.6	MS	4.6	MR	92	132	12	25.28
38	RGD05011-28-27-3-B-3-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	18.8	3.8	MR	6.9	MS	5.6	MR	97	150	11	26.70
39	RGD05011-28-27-3-B-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	16.7	1.9	R	6.6	MS	8.4	MS	97	147	17	25.25
40	RGD05011-28-27-3-B-6-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	21.7	-		6.2	MS	6.2	MS	97	139	12	27.07
41	RGD05011-28-27-3-B-7-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	27.1	-		5.5	MR	6.9	MS	97	145	17	25.60
42	RGD05011-28-27-3-B-8-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	21.1	-		6.4	MS	6.0	MR	94	146	13	25.33
43	RGD05011-28-27-3-B-9-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	34.7	2.7	R	7.8	MS	3.7	MR	97	145	19	27.16
44	RGD05011-28-27-4-B-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	24.9	2.3	R	6.1	MS	4.8	MR	100	138	33	25.98
45	RGD05011-28-27-4-B-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	28.4	3.7	MR	3.7	MR	-		106	149	28	24.42
46	RGD05010-15-72-4-3-4-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	38.8	-		4.2	MR	6.6	MS	97	133	18	23.92
47	RGD05010-15-72-4-3-8-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	15.2	2.5	R	5.7	MR	6.5	MS	94	137	13	25.62
48	RGD05010-15-72-4-6-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	35.1	2.4	R	5.0	MR	6.2	MS	94	144	13	25.08
49	RGD05010-15-72-4-6-3-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	28.8	3.1	MR	5.6	MR	6.2	MS	92	139	13	24.58
50	RGD05010-15-72-4-6-4-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	42.4	3.4	MR	3.2	MR	7.6	MS	100	139	15	25.88
51	RGD05010-15-72-4-6-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	24.1	2.9	R	4.6	MR	5.3	MR	94	136	12	25.06
52	RGD05010-15-72-4-6-7-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{KDIII}$	20.2	1.8	R	3.3	MR	4.0	MR	97	134	12	24.75

Appendix Table 5 (Continued)

Entries	Pedigree	Gene(s)	PPS	Lesion length (cm)						DF	PH	NETP	TGW
			(%)	TXO85		TXO95		TXO153		(d)	(cm)		(g)
53	RGD05011-18-24-B-2-2-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	35.6	8.2	MS	20.9	S	22.1	S	97	134	14	26.34
54	RGD05011-18-24-B-3-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	38.6	-	MR	13.3	S	10.8	S	102	146	12	25.23
55	RGD05011-18-24-B-4-2-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	43.6	7.1	MS	11.2	S	16.5	S	97	142	13	25.91
56	RGD05011-18-24-B-7-2-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	14.3	8.8	MS	17.2	S	17.2	S	97	142	13	26.51
57	RGD05011-18-24-B-10-1-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	42.0	10.6	S	18.0	S	15.8	S	97	144	18	28.50
58	RGD05011-18-24-B-10-2-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	32.0	9.8	S	17.9	S	18.1	S	97	145	16	25.53
59	RGD05010-15-7-4-6-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	40.2	9.0	S	18.3	S	16.6	S	97	144	13	24.28
60	RGD05010-15-7-4-6-6-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	23.8	-		-		-		100	135	13	23.18
61	RGD05010-15-7-4-6-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	21.1	12.2	S	14.4	S	12.0	S	100	140	15	22.92
62	RGD05010-15-7-4-6-10-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	38.3	9.0	MS	19.6	S			100	135	15	23.57
63	RGD05010-15-7-8-1-3-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	26.3	8.2	MS	14.4	S	18.3	S	100	146	9	23.61
64	RGD05010-15-7-8-1-5-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	23.9	8.6	MS	14.4	S	13.4	S	100	147	14	22.67
65	RGD05010-15-7-8-1-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	27.5	7.3	MS	14.9	S	15.8	S	100	137	10	24.74
66	RGD05010-15-7-8-1-10-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	41.9	8.1	MS	13.7	S	12.9	S	100	131	15	22.48
67	RGD05010-15-58-1-2-1-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	31.1	10.7	S	16.2	S	14.1	S	102	146	16	23.02
68	RGD05010-15-58-1-2-1-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	41.3	9.6	S	17.8	S	17.1	S	104	154	8	23.05
69	RGD05010-15-58-1-2-2-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	39.9	9.8	S	16.6	S	17.1	S	102	145	11	22.73
70	RGD05010-15-58-1-2-3-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	54.1	10.8	S	16.4	S	17.3	S	100	151	9	21.93
71	RGD05010-15-58-1-2-4-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	40.4	9.2	S	18.8	S	15.9	S	100	153	9	23.22

Appendix Table 5 (Continued)

Entries	Pedigree	Gene(s)	PPS	Lesion length (cm)						DF	PH	NETP	TGW
			(%)	TXO85		TXO95		TXO153		(d)	(cm)		(g)
72	RGD05010-15-58-1-2-5-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	58.7	7.8	MS	18.8	S	17.3	S	100	152	13	23.30
73	RGD05010-15-58-1-2-7-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	51.0	15.5	S	21.5	S	16.4	S	100	151	14	21.39
74	RGD05010-29-7-1-3-8-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	22.2	8.0	MS	19.2	S	21.8	S	100	141	11	26.45
75	RGD05010-29-7-1-3-9-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	32.5	11.1	S	18.2	S	16.8	S	100	136	16	26.45
76	RGD05010-29-7-1-4-9-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	22.2	-		19.4	S	16.4	S	100	133	10	26.92
77	RGD05010-29-7-1-4-9-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	32.4	8.2	MS	16.6	S	15.1	S	102	156	13	25.07
78	RGD05010-29-7-1-4-10-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	28.5	9.8	S	13.1	S	16.0	S	102	148	12	26.30
79	RGD05010-29-7-1-4-10-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	39.2	-		-		-		100	145	13	27.26
80	RGD05010-29-7-2-2-1-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	30.6	7.5	MS	14.0	S	17.0	S	102	149	12	-
81	RGD05010-29-7-2-2-1-3	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	23.2	9.5	S	15.2	S	17.3	S	102	159	12	23.57
82	RGD05010-29-7-2-2-1-4	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	25.0	8.1	MS	15.3	S	18.4	S	102	169	9	23.90
83	RGD05010-29-7-2-2-3-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	30.3	8.2	MS	16.2	S	15.7	S	102	171	12	23.50
84	RGD05010-29-7-2-2-3-3	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	22.5	7.8	MS	16.3	S	20.0	S	102	158	10	24.80
85	RGD05010-29-7-2-2-3-4	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	27.6	9.0	MS	17.5	S	17.5	S	102	155	11	23.87
86	RGD05010-13-50-B-10-2-B	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	31.3	10.3	S	-		15.7	S	97	124	15	25.22
87	RGD05010-15-7-8-2-1-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	36.1	-		14.5	S	-		97	139	10	24.21
88	RGD05010-15-7-8-2-1-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	31.0	8.2	MS	13.4	S	20.5	S	97	133	10	25.03
89	RGD05010-15-7-8-2-3-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	53.3	8.6	MS	14.3	S	16.5	S	100	141	10	23.27
90	RGD05010-15-7-8-2-3-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	32.6	7.4	MS	15.9	S	13.7	S	100	146	11	23.21

Appendix Table 5 (Continued)

Entries	Pedigree	Gene(s)	PPS	Lesion length (cm)						DF	PH	NETP	TGW
			(%)	TXO85		TXO95		TXO153		(d)	(cm)		(g)
91	RGD05010-15-7-8-2-4-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	21.5	8.9	MS	15.6	S	13.9	S	100	146	10	24.31
92	RGD05010-15-7-8-2-5-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	21.5	10.0	S	27.5	S	14.9	S	100	147	10	24.41
93	RGD05010-15-7-8-2-7-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	45.0	11.6	S	15.7	S	14.0	S	100	143	11	23.29
94	RGD05010-15-7-8-2-7-2	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	33.5	9.2	S	14.6	S	15.7	S	97	145	10	23.90
95	RGD05010-15-7-8-2-9-1	$Wx^{RD6}/Sub1^{KDIII}/Xa21^{RD6}$	23.8	11.6	S	17.0	S	18.1	S	97	139	9	25.27

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