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Development of Disease Resistant Rice Using Whole Genome Sequencing and Standard Breeding Methods

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DEVELOPMENT OF DISEASE RESISTANT RICE USING WHOLE GENOME SEQUENCING AND STANDARD BREEDING METHODS

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ………………………………………………………… …….…ii

LIST OF TABLES…………………………………………………………………...v

LIST OF FIGURES ……………………………………………………………..vi

ABSTRACT ………………………………………………………………………viii

CHAPTER 1 GENERAL INTRODUCTION………………………………………. ……...1
1.1 Research Objectives ……………………………………………………… …..7
1.2 References……………………………………………………………………8

CHAPTER 2. DEVELOPMENT OF CANDIDATE MOLECULAR MARKERS FOR SHEATH BLIGHT RESISTANCE ……………………………………………… ….13
2.1 Introduction …………………………………………………………………...13
2.2 Materials and Methods ………………………………………………… ….16
2.2.1 Plant Material ……………………………………………………. ….16
2.2.2 nsSNPs-based Molecular Marker Design ……………………………17
2.2.3 Sanger sequencing ………………………………………………...17
2.3 Results ……………………………………………………………………..18
2.3.1 nsSNPs-based Molecular Markers …………………………………...18
2.3.2 Sanger Sequencing ……………………………………………… ….27
2.4 Discussion ……………………………………………………………………27
2.5 References ………………………………………………………………. …..34

CHAPTER 3 IDENTIFICATION OF CANDIDATE SNP-BASED MOLECULAR MARKERS FOR SHEATH BLIGHT RESISTANCE BY SELECTIVE GENOTYPING OF RICECAP SB2 MAPPING POPULATION ………………………………………………………..38
3.1 Introduction ………………………………………………………………. …38
3.2 Materials and Methods ……………………………………………………….40
3.3 Results ………………………………………………………………………. …41
3.4 Discussion ……………………………………………………………………47
3.5 References ……………………………………………………………………49

CHAPTER 4 EVALUATION OF RESISTANCE OF DOUBLED-HAPLOID LINES CONTAINING SELECTED SNPs UNDER FIELD AND GREENHOUSE CONDITIONS … 53
4.1 Introduction ……………………………………………………………..53
4.2 Materials and Methods ………………………………………………........56
4.2.1 Plant Material and Population Development …………………….....56
4.2.2 Marker-assisted Selection …………………………………………...58
4.2.3 Mist Chamber Evaluations …………………………………………..59
4.2.4 Field Evaluations ………………………………………………....61
4.2.5 Genotyping of DH Lines and SB Resistant Lines Previously Registered …..61
4.3 Results …………………………………………………………………..62
4.3.1 Marker-Assisted Backcrossing and Doubled-Haploids Production ………...62

4.3.2 Evaluation of SB-DH lines Under Field and Mist Chamber Environments . 64
4.3.3 Genotyping of DH Lines and Other SB Resistant Inbreed Lines …………..67
4.4 Discussion ……………………………………………………………………...... 73
4.5 References ………………………………………………………………….. 77
CHAPTER 5 SUMMARY AND CONCLUSIONS.…………………………………….…81
5.1 Development of nsSNP-based markers………………………………………........81
5.2 Selective genotyping for identification of candidate markers for SB resistance …….81
5.3 Production and evaluation of doubled-haploid lines for SB resistance………………82
APPENDIX A. SEQUENCES CONFIRMING nsSNPs IN CANDIDATE GENES FOR SHEATH BLIGHT RESISTANCE …………………………………………………………..84
APPENDIX B. DESIGN OF ALLELE SPECIFIC SNP-BASED MARKERS FOR FIVE IMPORTANT GENES IN RICE ……………………………………………………………..90
VITA ……………………………………………………………………………………….93

LIST OF TABLES

2.1 Primers flanking nsSNPs in 12 different candidates genes reported by Silva *et al*. (2012) ………………………………………………………………………………...19

2.2 Primer sequences for SNP-based markers located in previously reported QTLs for sheath blight resistance……………………………………………………………………….21

2.3 Comparison of four methods for SNP-based markers including number of primers required, PCR reactions, number of gels, time consumed, and disadvantages…………….30

3.1 Genotypes for extreme phenotypes from the DH SB2 population………...……………….42

3.2 Ranking of 136 genotyped SB markers in SB2 mapping population based on raw P, Hochberg, Bonferroni, False Discovery, and R-squared values…………………………..45

4.1 List of crosses and number of F_1, BC_1F_1, and BC_2F_1 and DH lines produced from each cross……………………………………………………………………………….….57

4.2 Pedigree and SB rating of the 45 selected DH lines ……………………………….…...63

4.3 SB rating (0-9 scale), plant height (PH), and heading date (HD) for 45 DH-lines, susceptible Cocodrie (CCDR), resistant MCR10277, and resistant Oryzica Llanos 5. Data obtained from field evaluations, Summer 2014, 2015, mist chamber (MC) in Fall 2014 ……………………………………………………………………………..… 66

4.4 Genotypes and average SB scores from three environments for DH lines derived from backcrossed lines selected by candidate resistant markers………..……………………….68

4.5 R^2 for the 30 SB markers screened in 45 DH lines compared with R^2 for the same markers in SB population (Chapter 3) ……………………………………………………70

4.6 Genotypes for the 10 most resistant lines from SB2 population (Chapter 3) and 25 SB resistant inbreed lines (Rush *et al*, 2011) plus MCR10277 (MCR) and Cocodrie (CCDR). Green cells indicate resistant alleles and red cells indicate susceptible alleles….72

4.7 Additional markers from chromosomes 1, 3, 5, and 11. Markers from chromosome 8, 9 and12 that were evaluated initially were screened again as controls and to evaluate the genotype of the donor parent LSBR-5 and its assumed origin from the variety Labelle. Susceptible alleles (red) resistant alleles (green)…………………………………73

LIST OF FIGURES

2.1 Distribution of the 136 nsSNP-based markers in the rice genome. Yellow bands indicate the regions covered by the markers which reported as QTLs for SB resistance…………..27

2.2 Schematic representation of the SNP-based markers used by Drenkard *et al*. (2000).……31

2.3 Schematic representation of the modified method by Hayashi *et al.* (2004)………………31

2.4 Schematic representation of the modified Drenkard *et al*. (2000) procedure…….………32

2.5 Schematic representation of the method by Ramkumar *et al*. (2010)……….………….32

4.1 Schematic representation of the backcrossing and doubled-haploid development……......58

4.2 Mist chambers with capacity for 48 per chamber. Dimensions: 1.32 m wide, 2.70 m length, and 1.42 m height…………………………………………………………….60

4.3 Inoculation of rice plants with *R. solani* in mist chamber………………..………………61

4.4 Lesions produce by *R. solani* infection of six DH lines 21 days after inoculation in mist chamber……………………………………..………………………………...65

4.5 SB ratings and chromosomal locations of selected markers for two resistant and susceptible DH lines 21 days after inoculation. ……………………….……………………..69

A1 Comparison of sequences from the locus LOC_Os09g37590 confirming the presence of the nsSNP located in the position 21666818 on chromosome 9………….……………….84

A2 Comparison of sequences from the locus LOC_Os04g58910 confirming the presence of the nsSNP located in the position 34856814 on chromosome 4………..………………84

A3 Comparison of sequences from the locus LOC_Os02g54330 confirming the presence of the nsSNP located in the position 33307448 on chromosome 2………..………………85

A4 Comparison of sequences from the locus LOC_Os01g52880 confirming the presence of the nsSNP located in the position 30406859 on chromosome 1………………………..85

A5 Comparison of sequences from the locus LOC_Os03g37720 confirming the presence of the nsSNP located in the position 20914617 on chromosome 3……….………………86

A6 Comparison of sequences from the locus LOC_Os04g59540 confirming the presence of the nsSNP located in the position 35230058 on chromosome 4………………….……….86

A7 Comparison of sequences from the locus LOC_Os02g02650 confirming the presence of the nsSNP located in the position 975892 on chromosome 2…………….……….…87

A8 Comparison of sequences from the locus LOC_Os06g29700 confirming the presence of the nsSNP located in the position 17044919 on chromosome 6……….……………87

A9 Comparison of sequences from the locus LOC_Os06g28124 confirming the presence of the nsSNP located in the position 15968674 on chromosome 6………..……………88

A10 Comparison of sequences from the locus LOC_Os09g17630 confirming the presence of the nsSNP located in the position 10792494 on chromosome 9………….…………88

A11 Comparison of sequences from the locus LOC_Os02g35210 confirming the presence of the nsSNP located in the position 21160861 on chromosome 2……….………….…89

A12 Comparison of sequences from the locus LOC_Os12g10180 confirming the presence of the nsSNP located in the position 5378630 on chromosome 12…………..…………89

B1 Primer sequences for the sterility genes pms3 (LOC_Os12g36030). Image below shows the polymorphism between the sterile line 08S and the fertile varieties Lemont and Cocodrie ……………..………………………………………………………..90

B2 Primers sequences for the sterility gene pms1 (LOC_Os07g12130). Image below shows the polymorphism between the sterile line 08S and the fertile varieties Lemont and Cocodrie ……………………………………………………………..….. .90

B3 Primer sequences for the imidazolinone herbicide resistance gene ALS (BGIOSGA008288) Image below shows the polymorphism between the imidazolinone resistant varieties CL131 and CL152, and the imidazolinone susceptible varieties Cocodrie and Jupiter ………………………………………………..91

B4 Primer sequences for the gene waxy (OS06G0133000). Image below shows the polymorphism between the high amylose content varieties IR8 and Cocodrie, and the low amylose content varieties Jupiter and 69S ………………………………………91

B5 Primer sequences for resistance to herbicide (Provicia). Image below shows the polymorphism between the resistant mutant, an heterozygous an the susceptible wild and the susceptible variety Jupiter ……………………………………………………..92

ABSTRACT

Cultivated rice is the most important staple crop in the world, but diseases cause substantial losses in grain yield and quality. Sheath blight disease caused by the fungus *Rhizoctonia solani* is the second most important disease in rice. Most U.S varieties are tropical japonica type, but known sources of resistance in this subspecies are rare. Silva *et al*. (2012) identified candidate SNP associated with resistance to sheath blight by whole genome sequencing. The objectives of this study were to develop SNP-based markers from the information reported by Silva *et al*. (2012), to validate the markers by selective genotyping in the RiceCAP SB2 mapping population, and to develop and evaluate breeding lines resistant to sheath blight by marker-assisted selection coupled with backcrossing, anther culture, and field assessment methods. A total of 136 SNP-based markers were developed and screened in extreme resistant and susceptible phenotypic groups from the RiceCAP SB2 mapping population. SNPs in reported genomic regions for sheath blight resistance were identified including eight markers located on chromosomes 6, 8, 9, and 12 that were used in a marker-assisted backcrossing strategy by crossing seven different resistant lines to four susceptible U.S. commercial varieties. A total of 45 doubled-haploid (DH) lines were developed from 28 BC_2F_1 individuals containing different combinations of selected SNPs. Field evaluation of selected DH lines was carried out in 2014 and 2015. Additional evaluations were performed using a mist chamber to reproduce optimal conditions for disease development. Fourteen DH lines containing different combinations of resistant alleles from chromosomes 2, 6, 8, 9 and 12 showed high levels of resistance after inoculation with *R. solani*. Results from this research suggest that development of disease resistant rice can be successfully accomplished using whole genome sequencing information combined with standard breeding approaches.

CHAPTER 1. GENERAL INTRODUCTION

Rice is the most important staple crop in the world. Approximately 20% of the calories consumed by half of the world's population are derived from rice (www.IRRI.org). Cultivated rice (*Oryza sativa* L.) originated in China ~ 100,000 years ago and was domesticated ~10,000 years ago (Wei *et al.*, 2012). In the United States, rice was first cultivated in South Carolina in the mid-seventeenth century (Dethloff, 2003). After the Civil War in the nineteenth century, acreage rapidly expanded to the southern Mississippi river valley states, becoming one of the most important crops in the region. Although rice was grown since 1718, the crop was not really economically important in the region until 1880. Currently, the U.S. rice industry is one of the major exporters of the grain in the world, ranked fifth according to the 2015 USDA report "Grain: World Markets and Trade". Because the world population has reached ~ 7 billion and will continue to rise, a substantial increase in food production is a priority. According to FAO, food production must be increased by 70% in 2050 when the global population will be about 9.1 billion people (FAO, 2009). However, recent trends in crop production show that by 2050 yield increases will be insufficient to satisfy demand. In the case of rice, with the actual annual rates of yield increase, production is expected to rise only by 42% using the same cultivated area (Ray *et al.*, 2013). Therefore, more research and new methodologies to resolve food production challenges are needed.

One of the main issues affecting rice productivity is biotic stress. Except for the bacterium *Burkholderia glumae* Kurita and Tabei, causing panicle blight, fungi produce the most significant yield loss in the U.S. (Groth *et al.*, 2014). Rice sheath blight disease, caused by the basidiomycete fungus *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris*), is the second most important rice disease in the world (Lee and Rush, 1983). Reduction in productivity

due to sheath blight can reach 50% under southeast U.S. field conditions (Lee and Rush, 1983). Fungicides are used to reduce losses, but prolonged chemical use can cause negative effects to the environment, create adverse consequences for human and animal health, and increase production costs (Slaton *et al*., 2003). Therefore, more efforts are needed to develop resistant varieties by breeding.

Although genotypes immune to sheath blight have yet to be identified, cultivars and wild rice accessions with high levels of "partial resistance" have been reported (Srinivasachary *et al*., 2011). However, none of these accessions is well adapted to southeast U.S conditions. Efforts using traditional breeding methods have resulted in the release of partially resistant germplasm (Rush *et al*., 2011). This germplasm could be used as a source of resistance to produce new adapted resistant varieties. However, when populations have been created using these lines as parents, the resistance is rarely maintained in succeeding generations (J. Oard unpublished results) due to the polygenic or quantitative nature of the inheritance (Li *et al*., 1995). Moreover, favorable alleles segregate in the progeny derived from new crosses, and gene combinations required to produce commercial levels of resistance are lost. To recover the resistance in the progeny, it is necessary to identify genomic regions involved in resistance to create molecular markers for an efficient marker-assisted selection strategy (Lande and Thompsom, 1990).

To identify genomic regions associated with resistance to sheath blight, quantitative trait loci (QTLs) have been identified using different mapping populations and strategies (Kunihiro *et* al., 2002; Pinson *et al*., 2005; Liu *et al*., 2009; Channamallikarjuna *et al*., 2010; Jia *et al*., 2012). Results from these studies have shown that resistance to sheath blight is quantitative where each of several regions in the genome explains a relatively small portion of the observed phenotypic variation (Srinivasachary, 2011). Some reported QTLs have been detected across different

studies. For example, a QTL on chromosome 9 was reported independently by Pinson *et al*. (2005) Tan *et al*. (2005), Zuo *et al*. (2008), Liu *et al*. (2009), and Nelson *et al*. (2012). Most QTLs have been identified using SSRs (Simple Sequence Repeats) or RFLP (Restriction Fragment Length Polymorphism) markers. Although these markers may be associated with a QTL region controlling the trait, their level of precision has not been sufficient, due primarily to low marker polymorphism to identify causal gene(s) for sheath blight resistance.

Another strategy used to detect QTLs is selective genotyping. Selecting only individuals with extreme phenotypes from a population for genotyping, and including the phenotypic information of the remaining individuals in the analysis, it is possible to detect QTLs efficiently reducing time and cost (Darvasi and Soller, 1992; Lee *et al*., 2014). For example, selective genotyping has demonstrated to be effective in QTL detection associated with rheumatoid arthritis in humans (Xing and Xing, 2009), detection of QTLs in cattle affecting milk yield and quality (Bagnato *et al*., 2008), and for submergence tolerance (Nandi *et al.*, 1997) and drought resistance (Subashri *et al*., 2009) in rice.

Doubled haploids (DH) generated by *in vitro* culture methods have been used to assist in QTL detection in different cereal species as wheat (Zhang *et al*., 2009) and rice (Ma *et al*., 2009). To study the genetic basis of resistance to *R. solani,* the SB2 DH mapping population was developed by the LSU AgCenter as part of the USDA-funded RiceCAP research effort (Chu *et al*., 2006). Breeding line MCR10277 (GSOR 200327) was used as the resistant donor, and LSU AgCenter long-grain variety Cocodrie (Linscombe *et al.*, 2000) was used as the susceptible recipient. Phenotypic evaluation was carried out for this population under replicated greenhouse and field trials (Louisiana, Arkansas, 2006, 2007; Nelson *et al*., 2012). A QTL detected at the bottom of chromosome 9 by previous research (Pinson *et al*., 2005; Tan *et al*., 2005; Zou *et al*.,

2000; Liu *et al*., 2009, Zuo *et al*., 2014) was also found in this study along with additional QTLs on chromosomes 2, 3, 5, 6, 8 and 12.

SNPs (Single Nucleotide Polymorphisms) represent an abundant source of variation in the rice genome, so their use as molecular markers should result in greater coverage and more accurate analysis vs. SSRs or RFLPs (Feltus *et al.*, 2004). The completed genome sequencing of rice (Goff *et al*., 2002; International Rice Genome Sequencing Project, 2005) provides an important reference in the search for SNPs and other variants. Moreover, the advent of next-generation sequencing has been useful for study of multiple traits in rice along with different SNP databases that are now available to the scientific community (Huang *et al*., 2010; Zhao *et al*., 2011). Recently, coordinated efforts from the international rice research community produced whole genome sequences of 3000 rice accessions representing indica, tropical japonica, and temperate japonica subspecies (The 3,000 Rice Genome Project, 2014). Data from ~20 million SNPs from the 3000 accessions are available for public access at http://www.oryzasnp.org/iric-portal/ (Alexandrov *et al.*, 2015). Next-generation sequencing technology permits whole genome sequencing in a rapid and cost effective manner that allows identification of SNPs that may be associated with certain traits including those involved in disease resistance. Whole genome sequencing (WGS) has demonstrated efficiency in the identification of variants associated with different Mendelian disorders in humans. Rios *et al*. (2010) demonstrated the efficacy of WGS for identification of genes involved in severe hypercholesterolemia. Lupski *et al*. (2010) used WGS to discover genes responsible for Charcot–Marie–Tooth disease and used it for diagnosis, and WGS has demonstrated utility in diagnosis for cancer in humans (Foley *et al*., 2015).

The new sequencing technologies have also been applied to rapid and cost effective development of molecular markers in plants. For instance, in the legume lupin (*Lupinus angustifolius* L), sequencing of resistant and susceptible varieties resulted in development of molecular markers for breeding against anthracnose disease (Yang *et al*., 2012), and phomosis stem blight disease (Yang *et al*., 2013). Terauchi *et al*. (2012) proposed the application of WGS for rice breeding using mutant populations.

Successful identification of variants associated with various human disorders by WGS prompted Silva *et al*. (2012) to evaluate the potential of genome technology to identify non-synonymous (ns) SNPs associated with resistance to sheath blight by whole genome sequencing of 13 rice inbred lines. They evaluated variants between resistant and susceptible lines, identifying 333 nsSNPs (non-synonymous SNPs), of which ~ 200 were present in genes belonging to protein families involved in resistance, such as the nucleotide binding site-leucine rich repeat (NBS-LRR) gene class. Moreover, many of the selected SNPs were located in regions where QTLs have been reported in previous studies (Zeng *et al*., 2011; Nelson *et al*., 2012).

It is clear that advances in genomics can assist in the improvement and breeding of different crops such as rice. This is particularly true where transfer of a single gene or major QTL that expresses the desired trait can be carried out with a straightforward marker-assisted selection (MAS) breeding scheme such as that reported by Neeraja *et al*. (2007). This study demonstrated that transfer of the rice *Sub*1 gene, aided by use of SSR markers, can result in development of submergence tolerance in elite Asian cultivars. For some rice diseases, certain gene combinations may be needed to maintain durable resistance, as in the case of bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* and blast disease caused by *Magnaporthe oryzae* (Singh *et al*., 2001; Jiang *et al*., 2012). In these instances, resistance was achieved by

combining (pyramiding) different resistance factors through crossing into one individual or population. However, using MAS for quantitative traits is more complex due to the high number of minor QTLs that may or may not be detected depending on the environment, different statistical approaches used for analysis, and the particular parents used to develop different mapping populations (St. Clair, 2010). Steele *et al*. (2006) introduced five QTLs for drought tolerance from the rice variety Azucena to the susceptible variety Kalinga III. Molecular markers were used in three backcrosses to select plants containing QTLs for further crossing between them to combine the five QTLs. However, in contrast with the positive effects generated by the introgressed QTLs, the selected lines showed disadvantages in other agronomic traits. Therefore, more research is needed to utilize MAS in applied breeding of quantitative traits.

Recent publications propose genomic selection (GS) as a more effective alternative to traditional MAS for quantitative traits (Xu *et al*., 2014; Wang *et al*., 2014), since GS is based not only on a few selected markers, but on the entire genome from individuals in the population. However, the successful models applied initially in animal breeding have not been readily adapted to crops, and the methodologies and statistical approaches are still being studied to implement GS in plants for successful and effective outcomes (Jonas and de Koning, 2013). For wheat, a GS model was proposed to breed for quantitative disease resistance (Rutkoski *et al*., 2014). However, these results have not been validated in other wheat populations. Recently, Spindel *et al*. (2015) reported the first GS study in rice showing promising results for prediction of grain yield, plant height and flowering time. The research was carried out in collaboration between Cornell University, the International Rice Research Institute (IRRI), the International Center for Tropical Agriculture (CIAT), and the US Department of Agriculture (USDA), supported by the Bill and Mellinda Gates Foundation. This study required the development of

training and test populations, sequencing and bioinformatics platforms and sophisticated statistical analyses typically not accessible for most small breeding programs. Therefore, alternative strategies for breeding for quantitative traits using molecular markers that fit the capacity of small research programs should be investigated.

Identification of variants in genes controlling desired agronomic traits is a useful tool in marker-assisted breeding. For example, identification of the ALS herbicide resistant gene in rice allowed development of allele-specific markers based on variation of a single nucleotide (Kadaru *et al*., 2008). Based on the SNPs reported by Silva *et al*. (2012) in candidate genes for resistance to sheath blight, it may be possible to develop allele-specific markers that facilitate mapping of exact positions of genes involved in quantitative resistance. These efforts should increase our understanding of the genetic basis of resistance and help to create efficient and low-cost strategies for marker-assisted breeding of sheath blight and other complex diseases.

1.1 Research Objectives

1. Development of candidate molecular markers for sheath blight resistance.
2. Identification of candidate SNP-based molecular markers for sheath blight resistance by selective genotyping of SB2 mapping population.
3. Evaluation of resistance levels of doubled-haploid lines containing selected nsSNPs under field and greenhouse conditions.

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CHAPTER 2. DEVELOPMENT OF CANDIDATE MOLECULAR MARKERS FOR SHEATH BLIGHT RESISTANCE

2.1 Introduction

Sheath blight disease, caused by *Rhizoctonia solani* is a major challenge for high grain yield and grain quality for rice growing regions in the southern U.S. Most rice grown in Louisiana such as the variety Cocodrie (PI 606331), are tropical japonica type known to be more susceptible to *R. solani* than most *indic*a-derived varieties such as Teqing (PI 536047) or Jasmine 85 (PI 595927) (Jia *et al*., 2012). Azoxystrobin and flutolanil fungicides are widely used as control agents in commercial U.S. rice production, but these chemicals are expensive and sometimes, when applied at the wrong stage, are not cost-effective (Groth and Bond, 2007). Moreover, these fungicides may result in environmentally toxic conditions (Gustafsson *et al*., 2010). Therefore, development of resistant varieties adapted to the Louisiana growing conditions is needed.

Some researchers have reported increased resistance to sheath blight disease by genetic engineering approaches. Strategies such as constitutive expression of rice chitinase genes (Shah *et al*., 2013; Lin *et al*., 1995), overexpression of polygalacturonase inhibiting proteins (Wang *et al*., 2015), oxalate oxidase genes (Molla *et al*., 2013), and transformation using fungal chitinase genes (Shah *et al*., 2009), have demonstrated enhanced resistance both in *indica* and *japonica*-derived varieties. Nevertheless, acceptance of genetically modified rice by farmers and consumers is still uncertain. Thus, an acceptable option is to transfer resistance from the most resistant varieties to Louisiana-adapted *japonica* varieties using traditional breeding methods supported by molecular markers. However, it has been demonstrated in several studies that resistance to sheath blight is a quantitative trait conferred by multiple loci, each with small

effects (Li *et al*., 1995; Srinivasachary *et al*., 2011; Yadav *et al*., 2015). Various quantitative trait loci (QTL) have been identified on all 12 chromosomes across different mapping populations (Srinivasachary *et al*., 2011) and association mapping panels (Jia *et al*., 2012; Yan *et al*., 2014). These studies have identified several candidate chromosomal regions and markers for development of resistance against *R. solani*. However, progress in the use of QTL-based markers to introgress resistance into susceptible lines has been slow. For example, three recent studies reported the pyramiding of QTLs which resulted in only modest reductions in disease severity. Chen *et al*. (2014) pyramided two QTLs identified in the variety TeQuing, *qSB-9TQ* and *qSB-7TQ*, located on chromosome 9 and 7, respectively. Also, Zuo *et al*. (2014) combined sheath blight QTL (*qSB-9*^{TQ}) and a tiller angle QTL (*TAC1*^{TQ}) located on chromosome 9 and 1, respectively.

Identification and application of additional markers to increase resistance are needed. Genomic information generated by Next Generation Sequencing (NGS) of 33 elite U.S. and South American rice varieties was recently made available to the public (Duitama *et al*., 2015). Silva *et al*. (2012) previously used sequence data from 13 of the 33 varieties to identify non-synonymous SNPs (nsSNPs) present in three resistant varieties that were absent in three susceptible varieties. This approach resulted in the identification of 333 candidate nsSNPs, the majority of which were located in various QTLs previously reported in the literature. Approximately 50 QTLs related to sheath blight resistance have been reported for all 12 chromosomes (Yadav *et al*., 2015). For instance, Pinson *et al*. (2005) and Channamallikarjuna *et al*. (2009) have reported QTLs on Chromosome 1, Sharma *et al.* (2009), Liu *et al.* (2009), Pinson *et al.* (2005), Zou *et al.* (2000), Kunihiro *et al.* (2002) and Nelson *et al.* (2012) reported QTLs for chromosome 2, Nelson *et al.* (2012), Channamallikarjuna *et al*. (2009), Liu *et al.* (2009) reported

QTLs on chromosome 3, Pinson *et al.* (2005), Sharma *et al.* (2009), Li *et al*. (1995), Xie *et al*. (2008) reported QTLs on chromosome 4, Nelson *et al.* (2011), Che *et al.* (2003) and Ha *et al*. (2002) reported QTLs on chromosome 5, Nelson *et al.* (2011), Liu *et al.* (2009), Pinson *et al.* (2005), and Xie *et al*. (2008) reported QTLs in chromosome 6, Yadav *et al*. (2015), Liu *et al*. (2009), and Kunihiro *et al.* (2002) reported QTLs on chromosome 7, Pinson *et al*. (2005), Nelson *et al.* (2011), Channamallikarjuna *et al*. (2009), and Xie *et al*. (2008) reported QTLs on chromosome 8, Nelson *et al.* (2012), Pinson *et al.* (2005), Tan *et al*. (2005), Liu *et al.* (2009), Sharma *et al.* (2009) and Tagushi-Shiobara *et al*. (2013) reported QTLs on chromosome 9, Pinson *et al.* (2005), and Sharma *et al*. (2009) reported QTLs on chromosome 10, Channamallikarjuna *et al*. (2009), Zou *et al.* (2000), and Xie *et al*. (2008) reported QTLs on chromosome 11, and Nelson *et al.* (2011) and Li *et al.* (1995) reported QTLs on chromosome 12.

Molecular markers such as RAPDs, RFLPs, AFLPs and SSRs have been widely used in genetics research. However, RAPDs and RFLPs have problems of repeatability. RAPDs, RFLPs and AFLPs are time consuming methods. For all the four types of markers, polymorphism and genome coverage is an issue especially between closed related individuals (Mammadov *et al*., 2012). Therefore, use of SNP-based molecular markers is a valuable alternative to greater abundance with high levels of polymorphism to obtain millions of data points in less time and lower cost vs other approaches (Kumar *et al*., 2012). It has been demonstrated that single point mutation in genes may change structure and function of proteins. In rice, single mutations have been associated with important traits. For instance, a single mutation in acetolactate synthase (*ALS*) gene confers resistance to imidazolinone herbicides (Tan *et al*., 2005), grain length is affected by a single variation in the QTL GS3 (Fan *et al*., 2006), amylose producing postranscriptional splicing in mRNA (Hirano *et al*., 1998; Issiki et al., 1998), gel consistency of

cooked rice due to single mutations in the *ALK* gene (Gao *et al*., 2011). Thus, developing molecular markers based on these variations permits rapid identification and effective selection of desired traits. Drenkard *et al*. (2000) developed a procedure based on single nucleotide variation for marker-assisted selection in *Arabidopsis*. A variation in the 3' end of the forward primer based on a specific SNP allows the identification of allele-specific variants by absence/presence of PCR products. This type of marker can be readily scored in agarose gels and does not require sophisticated and expensive equipment.

The specific objective of this research was to design and carry out initial characterization of polymorphic molecular markers based on the SNP variation information generated for Silva *et al* (2012), and using the methodology proposed by Drenkard *et al*. (2000), to identify sheath blight resistant alleles located in chromosomal regions containing reported QTLs for resistance.

2.2 Materials and Methods

2.2.1 Plant Material

Plant material used for the optimization of markers includes the susceptible varieties Cocodrie (PI 606331), Cypress (PI 561734), and Lemont (PI 475833) and the resistant variety Jasmine 85 (PI 595927), the breeding line MCR10277 (GSOR 200327), and the variety Teqing (PI 536047). These six lines were the selected susceptible and resistant material for identification of SNPs described by Silva *et al*. (2012). To validate 12 of these SNPs identified by NGS sequencing (Silva et al. 2012), fragments of candidates genes containing the variants were sequenced by the Sanger method using the variety Cocodrie, widely cultivated in Louisiana, but highly susceptible to sheath blight, and Araure-3 (F. Correa, personal communication), a traditional variety cultivated in Venezuela with resistance to sheath blight.

2.2.2 nsSNPs-based Molecular Marker Design

From the identified nsSNPs in candidate genes for sheath blight resistance reported by Silva *et al*. (2012), primers for ~ 200 genes were designed to identify specific resistance or susceptibility alleles. Forward primers for each nsSNP were designed to contain a mismatch pair at the 3' end of one allele (the resistant allele), and a 3' end matching with the susceptible allele. Additional primers were designed to mismatch the 3' end of the susceptible allele and match the resistant allele to obtain co-dominant markers. In both cases, additional mismatches were included in two more nucleotides before the last 3' to increase specificity (Drenkard *et al*., 2000). Reverse primers in both cases were designed by standard methods, matching all nucleotides in the sequence. Reverse primers were designed to amplify fragments of different sizes between ~ 50 to 100 bp for resistant and susceptible alleles to allow reliable scoring of markers on 1.5% agarose gels. This size-based polymorphism was developed to visualize both PCR products in the same gel, to detect resistant and susceptible alleles, and to identify heterozygous markers, thus reducing cost and increasing efficiency. Primers were designed using the SNAP program (http://ausubellab.mgh.harvard.edu/) based on the variants found between the resistant and susceptible groups from the RiceCAP project (www.ricecap.uark.edu/) and the reference Nipponbare sequence posted at the Gramene website (http://www.gramene.org). PCR conditions were optimized based on conditions described by Kadaru *et al*., (2008).

2.2.3 Sanger Sequencing

Primers flanking the SNPs in 12 of the ~200 genes identified by Silva *et al*. (2012) were designed to amplify a ~500-600 PCR product to confirm presence of the nucleotide variant between susceptible (Cocodrie) and resistant (Araure 3) varieties by Sanger sequencing. DNA samples were taken from 3 different plants per variety to be sequenced. Design of the primers

was carried out using the tool primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Primer sequences for the selected genes and PCR product sizes are showed in Table 2.1. PCR reactions (20 µL) consisted of: 3 µL 4ng/µL DNA template, 2 µL 10X PCR buffer (containing 1mM MgCl2), 1.6 µL of 10mM dNTPs mix, 0.4 µL each of 20 µM forward and reverse primers, and 0.16 µL of 5U/µL of Taq polymerase, and 14.44 µL of dH2O. PCR reactions were run on a BioRad ICycler. The PCR program consisted of the following steps: 95° C, 3 min, 95° C, 30 sec; 62° C, 30 sec; 72° C, 30 sec, repeat 30X previous three steps, 72° C, 5 min. Amplified PCR products were visualized by running on a 2% agarose gel and stained with ethidium bromide. Specific bands were removed from the gel and purified using QIAquick Gel Extraction Kit (Qiagen) and sequenced using an Applied Biosystems 3130XL sequencer in the Genomics Core Facility at Pennington Biomedical Research Center (Baton Rouge, LA). The sequences were extracted and edited using Sequence Scanner Software 2 (Applied Biosystems). The Clustal Omega software (EMBL-EBI: http://www.ebi.ac.uk/Tools/msa/clustalo/) was used for sequence alignment to identify SNPs between varieties.

2.3 Results

2.3.1 nsSNPs-based Molecular Markers

In total 136 SNP-based molecular markers were designed and validated to discriminate between the resistant lines MCR10277, Teqing and Jasmine 85 and the susceptible varieties Cocodrie, Cypress and Lemont. A majority of the markers (134) were based on the nsSNPs located in candidate genes for disease resistance reported by Silva *et al*. (2012). Thus, these markers represented near total coverage of the regions reported by Silva *et al*. (2012)

Table 2.1 Primers flanking nsSNPs in 12 different candidates genes reported by Silva *et al*. (2012)

Gene	Function	Primer Forward	Primer Reverse	Product Size
LOC_Os02g35210	resistance protein, putative	TTTTGGGACGGAGAGTGTAA	GGCTCATCTTTGAGGTGGAC	537
LOC_Os09g17630	receptor-like protein kinase 2, putative, expressed	TCCCAATTCATGCTACATTCA	CATGTACAGAGCTCATGAAACACTT	474
LOC_Os12g10180	NBS-LRR type disease resistance protein Rps1-k-2, putative, expressed	TCTTTGGTTGGAGTGCCTTC	TGCGTGAGTCCTTGTAGTGC	558
LOC_Os09g37590	OsFBDUF47 - F-box and DUF domain containing protein, expressed	TACCACATGGGACGAAGACA	CGAGACTGCAGAATCGTCAA	553
LOC_Os04g58910	receptor protein kinase TMK1 precursor, putative, expressed	CCGGCAATCTCAACTTCAAT	GGTGCCGTACCTTGGTTAGA	612
LOC_Os02g54330	OsFBDUF14 - F-box and DUF domain containing protein	GCTCGTATCAGGACGAGGAC	GAAGAATAGACGCCCATCCA	575
LOC_Os01g52880	leucine-rich repeat family protein, putative, expressed	ACCATCTCCCAGAACGGATT	ACATCCTTGTCAGCCTGGTC	571
LOC_Os03g37720	NBS-LRR type disease resistance protein Rps1-k-1, putative	CAGATGATCCATGGTGTTGC	AGGCATGGCTACATGGAAAC	634
LOC_Os04g59540	phosphatidylinositol-4-phosphate 5-Kinase, putative, expressed	GCAGGGGATCATTACTGGAA	GGCTCTCCTCACAGACAACC	537
LOC_Os02g02650	THION21 - Plant thionin family protein precursor, putative	CTTAGGCGCTGCTCATAGGT	GGTTCTTGGTGCAACCATCT	596
LOC_Os06g29700	OsFBD11 - F-box and FBD domain containing protein, expressed	TGCAGTGCGAGACCACTATC	ACAAGTGGTTCAGGCTTTCG	574
LOC_Os06g28124	glycosyltransferase, putative, expressed	AATGGAGCATCCGAGATCAG	CCGTTGCATACTGGACTCCT	500

and are closely/moderately linked (≤ 1 cM) to the ~200 candidate genes detected in that study. Two additional markers were designed for nsSNPs not identified by Silva *et al*. (2012) in LOC_Os09g37230 and LOC_Os09g37240 both on chromosome 9 as reported in a Lemont x Teqing mapping population by Zuo *et al.* (2014). These nsSNPs were identified by comparing DNA sequences of Lemont and Teqing at http://oryzasnp.org/iric-portal/. All the primers that showed polymorphism between susceptible (Cocodrie, Lemont and Cypress) and resistant (MCR10277, Teqing and Jasmine 85) varieties are presented in Table 2.2. These new molecular markers are: 10 SNPs on chromosome 1 located in the QTLs reported by Pinson *et al*. (2005) and Channamallikarjuna *et al*. (2009), 26 on Chromosome 2 located within QTLs reported by Sharma *et al.* (2009), Liu *et al.* (2009), Pinson *et al.* (2005), Zou *et al.* (2000), Kunihiro *et al.* (2002) and Nelson *et al.* (2012), 10 on chromosome 3 located within QTLs reported by Nelson *et al.* (2011), Channamallikarjuna *et al*. (2009), Liu *et al.* (2009), 17 on chromosome 4 located within QTLs reported by Pinson *et al.* (2005), Sharma *et al.* (2009), Li *et al.* (1995), Xie *et al.* (2008), six on chromosome 5 located with QTLs reported by Nelson *et al.* (2011), Che *et al.* (2003) and Ha *et al*. (2002); 15 on chromosome 6 located within QTLs reported by Nelson *et al.* (2011), Liu *et al.* (2009), Pinson *et al.* (2005), and Xie *et al*. (2008), 11 on chromosome 8 located within QTLs reported by Pinson *et al*. (2005), Nelson *et al.* (2012), Channamallikarjuna *et al.* (2009), and Xie *et al*. (2008), 23 on chromosome 9 located within QTLs reported by Nelson *et al.* (2012), Pinson *et al.* (2005), Liu *et al.* (2009), Sharma *et al.* (2009), Tagushi-Shiobara *et al.* (2013) and Zuo *et al*. (2014), six on chromosome 11 located within QTLs reported by Channamallikarjuna *et al*. (2009), Zou *et al.* (2000), and Xie *et al*. (2008), and finally, 13 on chromosome 12 located within QTLs reported by Nelson *et al.* (2012) and Li *et al.* (1995). Chromosomal locations of the 136 markers are shown in Figure 2.1.

Table 2.2 Primer sequences for SNP-based markers located in previously reported QTLs for sheath blight resistance.

Gene	Function	SNP Position	Ref. Allele	Var. Allele	Ref. AA	Var. AA	Primer Ref Forward (Susceptible Allele)	Primer Ref Reverse (Susceptible Allele)	Primer Alt Forward (Resistant Allele)	Primer Alt Reverse (Resistant Allele)
LOC_Os01g13300	B3 DNA binding domain containing protein, expressed	7420797	A	C	I	L	TGCTCGGGGAGGCCGAGGAT	AACTTCAACATCGTCGTGGACGGC	TGCTCGGGGAGGCCGAGAGG	GGTTTTCAGACTCAGAGATGAGCTTTGACG
LOC_Os01g52330	NB-ARC domain containing protein, expressed	30075242	G	A	E	K	CATGGAGAAGTATTTACGCACCCGGTC	GTTTCTCCACTAGAACAAGGAAAATTGCATCC	AATCATGGAGAAGTATTTACGCACCCGATT	GTTTCTCCACTAGAACAAGGAAAATTGCATCC
LOC_Os01g52880	leucine-rich repeat family protein, putative, expressed	30406859	G	A	C	Y	GGCCTCCGAAACCTCCAGCG	CCATCCGGTCATCCAGGCACA	CCGGCCTCCGAAACCTCCACTA	CCATCCGGTCATCCAGGCACA
LOC_Os01g53420	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed	30689063	T	C	R	G	AGCAAGGGAAGCAGATCAGGCAGA	CTGACTTGTTACGACCGGAAAAATCCAAATA	GAGCAAGGGAAGCAGATCAGGGATG	CTGACTTGTTACGACCGGAAAAATCCAAATA
LOC_Os01g54350	protein kinase domain containing protein, expressed	31276574	A	G	V	A	CGAGCAGGAGCCCTCCTCACGT	TAGGATGGTTCAGGCGGGACAGTG	CCGAGCAGGAGCCCTCCTCATTC	TAGGATGGTTCAGGCGGGACAGTG
LOC_Os01g54515	peptide transporter PTR2, putative, expressed	31358087	A	G	K	E	GACAGGACCATCGGCACGATCA	GTAGAAGAACTCGAGGAGCCCGATGAAG	CAGGACCATCGGCACGCTCG	GTAGAAGAACTCGAGGAGCCCGATGAAG
LOC_Os01g55050	protein of unknown function DUF1421 domain containing protein, expressed	31652519	A	C	T	P	AGTGCAACCGCAGCAATCTCACA	GTCCGAAAGAGCCCTGGCTTGG	CAGTGCAACCGCAGCAATCTAAGC	TAGCCATATGCAGTGTTGTAGCCATGTGA
LOC_Os01g56040	Zinc finger A20 and AN1 domain-containing stress-associated protein 3	32266156	C	A	S	Y	TTGGCCTACTTATCTGGTGTCGCCTG	TCAGCATGCAATTTTATTTGTCACGCTTACT	TTGGCCTACTTATCTGGTGTCGCGAA	TCAGCATGCAATTTTATTTGTCACGCTTACT
LOC_Os01g57230	BTBN1 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain, expressed	33065796	T	G	E	A	TGCTGCAGCGGATGATCAACGA	GTCCTTCCAGTTGGCGAGCACG	GCTGCAGCGGATGATCAGGGC	GCGGTGATGCAGCGGGAGAC
LOC_Os01g57900	PPR repeat domain containing protein, putative, expressed	33479245	G	A	R	C	GATTTAGAAAGCTATACAGAGGTTGCACCGC	CATCAATAAGAGCATCATAACTGCCAGCATC	TTGATTTAGAAAGCTATACAGAGGTTGCACGGT	CATCAATAAGAGCATCATAACTGCCAGCATC
LOC_Os02g02650	THION21 - Plant thionin family protein precursor	975892	T	G	N	T	GATGACTGCAGCCCCAACACGAA	TGATATGTTGTTGACAGACATCAGCGTGAAT	GGATGACTGCAGCCCCAACAACAC	TGATATGTTGTTGACAGACATCAGCGTGAAT
LOC_Os02g09820	zinc finger, C3HC4 type domain containing protein, expressed	5065045	A	G	T	A	TGGGGACTGTATCTGGCCATGGTTTA	ATCCAGCACACCAATAATTACATTAGCATTGAAA	GGGACTGTATCTGGCCATGGTTCG	ATCCAGCACACCAATAATTACATTAGCATTGAAA
LOC_Os02g10120	lipoxygenase, putative, expressed	5277344	T	G	K	N	CGGCCGACGGTGATGAGGAATAAA	GGGCGTGGAGAACGGCTTGAG	GCCGACGGTGATGAGGAAGACC	GGGCGTGGAGAACGGCTTGAG
LOC_Os02g10900	NB-ARC domain containing protein, expressed	5786160	G	A	A	V	AGGGGTGCATGGGACCTGGATC	ATATGGTTCCCCTCCAGTGCAGGC	GGGGTGCATGGGACCTGGTGT	ATATGGTTCCCCTCCAGTGCAGGC
LOC_Os02g11820	GTPase-activating protein, putative, expressed	6114451	T	A	V	E	AGCCTGCTAGTGCACAGCCCGT	AAAAATGAAGAATGTGACTGCCCCATCA	CAGCCTGCTAGTGCACAGCCACA	AAAGAAATGGAAGGTACACAGATCGACTTCTGATA
LOC_Os02g34490	Leucine Rich Repeat family protein, expressed	20661950	G	C	W	S	TTGAAGCTCTGAGAGGGAGGTGATCTCTC	ATGTGTATCGGCTCCCATATTGCTTGTTATC	AGCTCTGAGAGGGAGGTGATCTGCG	ATGTGTATCGGCTCCCATATTGCTTGTTATC
LOC_Os02g34850	histone-lysine N-methyltransferase ASHH2, putative, expressed	20899450	T	A	V	D	CAAGTACTTCCTCAGCATCCTCCTGCA	GAGATAATTTGTGCAAATGAAACGTATCCTTCAGT	GCACCAAGTACTTCCTCAGCATCCTCCTTAT	CCACATGCAAAAGACCCCAATTCAAG
LOC_Os02g35210	resistance protein, putative	21160861	G	A	D	N	GGACTCTGTCCTCAGCAAGCTCATCG	CATCTCCTTGGCAATTTGGTAGTGATTCC	ATGGACTCTGTCCTCAGCAAGCTCAACA	CATCTCCTTGGCAATTTGGTAGTGATTCC
LOC_Os02g39590	GDSL-like lipase/acylhydrolase, putative	23887432	T	A	K	M	CATGGCCGAGCTTCTGTACCACCA	AGCTGAAGTGTTCATTCTTCAGTAGTACATTGTGG	TGGCCGAGCTTCTGTACCGCAT	TATGGTTGTAAGCCTGGTGCTCCTGTGTAAT
LOC_Os02g42412	F-box/LRR-repeat protein 2, putative, expressed	25509520	G	T	L	M	TGAGGTCTCTTCATCGTCATTGGTATCATTAAATC	GATTGAAATCCATTTGCATCCATATCCTGA	CTGAGGTCTCTTCATCGTCATTGGTATCATTATAAA	GATTGAAATCCATTTGCATCCATATCCTGA
LOC_Os02g43460	required to maintain repression 1, putative	26228789	C	G	A	G	TCGGCGTACGGTGGGGATTGTAC	TTTGGTCCATTGTTTCTGACGCATTGT	GGCGTACGGTGGGGATTGAGG	TTCCTCCTCTGAGCAATCTTTACATTTCTCTTG
LOC_Os02g44730	tetracycline transporter protein, putative, expressed	27099654	T	A	M	K	GGGCAATCTGGTGCAAGTGGGAT	TACAACAAGCTGGGCTGGCTTCTATGAC	AGGGCAATCTGGTGCAAGTGAGGA	TGGCATTAACTCATGTCTGAAGCTCCG
LOC_Os02g45160	aluminum-activated malate transporter, putative, expressed	27387949	A	G	S	P	TGACGGTGCCGGAGGGCTAGT	TCGAAGACCACGACAACCGTCATG	GACGGTGCCGGAGGGCTCAC	AAACACGGTGAGGAACACAGCAAACTG
LOC_Os02g45980	ZR1 protein, putative, expressed	28014024	C	T	T	M	CGTTGGAAGTTCTTCTGCAATTCTGATGAG	TGCCCAATTGTGCAGCATGATTTTT	TGGAAGTTCTTCTGCAATTCTGAGGCA	GACCTATCTCCATTTGACAGATTTACCGGC
LOC_Os02g48210	lectin-like protein kinase, putative, expressed	29516606	T	C	N	S	GAACGACACCAGAAAGCCCTCGGT	CACACCCGGGATGCTCCTGAAAT	CGACACCAGAAAGCCCTCGCC	CACACCCGGGATGCTCCTGAAAT

Table 2.2 Continued

Gene	Function	SNP Position	Ref. Allele	Var. Allele	Ref. AA	Var. AA	Primer Ref Forward (Susceptible Allele)	Primer Ref Reverse (Susceptible Allele)	Primer Alt Forward (Resistant Allele)	Primer Alt Reverse (Resistant Allele)
LOC_Os02g49986	MYB family transcription factor, putative, expressed	30540363	A	C	H	Q	GAAGCAGCAGCAGCAGCACCAT	AGCCAGCCCAGTCAATGTCAAGAATG	CCTGAAGCAGCAGCAGCAGCAGTAG	TGATCAAAGCCAACGTCAGAGCATTG
LOC_Os02g51900	cytokinin-O-glucosyltransferase 2, putative, expressed	31782340	G	C	H	D	CAGGCCCGACCTCGTCGCTA	TTGCTTTCGCTCTCATATCCTTGCTTTTC	CAGGCCCGACCTCGTCGCTG	TTGCTTTCGCTCTCATATCCTTGCTTTTC
LOC_Os02g52060	peptide transporter like protein, putative	31859549	G	A	T	M	AGCGCGCGATGGTGCGCTAC	CACAACCATGTGGTCGTACACCGG	AGCGCGCGATGGTGCGCTAT	CGTGGTGAGGCAGGCTGTTGC
LOC_Os02g53970	OsSub24 - Putative Subtilisin homologue, expressed	33040089	T	C	S	G	AAACCAGATAGCGATTTTCACAAGGGAGA	CGAGTTGCTTGACCCTGCCGAC	AAACCAGATAGCGATTTTCACAAGGGATG	ATTTCCAGGTGGCCACGACGG
LOC_Os02g54330	OsFBDUF14 - F-box and DUF domain containing protein	33307448	C	G	R	T	GGATACAGGTGACGAGGAATCCCCTTC	CACGCCATGATCAACCTCCGGT	TACAGGTGACGAGGAATCCCCACG	CACGCCATGATCAACCTCCGGT
LOC_Os02g54500	WD40-like, putative, expressed	33367587	A	G	T	A	CACCCTGCTGCACAGGGAATTACA	CAACTATCCACCAGAAAATTAGGCAGTAACAGCTAT	CCTGCTGCACAGGGAATTCGG	CAACTATCCACCAGAAAATTAGGCAGTAACAGCTAT
LOC_Os02g55180	ubiquitin carboxyl-terminal hydrolase domain containing protein, expressed	33794880	C	T	T	I	CTGTTAACTTGCCCATGAAAACAGAGCATAC	AGTGAGCTTCAGATGCCTGGCCATT	TGTTAACTTGCCCATGAAAACAGAGCAACT	AGTGAGCTTCAGATGCCTGGCCATT
LOC_Os02g56380	OsWAK21 - OsWAK receptor-like cytoplasmic kinase OsWAK-RLCK	34511349	C	A	A	S	GATGACAAGCTCAACGCCAAAGTCG	CATGAGGAGGTCTGCAATCTCTGTTGC	TTGATGACAAGCTCAACGCCAAAGTCT	CATGAGGAGGTCTGCAATCTCTGTTGC
LOC_Os02g56480	PB1 domain containing protein, expressed	34568863	T	C	H	R	GGAGGACGCTGGTGTAGTAGATGGGGT	TCCTACCGGTGCCGGGAAGGTATA	GGAGGACGCTGGTGTAGTAGATGGCTC	AGTACTACTTGCCCAAGTACCAGGAGAAGCC
LOC_Os02g57960	Leucine Rich Repeat family protein, expressed	35493288	G	a	R	C	GCCACATGCAAACGGCTAGAGTATCTTC	AAAGTAATTACCTTTTCGCTCAAGAAATTGAGGTG	GCCACATGCAAACGGCTAGAGTATGTGT	AAAGTAATTACCTTTTCGCTCAAGAAATTGAGGTG
LOC_Os02g58540	RING-H2 finger protein, putative, expressed	35778055	G	A	A	V	ATCTGCGTCGCCGGCCTGTC	CGTGGGTCCCCAGCCACGTA	ATCTGCGTCGCCGGCCTTGT	GGCCGGGGAGAGGGAGGAATAAT
LOC_Os03g30130	phospholipase C, putative, expressed	17206912	C	T	R	K	TGTGTGGATGCCAGGATGTCCG	ATAAACACTGCAGAAATTTGTTAAAGGCCAAGTC	CTGTGTGGATGCCAGGATGTCGA	ATAAACACTGCAGAAATTTGTTAAAGGCCAAGTC
LOC_Os03g37720	NBS-LRR type disease resistance protein Rps1-k-1, putative	20914617	A	G	L	P	GCCAAGAAGATGGGCGGCGT	AACCAAATCTTCAAAGAACTTGCTTCCAATGT	CTAGCCAAGAAGATGGGCGGACC	AACCAAATCTTCAAAGAACTTGCTTCCAATGT
LOC_Os03g39150	protein kinase domain containing protein	21745084	A	C	M	L	CGACCTCAAGCCGGAGAACGTGA	GACGCCTCGCTGGTTCGGTG	CGACCTCAAGCCGGAGAACATGC	GACGCCTCGCTGGTTCGGTG
LOC_Os03g40250	Leucine Rich Repeat family protein, expressed	22369241	C	T	R	K	AGCGGCAAGGCGATCAAGCG	TATCGCTGGCTCAGGTTGTACACCG	CGAGCGGCAAGGCGATCAAGTA	TATCGCTGGCTCAGGTTGTACACCG
LOC_Os03g43684	KIP1, putative, expressed	24429583	T	C	I	V	CAGTCGATTGTTGGCATTGCAAAACTCT	GATGGACAATCCAGACATGGCTCCA	TCGATTGTTGGCATTGCAAAACGAC	GATGGACAATCCAGACATGGCTCCA
LOC_Os03g53220	U5 small nuclear ribonucleoprotein 200 kDa helicase, putative	30523344	A	T	Q	L	TTCTCCTTCCTGCTCATAGATTGCTCGA	CGACGATGTCCTGCAGCTGGG	GAGATTCTCCTTCCTGCTCATAGATTGCTCTT	TCAAGAACTTGGAAAGCCATGTCGATG
LOC_Os03g56400	pentatricopeptide, putative, expressed	32144849	T	C	Q	R	CAGGGCTATCAGATCATTGCTAGGGCA	CCCTTCATTGACAAAACCGGCATG	AGGGCTATCAGATCATTGCTAGGGCG	CCATGCAGGCGTAATGGCTCATCT
LOC_Os03g57160	zinc ion binding protein, putative, expressed	32586703	C	T	G	D	ACAGAAATATTGAAATGGATGATGTCTCGGG	TTTCAGCCCTTTGCAAAGAAACATCAATT	CACAGAAATATTGAAATGGATGATGTCTGGGA	GTATGACCCATCGTCTCTGTTAGCTCAGTATTAGG
LOC_Os03g58390	zinc finger, C3HC4 type domain containing protein, expressed	33260375	A	G	T	A	TGGAGGCACTCGGAGATGGTCGT	AGGCGGCGTAGGGGAAGGAGA	GGAGGCACTCGGAGATGGTGCC	TACTACTGCAGTTGCTCACACACCCACAC
LOC_Os03g63110	prefoldin, putative, expressed	35667086	A	G	V	A	GGAGTCTGAAGATCACGAGAGGACGGT	GCACACACTAGTACATCAACCCAACACCAC	GGAGTCTGAAGATCACGAGAGGAAGCC	TGAACTGATACCAGATTCATAACCATTTCACCATAT
LOC_Os04g05030	serine-rich 25 kDa antigen protein, putative, expressed	2441294	G	A	D	N	GAGCTGCCAAAGAAAGGTGCCG	TTGCACAGCAACAGAAGATGACAAATCTG	CGTGAGCTGCCAAAGAAAGGTGCTA	TTGCACAGCAACAGAAGATGACAAATCTG
LOC_Os04g10460	amidase, putative, expressed	5684447	C	G	H	D	GCGATTGTCACCCCCAACTCCC	ATCAATCCGTAAGAAAGGATTAAGTATGCGTCC	ATGCGATTGTCACCCCCAACTGTG	ATCAATCCGTAAGAAAGGATTAAGTATGCGTCC
LOC_Os04g11640	methyl-CpG binding domain containing protein	6377725	A	G	Q	R	ACACGGCGCTTGAGGCAGTGTA	CCTTTGCTTGAATGGTCACATAAGACAACC	CACGGCGCTTGAGGCAGTGAG	CCTTTGCTTGAATGGTCACATAAGACAACC
LOC_Os04g11970	O-methyltransferase, putative, expressed	6560546	G	A	A	T	CGTGGTTCAGGGATGGACGAAATG	TATCCTCAAACATGTCGCCAGCGATAA	CGTGGTTCAGGGATGGACGAAAGA	TATCCTCAAACATGTCGCCAGCGATAA
LOC_Os04g15650	Leucine Rich Repeat family protein, expressed	8505140	G	T	G	C	CAGTGGCATGCCCAGTATGCCTG	GGTTTTCGTGGTCCAATGTTGAGCATAG	GCAGTGGCATGCCCAGTATGCTCT	GGTTTTCGTGGTCCAATGTTGAGCATAG
LOC_Os04g20680	wall-associated receptor kinase 3 precursor, putative, expressed	11560624	A	G	Y	H	AAGAAATACTACATGAGGATAACATGGAACTGCTGT	CATAGAAGCCAAATGTAGCTCAGACAAAAACTTTC	AAGAAATACTACATGAGGATAACATGGAACTGCTTC	CATAGAAGCCAAATGTAGCTCAGACAAAAACTTTC

Table 2.2 Continued

Gene	Function	SNP Position	Ref. Allele	Var. Allele	Ref. AA	Var. AA	Primer Ref Forward (Susceptible Allele)	Primer Ref Reverse (Susceptible Allele)	Primer Alt Forward (Resistant Allele)	Primer Alt Reverse (Resistant Allele)
LOC_Os04g21890	disease resistance protein RPM1, putative, expressed	12387967	A	C	Q	P	AGGATATTATGAAGTGGTGTTGTGGTCGACA	ATAAGTAATCATGCGCTAGCTCTTCCATCGTAGT	AAAGGATATTATGAAGTGGTGTTGTGGTCTTCC	ATAAGTAATCATGCGCTAGCTCTTCCATCGTAGT
LOC_Os04g23620	D-mannose binding lectin family protein	13514379	A	C	S	A	CTGCGCCCCACCCTGCCTAT	GCAATGACTGCCCAGGGACCAAT	CTGCGCCCCACCCTGCCTTG	GCAATGACTGCCCAGGGACCAAT
LOC_Os04g23890	AGC_PVPK_like_kin82y.10 - ACG kinases include homologs to PKA, PKG	13640560	T	C	Q	R	CAGGGAGGCCATCAGGGAGGA	TTGAAGCTCCCCCTGCACTCACA	CAGGGAGGCCATCAGGGAGGG	TTGAAGCTCCCCCTGCACTCACA
LOC_Os04g55760	OsWAK55 - OsWAK receptor-like protein kinase	33008803	G	A	E	K	CATCCATCACGGATGTAAGGATTGCCTAC	CCAGGTCACGTCTCTGATAGACCGAAATT	CCATCACGGATGTAAGGATTGCGTTT	CCAGGTCACGTCTCTGATAGACCGAAATT
LOC_Os04g56250	OsFBX152 - F-box domain containing protein, expressed	33349688	G	A	T	I	AAGGATTCCATCTTCTCCCACTCCAATG	GATTCAAGACGAGGACGGCGAGTG	CAAGGATTCCATCTTCTCCCACTCCAATA	GATTCAAGACGAGGACGGCGAGTG
LOC_Os04g57670	pentatricopeptide, putative, expressed	34150615	C	T	V	M	CTTCAGCTGCACAAAGGCATGGAC	GTCAGTGCTGAGTGGTTGTGGACGAG	CCTTCAGCTGCACAAAGGCATGAAT	GCACTGCAATGTGCGGTATGGCT
LOC_Os04g58720	anthranilate phosphoribosyltransferase, putative, expressed	34732078	A	G	T	A	TTGAAGCGTACGTCTACAACATCAACAGATACA	TGGTGCTGATGCTGCACCTCCTT	AGCGTACGTCTACAACATCAACAGATCGG	TGGTGCTGATGCTGCACCTCCTT
LOC_Os04g58820	ATOFP18/OFP18, putative, expressed	34804587	G	A	R	K	GGAGGAGATGCTCGGCTGGTACCTTAG	AACCCAATCAAACACACACACCAGTCAA	GAGATGCTCGGCTGGTACCGGAA	AACCCAATCAAACACACACACCAGTCAA
LOC_Os04g58910	receptor protein kinase TMK1 precursor, putative, expressed	34856814	T	C	N	D	TGGGTCGAACTACTGTTGCCATCATTTTT	GTGTGAAGGTGAATGTGACCGGCA	GGGTCGAACTACTGTTGCCATCATTCTC	GTGTGAAGGTGAATGTGACCGGCA
LOC_Os04g59060	heat shock protein DnaJ, putative, expressed	34943898	T	G	I	L	GCAGCCTTGAGGACATTGCGGAT	ATTTCTGTATGGCACTACAAGTAGGTGCTCCA	GCAGCCTTGAGGACATTGCCGAG	ATTTCTGTATGGCACTACAAGTAGGTGCTCCA
LOC_Os04g59540	phosphatidylinositol-4-phosphate 5- Kinase, putative, expressed	35230058	C	G	Q	E	CCGAAAGGATCAGGCTGTGACATTTTATG	TCATTACTGGAATACCATGATGGGGATCAC	CGAAAGGATCAGGCTGTGACATTTTCTC	TCATTACTGGAATACCATGATGGGGATCAC
LOC_Os05g37040	MYB family transcription factor, putative	21585027	A	G	S	P	TCGAGAGTGCACTCGTGGCATTGT	AAACAGTTCATCGATAATAGCAAGGGAAAATGAC	TGTTCGAGAGTGCACTCGTGGCATTAC	AAACAGTTCATCGATAATAGCAAGGGAAAATGAC
LOC_Os05g39760	VHS and GAT domain containing protein, expressed	23293209	G	A	S	N	TGTTTGGTGATTTGATTGATGTGAAGCG	TATACACATACTTCACAAACCAAAGCGGTGAAG	TTGTTTGGTGATTTGATTGATGTGAAGCA	TATACACATACTTCACAAACCAAAGCGGTGAAG
LOC_Os05g40790	CCR4-NOT transcription factor, putative, expressed	23860975	A	G	D	G	CATATGGACTCTGGACAAATCAGCGGA	GTTCACTTTAGTGCCATTTTCAACCTTACCAAA	TGGACTCTGGACAAATCAGCGGG	GTTCACTTTAGTGCCATTTTCAACCTTACCAAA
LOC_Os05g41130	OsFBX168 - F-box domain containing protein, expressed	24027934	C	A	G	C	GGTCGACCGCAAGCCTGTCG	GATATGGAGCGTATTTGAGCTTCATGTTGC	CAGGTCGACCGCAAGCCTGTCT	GATATGGAGCGTATTTGAGCTTCATGTTGC
LOC_Os05g41290	disease resistance RPP13-like protein 1, putative, expressed	24122910	T	G	N	K	TCATTCCACCCATTAGTTTCCCCACA	TGTCTACAATGATCCAAGAGTAAAGGAGTACTTCCA	AGTCATTCCACCCATTAGTTTCCCAAGC	TGTCTACAATGATCCAAGAGTAAAGGAGTACTTCCA
LOC_Os05g50660	PX domain containing protein, putative, expressed	28979361	A	G	N	D	GAGATCATCTTTATTGGTGGGGGATGATTAAA	ACAGAATATACAGCAAACATGCCAGATCCACT	CATCTTTATTGGTGGGGGATGATTTCG	ACAGAATATACAGCAAACATGCCAGATCCACT
LOC_Os06g13040	WD domain, G-beta repeat domain containing protein, expressed	7208678	C	A	G	C	CACCTCGTCCTGCACGTCCGA	CCAAGTTGATGTACGGCTCAGGGTTCT	CCACCTCGTCCTGCACGTCCTT	ATTTTATGCTTAACTAGCTTGATGTGATCATGCAAA
LOC_Os06g15170	3-ketoacyl-CoA synthase, putative, expressed	8598272	T	C	I	V	CAGGCTGCAGTTGACGACGAGGAT	CTCGAGCACGCGAGGCAGGT	CAGGCTGCAGTTGACGACGAGTCC	CGGCCACCGTGTACCTCGTGAT
LOC_Os06g19110	cadmium tolerance factor, putative, expressed	10871554	T	C	N	D	GGAGAACAAATGAAGCGGAAGTCACGA	CTACCAGGAGTCCAATCATGTCGAGAACA	GGAGAACAAATGAAGCGGAAGTCACCG	CTACCAGGAGTCCAATCATGTCGAGAACA
LOC_Os06g22020	cytochrome P450, putative	12751175	A	G	M	V	TGCCCCACATCTCCCTCCGAG	CGCCGCCTCAGTGATCCTGG	CTTGCCCCACATCTCCCTCCGTA	CGCCGCCTCAGTGATCCTGG
LOC_Os06g22460	disease resistance protein RPM1, putative, expressed	13056419	T	C	S	G	CATGGTGGTCAGTGTGTGGGGAATTA	CATCTTCAAATGCATCTTTGCTATCGAACC	TGGTGGTCAGTGTGTGGGGAATTG	CATCTTCAAATGCATCTTTGCTATCGAACC
LOC_Os06g23530	pre-mRNA-splicing factor ATP-dependent RNA helicase, putative, expressed	13725000	A	C	D	E	AGTGGCGTGATATCAGGAACGACGAT	ACTTGATTGTCACGAACAGCTTCGATAAGC	AGTGGCGTGATATCAGGAACGAGGTG	GTGTAACCTGGGTTGTTTTCCCTGAACC
LOC_Os06g28124	glycosyltransferase, putative, expressed	15968674	T	C	K	R	CATCGTCGACTTCAACCAGGACAGCTA	ACCACCCGGGAGAACTCCTCGA	TCGTCGACTTCAACCAGGACAGAGG	ACCACCCGGGAGAACTCCTCGA
LOC_Os06g28670	polygalacturonase, putative, expressed	16329889	G	T	V	F	CACGGTCACGTCCGACACCAC	ACCATACAGAACAGCGCCAGGTTCC	GCACGGTCACGTCCGACACAAA	AGAGCACGAACGTGGCGGTGA
LOC_Os06g29700	OsFBD11 - F-box and FBD domain containing protein, expressed	17044919	A	G	H	R	CGTCTTCAGCTGATCGTCCGCA	GGCTTTCGCATGACAAATAACACAGCTAAATA	CGTCTTCAGCTGATCGTCCGCG	GGCTTTCGCATGACAAATAACACAGCTAAATA
LOC_Os06g29844	MATE efflux family protein, putative, expressed	17195755	T	G	S	A	GCCCAGGAGATGATACTGCCGGTC	CTCACATATTTTCTCTGTCCAAGACTCTTCCTGTT	GCCCAGGAGATGATACTGCCGTGA	CAAGCAAATCCGTGTCGCAATTTTG

Table 2.2 Continued

Gene	Function	SNP Position	Ref. Allele	Var. Allele	Ref. AA	Var. AA	Primer Ref Forward (Susceptible Allele)	Primer Ref Reverse (Susceptible Allele)	Primer Alt Forward (Resistant Allele)	Primer Alt Reverse (Resistant Allele)
LOC_Os06g31070	PROLM24 - Prolamin precursor, expressed	18071409	T	A	K	N	CAAGAACCGCAATGACCAGTAGCACCT	GGGAGCAGTCACGCAGGCTACAAC	CAAGAACCGCAATGACCAGTAGCAAGA	AACCCGTGCAATGAGTTCGTGAGG
LOC_Os06g32350	THION12 - Plant thionin family protein precursor	18827854	A	C	N	K	GGACACAACGGTGACAGTCTGAGCTACA	CAATATTTCTGGCTCAATCATTCTTGCCTG	CACAACGGTGACAGTCTGAGCTGCC	CAATATTTCTGGCTCAATCATTCTTGCCTG
LOC_Os06g35850	lectin protein kinase family protein, putative, expressed	20916895	G	C	R	T	GCAAAGCAGTGTCATGACTAGATTAAGAGGGAG	TCCAGGATTTTGACCACCATGGACAT	GCAAAGCAGTGTCATGACTAGATTAAGAGGCTC	TCCAGGATTTTGACCACCATGGACAT
LOC_Os06g37500	cytokinin dehydrogenase precursor, putative	22193618	C	T	V	I	GATCACCGAGAGCCGACATGTGAAC	TGGCGTCCTCACTAGTTACGATGTTTCTTC	GATCACCGAGAGCCGACATGTCATT	TCGTCACTAGCTTCCTCTCTACTGTCCCCTA
LOC_Os06g44820	PPR repeat domain containing protein, putative	27075561	G	A	E	K	ATCAAAGATGCTCAGAGGATCCTACCCG	CAGACACTTAAGCTTTGGCGTAGTAGCTTATCTACC	GATCAAAGATGCTCAGAGGATCCTACCCA	CAGACACTTAAGCTTTGGCGTAGTAGCTTATCTACC
LOC_Os08g10560	histone-like transcription factor and archaeal histone family protein	6216207	A	T	I	N	TGGCGCGATTCGAGACCACA	CTACCACTTCGACCTGAGCGGCAC	ATGGCGCGATTCGAGACCCGT	CCGGGGCTCAACGACAAGCTC
LOC_Os08g12800	glucan endo-1,3-beta-glucosidase precursor, putative, expressed	7587176	T	C	V	A	CATGGCTGCCATCCTCGCAGT	AACATGCTATTTTCATAAAAAGAGATCATGGGACTC	ATGGCTGCCATCCTCGCCCC	AACATGCTATTTTCATAAAAAGAGATCATGGGACTC
LOC_Os08g13870	S-locus lectin protein kinase family protein, putative	8282993	C	G	G	A	CGTTCAGATGATGGAAGGAAGTGGGA	TTCCATCCTTTGGATGCATAGTTCGATTACT	GTCGTTCAGATGATGGAAGGAAGTGAGC	TTCCATCCTTTGGATGCATAGTTCGATTACT
LOC_Os08g19694	NB-ARC domain containing protein, expressed	11786501	A	C	D	E	AACAGGATACTGTCCCTGAGCTTCGGT	ATGGGAAGTAGTCACCATCATGCAGCTC	CAGGATACTGTCCCTGAGCTTCGCG	ATGGGAAGTAGTCACCATCATGCAGCTC
LOC_Os08g20020	octicosapeptide/Phox/Bem1p, putative, expressed	11987684	C	T	G	R	CAGCCGGAAGCAAATGGAGTCG	ATCTAATGTCCTCCCCAAAGACCAGCTTC	CCAGCCGGAAGCAAATGGAGTCA	TTGTCCTTATATTTAATAAGCAACGCTTTCAACGA
LOC_Os08g30850	YDG/SRA domain containing protein, expressed	19042526	G	A	G	D	AGTATGCCTCATCAGCCACCGCAC	GCAAATAGTGGTGAATTGCTGGGGTG	AGAGTATGCCTCATCAGCCACCTCGT	CAAATGTGCATCAAATCTTCAGCAAATCTTC
LOC_Os08g30910	YDG/SRA domain containing protein, expressed	19085103	T	C	I	T	TGGCGGGCAAGGACAACCTTCT	AGGTCAACTCTTCGAATCTTCAATGAACCC	TGGCGGGCAAGGACAACCTTTC	GCTCAACTCTGTACATAAATTCGTCACCAACTTC
LOC_Os08g35310	O-methyltransferase, putative	22277158	C	A	G	C	CAGAGCTCTGGTGCCACACTTTCG	CCAATACCATCAACAAGGAGGCGAGATAC	GCAGAGCTCTGGTGCCACACTTTCT	GGTTGATTGGCATGTGTGCTCAGTGT
LOC_Os08g36320	decarboxylase, putative, expressed	22876630	T	C	D	G	CCTTGGTGGCGTTCTCGCTCAAGTA	CAGTACGCGAAGATCTCCCTCTCGG	CTTGGTGGCGTTCTCGCTCAAGAG	CAGTACGCGAAGATCTCCCTCTCGG
LOC_Os08g36760	remorin C-terminal domain containing protein, putative, expressed	23212262	A	C	Y	S	GAACCATACTCATCAGTTTCGTCATCGTGATA	GCAATGACGGAGACGACTGAACTGC	CCATACTCATCAGTTTCGTCATCGTCAGC	AGTACTCCAAGACTAAGCACAAGAAGTACGGGTAAA
LOC_Os08g42930	disease resistance protein RGA1, putative, expressed	27130720	A	G	H	R	AAGTTTGGCCTGATGGAGCGCA	CAAGTCGCCTTCGCAACAACTCAATT	AAGTTTGGCCTGATGGAGCCCG	CAAGTCGCCTTCGCAACAACTCAATT
LOC_Os09g16540	protein kinase, putative, expressed	10153331	A	G	R	G	CTGACAAAGACCGACATCAGCGAGA	GACATGGTTGCCATCCTTCTCCCA	GCTGACAAAGACCGACATCAGCGAAG	GACATGGTTGCCATCCTTCTCCCA
LOC_Os09g17600	membrane protein, putative, expressed	10766714	G	A	R	K	GAGTTGGCATCTCAAACATGATTCATCG	CAATGTGAATATGTGATACATGCTGTACTGGCTT	GGAGTTGGCATCTCAAACATGATTCAAAA	CAATGTGAATATGTGATACATGCTGTACTGGCTT
LOC_Os09g17630	receptor-like protein kinase 2, putative, expressed	10792494	T	C	I	T	TTGAGCCTGCTTGAGGGGCAGAT	TCACTATCCTAAAGATTTAAGCAGAGTGTCCATCTT	TTGAGCCTGCTTGAGGGGCAAAC	TCACTATCCTAAAGATTTAAGCAGAGTGTCCATCTT
LOC_Os09g25620	CPuORF8 - conserved peptide uORFcontaining transcript, expressed	15385777	A	G	L	S	CATCACCGCATCGCAGCTTCAT	ACGGCGGGACCATAAATGCCAT	CATCACCGCATCGCAGCTTGTC	ACGGCGGGACCATAAATGCCAT
LOC_Os09g25890	trehalose-6-phosphate synthase, putative, expressed	15532799	T	A	F	I	CATGTCGACGCCGACGGAGAGTAA	CGCGTCGGGTTTTTCCTCCACT	CATGTCGACGCCGACGGAGAGTAT	GCGCGTCGTCGAGGTGCTCT
LOC_Os09g26300	hypro1, putative, expressed	15891490	A	G	V	A	GGTGGACGCGCAGCTGGTTGT	ACACGACGTAGCCCATCCCGTG	GTGGACGCGCAGCTGGTGAC	CGTGCGTGTCGTTGTACCGCA
LOC_Os09g27570	OsFBA3 - F-box and FBA domain containing protein, expressed	16748987	A	G	F	S	CACAGCAACAAATACAAGGTGGCTAGATGTTT	GCTAGCGTCTCACTGAAAAAGCAAGCAC	CACAGCAACAAATACAAGGTGGCTAGATGTTC	GCTAGCGTCTCACTGAAAAAGCAAGCAC
LOC_Os09g32020	ubiquitin fusion degradation protein, putative, expressed	19117102	C	T	V	I	AACAACAAGGAGTTCCTCATCGACATGG	CTAACTCCTGATGCTGCTGTCTCCTGATTC	CCTACAACAACAAGGAGTTCCTCATCGACATTA	CTAACTCCTGATGCTGCTGTCTCCTGATTC
LOC_Os09g32860	OsFBX336 - F-box domain containing protein, expressed	19591594	C	T	L	F	GGTTGATACACCAATATTGCCTAGCAAAGTCC	TAGAAACCGGTGATCTCCACACTCCG	ATGGTTGATACACCAATATTGCCTAGCAAAGTCT	TAGAAACCGGTGATCTCCACACTCCG
LOC_Os09g33710	Os9bglu33 - beta-glucosidase homologue, expressed	19913544	T	G	N	H	CCCAGCATTTGGGACACCTTCTTCA	TCATATCTGAACTTATCACTGACCTTGTAATGGTGA	CCAGCATTTGGGACACCTTCAACC	CCATGTCATACATAAGCTTTACATCCTCCTGAAA
LOC_Os09g34180	Formin, putative, expressed	20182171	T	G	L	R	GAACGAAAGAACAGCGATTGGATCTTAGCT	TTTATCACGAGATTCAAGCATTCAGCATGAT	CGAAAGAACAGCGATTGGATCTTCGTG	TTTATCACGAGATTCAAGCATTCAGCATGAT

Table 2.2 Continue

Gene	Function	SNP Position	Ref. Allele	Var. Allele	Ref. AA	Var. AA	Primer Ref Forward (Susceptible Allele)	Primer Ref Reverse (Susceptible Allele)	Primer Alt Forward (Resistant Allele)	Primer Alt Reverse (Resistant Allele)
LOC_Os09g36900	WD domain, G-beta repeat domain containing protein, expressed	21279866	C	T	P	L	GGCACGAGTCATCATCATTGTCACG	GCCCAACTGAAACTAAAGCCTGCATTCT	GGGCACGAGTCATCATCATTGTCAAA	CCCACTGACATGATAGATTGATAGATTCCTGC
LOC_Os09g37230	Putative serine/threonine-protein kinase ctr1	21490029	A	G	*	*	TGGGAATCAGACTGATGCTGATGCA	TAATTGACATTATCTGAGGTGCTATCATGGTCTTG	TGATGGGAATCAGACTGATGCTGATTTG	GAGCAAGGGCCTCAAGATTTGTAGATGAA
LOC_Os09g37240	Glutathione S-transferase, C-terminal domain containing protein, expressed	21502125	A	G	V	L	ATGGCTTTGATGAAACAAAGGCTCTCG	CTGGTGTCCTCTGCAGTTGGATATCTTGTT	AGATGGCTTTGATGAAACAAAGGCTCGTA	CTTCCAGCTTTGGTGGAGTAATTGCAGA
LOC_Os09g37590	OsFBDUF47 - F-box and DUF domain containing protein, expressed	21666818	T	C	M	T	AGTGACTTCCACGACGCCTCGC	CTCTGTGAACTGGATATTAACTTCCAAAAGCTCC	GACGTAAGTGACTTCCACGACGCCTACT	CTCTGTGAACTGGATATTAACTTCCAAAAGCTCC
LOC_Os09g37800	serine/threonine kinase, putative, expressed	21781200	T	C	H	R	CCGGAGTCGCTCAACAGGCAAT	TGGCAGAGCTTTAGCCAGCCGA	CCGGAGTCGCTCAACAGGGAAC	TGGCAGAGCTTTAGCCAGCCGA
LOC_Os09g37880	serine/threonine-protein kinase receptor precursor, putative, expressed	21841580	G	C	V	L	GAACACCAGCGCCATTGTCTTCC	TGCACGGCCAAGAAGCCGTC	CGTCGGTGTCGATGATCGCGTC	ATGAACACCGGCAACCTCGTCG
LOC_Os09g38700	STRUBBELIG-RECEPTOR FAMILY 5 precursor, putative, expressed	22245913	C	T	*	*	AATGCTCCAGTGACTTCATGTTGACCG	TGATAGCTGTGCTTCTTGCAGCTCTGATT	GAATGCTCCAGTGACTTCATGTTGGCTA	TGATAGCTGTGCTTCTTGCAGCTCTGATT
LOC_Os09g38710	HEAT repeat family protein, putative, expressed	22252462	G	A	*	*	GCAGCGCCACCATCCCCATATC	ATGGTTGGTCCCTTCTTGTCTTGCG	GCAGCGCCACCATCCCCATAAT	TCAACAAGATTGCAGACAGGGACACCTAC
LOC_Os09g38850	OsWAK91 - OsWAK receptor-like protein kinase, expressed	22317968	T	C	*	Q	GAACACTTTCGAGTGTCATCTCCACCAA	CATTCCAGCTGAACAAACTGGGATAACAAC	ACACTTTCGAGTGTCATCTCCACCCG	CATTCCAGCTGAACAAACTGGGATAACAAC
LOC_Os09g38970	zinc finger family protein, putative, expressed	22381404	T	A	S	R	ACGCTGGAAGGCATTAGGAGGATGA	ACATCTTATGTTCGGGAGAACCGGAGAG	CACGCTGGAAGGCATTAGGAGGAACT	ACATCTTATGTTCGGGAGAACCGGAGAG
LOC_Os09g39620	protein kinase family protein, putative, expressed	22736162	G	A	A	T	CCCTTGTCTCCTCAGCCGGTAGTACTTG	ATGGAAATACAACCGTTGTTGCCTGCT	CCCTTGTCTCCTCAGCCGGTAGTACATA	ATGGAAATACAACCGTTGTTGCCTGCT
LOC_Os11g13650	cellulose synthase, putative, expressed	7469515	G	T	P	H	GCCTCCGTCGACTCGTTGCC	CGTTCTGAGCGGTTTTGATTTGAGCTAGT	CGCCTCCGTCGACTCGTACCA	CGTTCTGAGCGGTTTTGATTTGAGCTAGT
LOC_Os11g19700	cycloeucalenol cycloisomerase, putative, expressed	11342380	C	A	N	K	CTTGCATGGTTCCAGGTGCAGATC	AGTATCTGTCCGGCTGTCGGCTCA	TGCATGGTTCCAGGTGCAGCAA	CTCCCTAAAACAGGGCGCAACGA
LOC_Os11g24060	permease domain containing protein, putative, expressed	13199356	T	C	V	A	CCGGCGTTCGTCACCATCGT	GCCCTGTCCAAATTCATCAGGGATCT	CCGGCGTTCGTCACCATGTC	GCCCTGTCCAAATTCATCAGGGATCT
LOC_Os11g24180	OsSCP50 - Putative Serine Carboxypeptidase homologue, expressed	13321629	A	T	V	E	CTGGTTCGAGGTGGACGTGGACA	CAGCAAGCTCGAAACTAATCCGGTGAT	TCTGGTTCGAGGTGGACGTGGTTT	ACGCAGGGTCCAGACTCCACCA
LOC_Os11g24770	ankyrin repeat domain containing protein	13648166	T	A	S	C	CGCTGCGTGGAAAGGGCAGA	CGCACTGACCCGCTCATCACTG	ATCGCTGCGTGGAAAGGGCTCT	CGCACTGACCCGCTCATCACTG
LOC_Os11g28950	pollen signalling protein with adenylyl cyclase activity, putative, expressed	16287232	T	C	E	G	GAAGGCACCTCAGTTTGCAACGGT	TGGCTTGTTGCACCCAACTACCTGATAT	GGAAGGCACCTCAGTTTGCAACGAC	TTTGGGTGTTCACTGCCAAATTGGA
LOC_Os12g03554	zinc finger C-x8-C-x5-C-x3-H type family protein	1411478	C	T	R	W	AGGGGTGTCCTTGATTGCATGCC	CCACCATGCGTTGAAGAAGTGGGT	TCATTAGGGGTGTCCTTGATTGCATTGT	CCACCATGCGTTGAAGAAGTGGGT
LOC_Os12g04660	zinc finger, C3HC4 type domain containing protein, expressed	1973059	G	C	T	R	ATGCGAGCAGGGCATCCACG	TCGCCCAGGTAGTCGGACGCT	AATGCGAGCAGGGCATCCACC	TCGCCCAGGTAGTCGGACGCT
LOC_Os12g06740	F-box domain containing protein, expressed	3280174	A	G	I	V	TCCCCGGCCACGAAAGACGTA	CCATGTATCCAATACCTGCGGAAAATCA	CTCCCCGGCCACGAAAGACAAT	CCATGTATCCAATACCTGCGGAAAATCA
LOC_Os12g06980	SAP domain containing protein, expressed	3410207	G	T	Q	K	CCCATGTACTTCTGAATTCACCCCCTG	TTTCTGACAGGCAAAAATCCAGGAAGC	ACCCATGTACTTCTGAATTCACCCGCTT	AAAACGGAGAAGAACTTCAATGGAAATGTCA
LOC_Os12g07800	S-locus-like receptor protein kinase, putative, expressed	3941715	T	C	M	T	CTACACAGAGCAACAAAGGAACGGGAAT	CATATCGCCCACGGCCAAGCT	CACAGAGCAACAAAGGAACGGGGAC	CTCGGCCTCACCTTGCTTCACATC
LOC_Os12g07950	transcriptional regulator Sir2 family protein, putative, expressed	4033132	C	T	R	H	GCAATTCAACTGGCTTACTCCCAGCTC	TGCTCTCCTCATTTGTCCAAATCAGCTTAC	GCAATTCAACTGGCTTACTCCCAGGAT	TGCTCTCCTCATTTGTCCAAATCAGCTTAC
LOC_Os12g09000	phosphomethylpyrimidine kinase/thiaminphosphate pyrophosphorylase, putative	4709578	T	C	L	S	GCAGATGGTGTCCATGTTGGTCAGTT	ATGCCGCCAATAGCGACCACAG	GCAGATGGTGTCCATGTTGGTCAAAC	TTTCTTGTTTCGGCTACGACACTCGG
LOC_Os12g09710	NBS-LRR disease resistance protein, putative	5128266	T	A	I	N	GACTTCTCCCACAAGCCTAGTGAAGCTATGA	GCGCAAGAGCAAAGATGTGGCTG	TCCCACAAGCCTAGTGAAGCTGGGT	GCGCAAGAGCAAAGATGTGGCTG
LOC_Os12g10180	NBS-LRR type disease resistance protein Rps1-k-2, putative, expressed	5378630	T	G	M	L	CCTCGAGACCAAGTCATCCAGGGTG	CTTCTCCAACACCAGCTCAGAAAGATGC	TCGAGACCAAGTCATCCAGGCCC	CTTCTCCAACACCAGCTCAGAAAGATGC

Table 2.2 Continued

Gene	Function	SNP Position	Ref. Allele	Var. Allele	Ref. AA	Var. AA	Primer Ref Forward (Susceptible Allele)	Primer Ref Reverse (Susceptible Allele)	Primer Alt Forward (Resistant Allele)	Primer Alt Reverse (Resistant Allele)
LOC_Os12g10330	NB-ARC domain containing protein, expressed	5468607	A	G	L	S	GAACCGAACTCTTCACGTTTCGCA	CATCCATTCAGAAAGGAGGAGTTGGTGA	TGTCGAACCGAACTCTTCACGTTTCTTG	TTGGAGCAGCAGTACCAAATATTATGGATGTC
LOC_Os12g10410	NB-ARC domain containing protein, expressed	5508921	G	C	A	G	GTTCAATTGGCAGCCTAGACATACTCCATG	GATTGTAAGGGGCCCTGGAGGTGA	GAGTTCAATTGGCAGCCTAGACATACTCCTTC	GATTGTAAGGGGCCCTGGAGGTGA
LOC_Os12g13100	WW domain containing protein, expressed	7284433	C	T	R	C	CTACCCAGCCAACCGTCGTCCTC	GCAAGCAAGCAAGCACCAACTGC	CTACCCAGCCAACCGTCGTCGAT	GCAAGCAAGCAAGCACCAACTGC
LOC_Os12g15460	pentatricopeptide, putative, expressed	8826281	A	G	*	*	GTTCCAGCATTCCATCAAACGCCT	GCCTGTGGAAAGGCCTGCGAC	TGTTCCAGCATTCCATCAAACAGCC	GCCTGTGGAAAGGCCTGCGAC

2.3.2 Sanger Sequencing

Twelve of the nsSNPs located within candidate genes for SB resistance previously identified by Silva *et al*. (2012) were confirmed in my research by Sanger sequencing between the resistant variety Araure 3 and the susceptible Louisiana variety Cocodrie. Those confirmed nsSNP variants are shown in Appendix A.

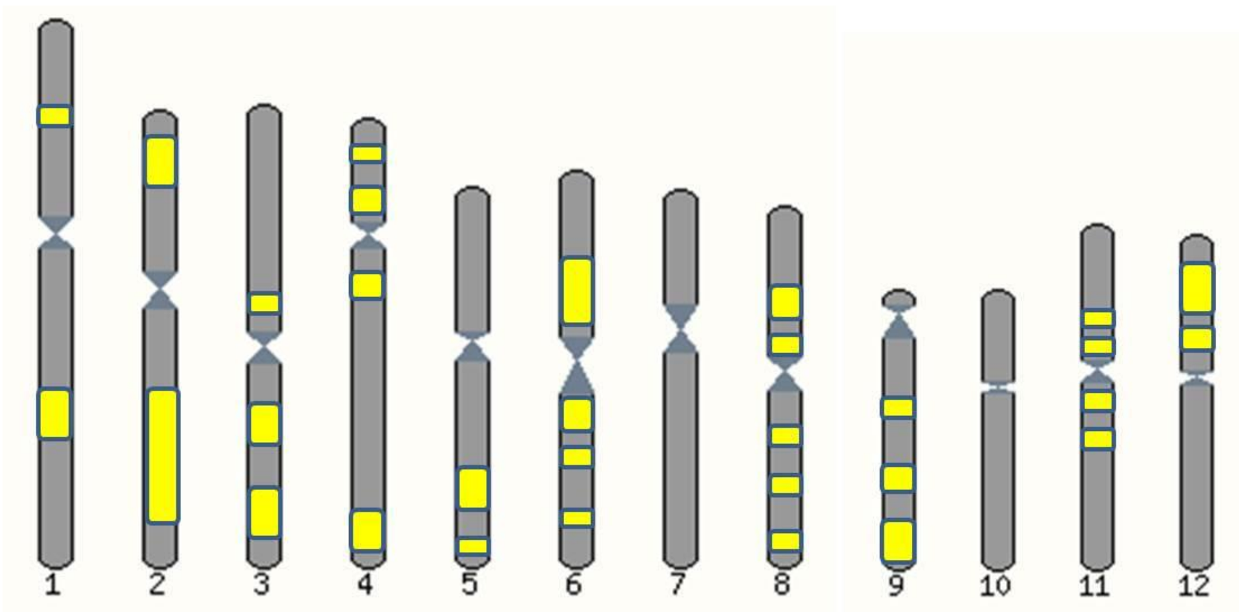

Figure 2.1 Distribution of the 136 nsSNP-based markers in the rice genome. Yellow bands indicate the regions covered by the markers reported in published QTLs for SB resistance.

2.4 Discussion

Understanding of the genetic mechanisms for complex traits requires the use of robust molecular markers such as SNPs that are abundant in rice with ~20 million SNPs available for the research community (Alexandrov *et al*., 2015). Using the SNPs located in candidate genes for resistance to sheath blight identified by Silva *et al*. (2012), and the procedure for SNP-based marker design proposed by Drenkard *et al*. (2000), 136 PCR-based molecular markers were designed and standardized for identification of specific alleles for a gel-based platform. Markers

were developed as described by Drenkard *et al*. (2000) where SNP variants were identified based on presence/absence of amplified PCR product(s) (Figure 2.2). However, this procedure required two different PCR reactions and three primers consisting of one forward primer for the reference allele, one forward for the alternative allele, and one common reverse primer. By this procedure, two PCR products of similar or the same size were obtained with two separate gel loadings required for each marker. However, this approach resulted in double the time and an additional primer to increase cost and efforts vs. SSR Markers. Hayashi *et al.* (2004) reported a modification to the Drenkard *et al.* (2000) method to detect SNP polymorphism based on difference on PCR product size instead of presence/absence. However, that method used the same reverse primer and the forward primers that are located in different SNPs in a region of interest (Figure 2.3). The advantage of the Hayashi *et al.* (2004) method is that only one multiplex PCR reaction containing the three primers was required, saving time and reagent costs. However, this type of marker can only be designed in regions with high SNP density to generate PCR products sufficiently different to detect polymorphism in agarose gels, and sufficiently close to amplify PCR products in multiplex PCR conditions. Moreover, this marker type may lack specificity because it is based on two different SNP in the same gene that may not be consistent among unrelated individuals. This type of marker may be informative for a specific bi-parental population with known genotypes, but not always for a diverse collection where haplotypes can be variable. To make the procedure more specific and efficient in terms of time, a modification of the procedure described by Drenkard *et al*. (2000) was included in my work. The design of forward primers was maintained as the initial procedure for one specific SNP, but two different reverse primers were designed, one for the reference and one for the alternative allele in different positions to obtain different size PCR products for each allele. Thus, my modification

did not use presence/absence as a design strategy, but rather implemented product size differences to define the genotype (Figure 2.4). Although my method still requires two separate PCR reactions, these can be mixed and products can be loaded in a single lane of an agarose gel saving time and expense vs. the method of Drenkard *et al*. (2000). The modified method was the most common approach used to design the 136 markers shown in Table 2.2. During the course of this research, I read a report by Ramkumar *et al*. (2010) that described an alternative design for allele specific SNP located in a major QTL controlling grain length. This method also targets one specific SNP, but design is based on allele-specific primers on different complementary DNA strands. Reverse primers for each allele in different DNA strands are designed with different distances in base pairs to the target SNP. Therefore, two PCR products with different size are detected in the same agarose gel. Moreover, the reaction for both alleles is carried out as a "multiplex" with all primers combined into one tube. Therefore, this method requires only four primers, one PCR reaction and one gel loading (Figure 2.5). Comparing the four methods described above, the procedure by Ramkumar *et al*. (2010) is the most efficient in terms of time and cost (Table 2.3). However, multiplex PCR is susceptible to problems of reproducibility and amplification, and multiplex primer design must be more accurate to avoid reaction inhibition by complementarity between primers (Henegariu *et al*., 1997). Therefore, all four methods have advantages and disadvantages and can be replaced depending on the sequence(s) of interest. Although most of the 136 markers for SB were designed using the method shown in Figure 2.3, the Ramkumar *et al.* (2010) procedure was recently evaluated for five SNP markers associated with four agronomic traits in rice (Appendix B). Based on these results, the Ramkumar method will be evaluated for SNP genotyping in future disease resistance and rice breeding research.

Table 2.3 Comparison of four methods for SNP-based markers including number of primers required, PCR reactions, number of gels, time consumed, disadvantages.

SNP- based marker design Method	Number of primers	PCR reactions	Number of gels[a]	Approximate price per sample (US $)[b]	Approximate time consumed[c]	Main disadvantage
Drenkard *et al.* 2000	3	2	2	0.48	8 hours 30 minutes	Time consuming.
Hayashi *et al*. 2004	3	1	1	0.24	4 hours 30 minutes	Additional SNP is required.
Proposed in this work	4	2	1	0.47	6 hours 30 minutes	One additional PCR reaction compared with Hayashi and Ramkumar methods.
Ramkumar *et al.* 2010	4	1	1	0.24	4 hours 30 minutes	Problems associated with multiplex PCR (lack of amplification or reproducibility)

[a] Agarose gels for 384 samples using a Horizontal Systems gel platform (26cm x 40cm; C.B.S Scientific).
[b] Prices calculated for 10 μL PCR using Jumpstart-Readymix (Sigma-Aldrich) in 384 PCR plates for C-1000 touch thermal cycler (Bio-Rad) and agarose gels for a Horizontal Systems gel platform (26cm x 40cm; C.B.S Scientific).
[c] Time determined from PCR preparation to gel picture, considering a 1 hour 20 minutes PCR program and only one C-1000 touch thermal cycler (Bio-Rad) and one Horizontal Systems gel platform (26cm x 40cm; C.B.S Scientific) available.

The total of 136 nsSNPs selected for design of markers located in chromosomal regions where QTLs for SB resistance have been reported, represents an initial effort for adequate coverage of these specific regions. High density of molecular markers is important for accurate mapping and gene identification. Polymorphism of markers and coverage has been a problem in QTL mapping research. For example, using SSRs Nelson *et al.* (2012) identified QTLs for sheath blight resistance in a Cocodrie x MCR DH population. Problems with coverage were

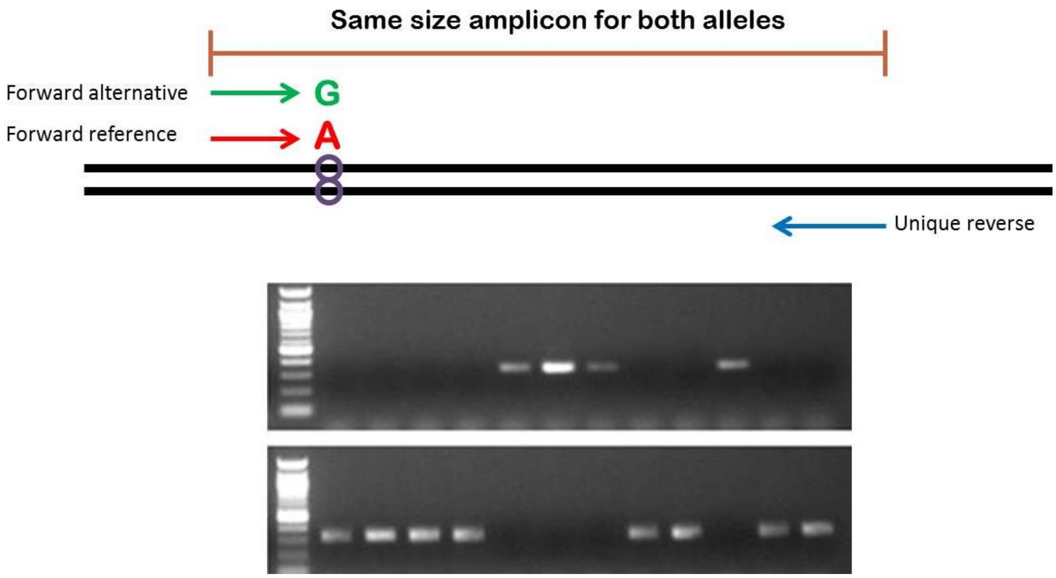

Figure 2.2 Schematic representation of the SNP-based marker used by Drenkard *et al*. (2000). Two forward primers (green and red arrows) were designed differing in the variable nucleotide in the 3' end (Green and red letters). Thus, each forward primer amplifies an allele. Reverse primer is the same for both alleles (blue arrow). Therefore, the band detected is similar in size (brown horizontal bar), and to identify the polymorphism, it is necessary to load the two PCR products in different gels or in the same gel at different times as shown in image below.

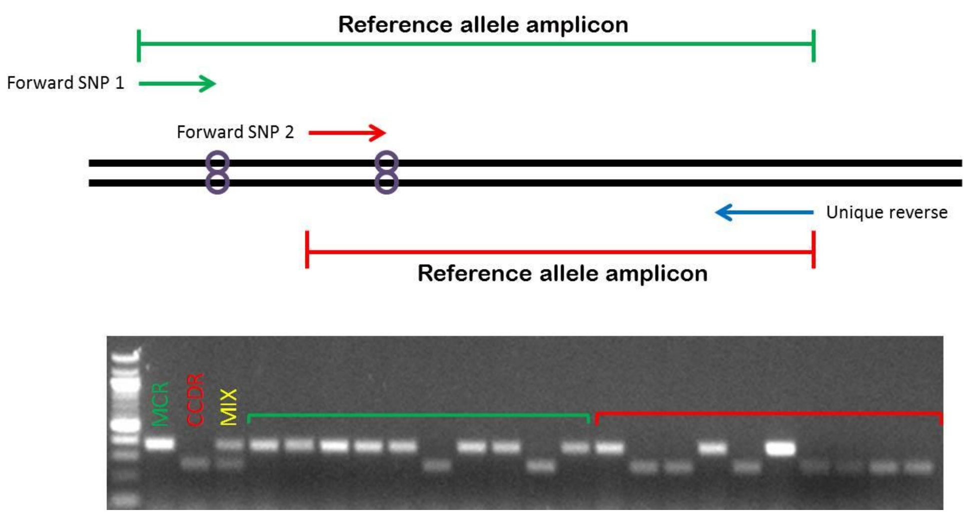

Figure 2.3 Schematic representation of the modified method by Hayashi *et al.* (2004). In this method forward primers for each allele are designed on different SNPs separated by ~ 100 bp (arrows green and red). Reverse primer is the same for both alleles (blue arrow). Thus, the polymorphic products (green and red horizontal bars) amplified in just one PCR reaction can be loaded in the same gel to determine the genotype of every individual as is shown in image below.

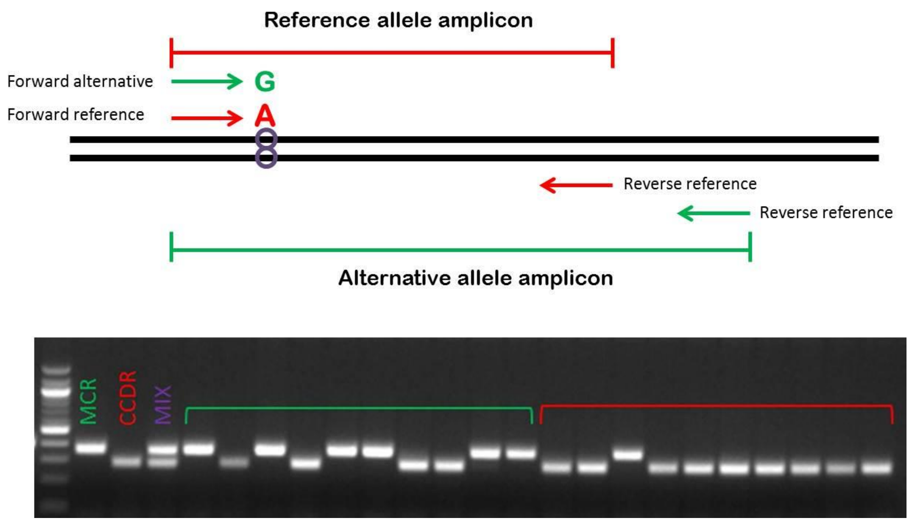

Figure 2.4 Schematic representation of the modified Drenkard *et al*. (2000) procedure. In this approach, two different reverse primers (green and red arrows toward left) were designed to complement each forward primer (green and red arrows toward right) and produce polymorphic bands (Green and red horizontal bars). PCR products using the two different sets of primers are mixed and loaded at the same time in the agarose gel. The polymorphism is evident as shown in the image below.

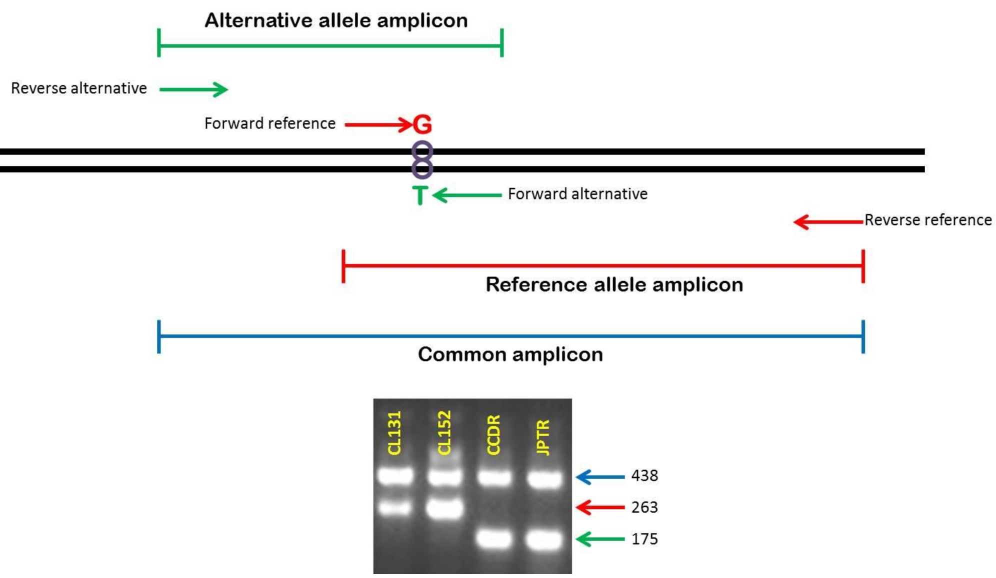

Figure 2.5 Schematic representation of the method by Ramkumar *et al*. (2010). One forward primer (red arrow toward right) is designed on the forward strand. The other primer for the alternative allele (green arrow toward left) is designed on the reverse strand with the specific 3' end to amplify each allele (green and red horizontal bars). Reverse primers are designed on the opposite strands with different distances to the SNP (green arrow toward right and red arrow toward left). Thus, the reverse primers define the size of the PCR product. All four primers are mixed in just one PCR reaction. Both reverse primers produce an additional fragment that is equal to the sum of the size of the two others PCR products minus the sum of the size of the two forward primers (see image below).

encountered for several chromosomes including 1,3,4,5 and 10. In chromosome 1 a large region of ~20 Mbp was reported by Nelson *et al*. (2012) with no polymorphic SSR markers that reduce accuracy and precision of QTL identification in that region. Using the nsSNP-based approach described here, nine markers were identified within a ~3.4 Mbp region of the SB QTL on chromosome 1 that substantially improved accuracy and precision for my study.

A strong effect QTL in the bottom of the long arm of chromosome 9 has been widely reported (Pinson *et al.*, 2005; Liu *et* al., 2009; Nelson *et al*., 2012; Taguchi-Shiobara *et al*. 2013; Zuo *et al*. 2014b). In total, 16 nsSNP-based SB markers located in candidate genes in that region were designed and validated during my research. Fourteen nsSNPs were previously identified by Silva *et al*. (2012) while two new SNPs were identified using the comparison tool available in the Rice SNP-seek Database (http://oryzasnp.org/iric-portal/) and the NGSEP pipeline for alignment reported by Duitama *et al*. (2015). These two additional SNPs are located in genes in a 145 kb region at the bottom chromosome 9 identified by Zou *et al.* (2014c) containing 18 candidate genes. Fine-mapping was achieved in that study using lines derived from a Teqing x Lemont cross. However, SNP variation was not reported in the 147 kb region or in the publication by Silva *et al.* (2012). With the comparison of the Teqing and Lemont sequences available in the Rice SNP-seek Database and those reported by Duitama *et al*. (2015), I detected a synonymous SNP in the locus LOC_Os09g37230 (putative serine/threonine-protein kinase ctr1) and a nsSNP in the locus LOC_Os09g37240 (glutathione S-transferase, C-terminal domain containing protein, expressed), both located in the fine-mapped region. A serine/threonine-protein kinase have been related to fungal disease resistance caused by *Blumeria graminis* in wheat (Cao *et al*. 2011), and glutathione S-transferase have been related to fungal disease resistance caused by *Botryosphaeria dothidea* (Liao *et al*. 2014). Primers were designed for these

two SNPs, and as the other SB markers, they were polymorphic between resistant (MCR10277, Teqing, and Jasmine 85) and susceptible (Cocodrie, Lemont, and Cypress) lines.

This new set of markers described here is efficient, cost effective and useful for discovery of genes involved in SB resistance. Adequate coverage and specificity of nsSNP-based markers is advantageous for mapping and other functional genomic research, but cost and availability of the required equipment for new SNP genotyping approaches is unachievable for many research groups. Thus, these new SB markers and the methodology used to find them, plus use of available online SNPs resources, constitute an important resource for rice researchers interested in cost-efficient, high coverage genotyping without using advanced platforms.

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CHAPTER 3. IDENTIFICATION OF CANDIDATE SNP-BASED MOLECULAR MARKERS FOR SHEATH BLIGHT RESISTANCE BY SELECTIVE GENOTYPING OF RICECAP SB2 MAPPING POPULATION.

3.1 Introduction

Different methodologies, populations and molecular markers have been used to identify genes associated with important traits in rice. Genetic maps to identify genes related with valuable traits in rice have been developed using RFLPs (Restriction fragment length polymorphism) (Wang *et al*., 1994). However, genotyping using this type of marker is time consuming, and expensive with potential exposure to radioactive elements. AFLPs (Amplified fragment length polymorphism) were also used at the beginning of gene mapping in rice (Mackill *et al*., 1996), but this dominant marker suffers from high cost, extended time periods required to screen markers, and reduced polymorphism vs SNPs (Single Nucleotide Polymorphism). SSRs (simple sequence repeat) have been exploited by researchers due to good reproducibility, abundance and polymorphism of these markers in the rice genome (McCouch *et al*., 2002). However, most SSR markers are not directly related to the gene function, and are not as abundant or polymorphic as SNPs (Mammadov *et al.*, 2012). A large amount of genomic information is currently available for rice research at the 3,000 Rice Genomes Project website (http://oryzasnp.org/iric-portal/) that facilitates research of specific DNA variants that may be associated with important agronomic traits (Alexandrov *et al*., 2015). Other approaches using genomic information from a smaller number of sequenced varieties have permitted rapid screening of variants between varieties with contrasting phenotypes such as resistance to sheath blight (Silva *et al*., 2012).

To identify QTLs (Quantitative Trait Loci) for sheath blight resistance, various populations, molecular markers, and strategies have been studied, including F_4 populations

genotyped with RFLPs (Li *et al*., 1995), and BILs (Backcrossed Inbreed Lines) genotyped with SSRs (Tagushi-Siobara *et al.*, 2013). Association mapping was used to identify QTLs in the USDA rice core collection genotyped with SSRs (Jia *et al*., 2012), F_2 clonal populations were genotyped with SSRs (Zou *et al*., 2000), and QTLs were identified from a BC_1F_1 population using SSRs and STS (Sequence-Tagged Site) (Sato *et al*., 2004). DH lines genotyped with SSRs were also used to identify QTLs for sheath blight resistance (Nelson *et al*., 2012). This DH population originated from the cross between the susceptible variety Cocodrie (PI 606331) and the partially resistant line MCR10277 (GSOR 200327) (Chu *et al*., 2006). However, all these strategies require genotyping a large number of individuals that increases cost and time to completion.

Two approaches to reduce time and cost for genotyping have been reported with similar results to those obtained with the methodologies described above. Bulk segregant analysis (BSA) groups extreme phenotype individuals from a segregating population for genotyping and subsequent QTL mapping (Michelmore *et al.*, 1991, Quarrie *et al*., 1999). BSA has successfully identified QTLs in rice for drought resistance (Salunkhe *et al.*, 2011), grain yield under abiotic stress (Venuprasad *et al.*, 2009), and confirmation of QTLs for sheath blight resistance (Yadav *et al*., 2015). Another strategy to increase efficiency is selective genotyping (SG) that detects QTLs for complex traits by genotyping only those individuals from contrasting phenotypes from a mapping population (Sun *et al*., 2010). A major QTL for resistance to *Fusarium oxysporum* in watermelon was identified using SNPs information and SG approach (Lambel *et al*., 2014). One advantage of SG is the low number of individuals that have to be genotyped. Simulations reported by Navabi *et al*. (2009) demonstrated that by genotyping only 20 individuals from the extreme phenotypes, efficient QTL detection is possible. Vikram *et al.* (2012) compared BSA,

SG and whole population genotyping and demonstrated the efficiency of the three methods for consistent identification of QTLs. BSA and SG are less time-consuming and cheaper than whole population genotyping. BSA requires less genotyping than SG because the extreme phenotypes are pooled. However, the estimation of allele frequencies was based on the intensity of the bands in a gel (Quarrie *et al*., 1999) which could generate false positives. Estimation may be more precise using capillary sequencing approaches, where allelic frequencies are determined by peak heights in a chromatogram (Xia *et al.*, 2010), but it does not apply for PCR-based markers run in regular agarose gels. Therefore, selective genotyping is potentially more precise than BSA in the estimation of allelic frequencies because every individual from each extreme group is genotyped.

The specific objective for this research is to identify the most important chromosomal regions involved in the resistance to sheath blight using the SG approach by genotyping extreme phenotypes from the RiceCAP SB2 mapping population with the candidate nsSNPs-based markers designed and validated in Chapter 2.

3.2 Materials and Methods

Extreme phenotypes for sheath blight resistance, consisting of the 10 most resistant and the 10 most susceptible lines from the SB2 mapping population (Cocodrie x MCR010277 double-haploid mapping population) (Chu *et al*., 2006), were selected according to previous field and greenhouse evaluations (Nelson *et al*., 2012; Silva *et al*., 2011) and subsequently screened for the 136 candidate nsSNP-based markers described in Chapter 2. The candidates were identified in QTLs identified in previous studies (Li *et al*., 1995; Sato *et al*., 2004; Pinson *et al*., 2005; Zeng *et al.*, 2011; Nelson *et al*., 2012; Jia *et al*., 2012; Tagushi-Siobara *et al.*, 2013). The candidate nsSNPs represent near total coverage of the regions where the ~200 candidate genes are located as reported by Silva *et al*. (2012). nsSNPs genotyping results of the 10 most resistant

and 10 most susceptible SB2 lines, and sheath blight resistance scores (0-9 scale, where 0 = not disease present and 9 = dead plant) were used to conduct a one-way ANOVA using PROC GLM in SAS, v. 9.1) for comparing "1" vs "0" alleles (1 = resistant allele, 0 = susceptible allele). This analysis returned F values with corresponding raw P-values. PROC MULTITEST in SAS, v.9.1 was used to adjust raw P-values to account for multiple testing. False Discovery Rate values ($P < 0.05$) were used to rank and identify the most important candidate nsSNPs associated with sheath blight resistance in selected group from the SB2 lines. Multiple regression was used to rank the markers according to the R-square results. Proc GLM, SAS, v.9.1 software was used. PCR products were evaluated using the Horizontal Systems gel platform (26cm x 40cm; C.B.S Scientific) to screen the 20 SB2 lines PCR conditions are as described in Chapter 2.

3.3 Results

Selective genotyping (SG) was carried out in twenty individuals with extreme phenotypes for sheath blight resistance from SB2 mapping population plus a susceptible reference Cocodrie and a resistant reference MCR10277 using the 136 SNP-based markers designed and validated in Chapter 2. Results of the genotyping are shown in Table 3.1. According to the statistical analysis performed (Table 3.2), the top ranked SNP marker, which represented the "resistant" allele in 100% of resistant lines and the "susceptible" allele in 100% of the susceptible lines, was based on the nsSNP located in the position 19591594 (bp) in the locus LOC_Os09g32860 (R^2=0.892) that encodes an F-box domain containing protein (OsFBX336). F-box proteins have been associated with the defense response in rice (Cao *et al*. 2008) and in Arabidopsis (Kim and Delaney, 2002). Other top-ranked markers were identified as 12 nsSNPs in exons in genes located at the bottom of chromosome 9, in the genomic region ranging from the locus LOC_Os09g33710 to LOC_Os0938970 (R^2=0.772268). These markers were found in disease

Table 3.1 Genotypes for extreme phenotypes from the DH SB2 population. Markers in first column on the left are highlighted using different colors depending on the chromosome they are located. Green cells represent "resistant" alleles and red cells represent "susceptible" alleles. MCR010277 was the resistant reference variety. Resistant SB2 lines: SB2-03, SB2-109, SB2-134, SB2-158, SB2-161, SB2-174, SB2-259, SB2-206, SB2-225, SB2-272. Cocodrie (CCDR) was the susceptible reference variety. Susceptible SB2 lines: SB2-99, SB2-13, SB2-48, SB2-88, SB2-125, SB2-144, SB-203, SB-255, SB-276, SB2-314.

SB2 POPULATION	MCR	SB2-03	SB2-109	SB2-134	SB2-158	SB2-161	SB2-174	SB2-259	SB2-206	SB2-225	SB2-272	CCDR	SB2-99	SB2-13	SB2-48	SB2-88	SB2-126	SB2-144	SB2-203	SB2-255	SB2-276	SB2-314
2011 SBR	3.5	4.7	5.5	5.5	5.0	5.7	4.5	5.7	6.0	6.0	6.0	7.5	7.5	7.5	8.0	8.0	8.0	8.0	7.5	8.0	8.0	7.5
LOC_Os01g13300	R	S	R	R	S	R	R	S	R	R	R	S	R	S	R	S	R	S	R	S	S	S
LOC_Os01g52330	R	S	S	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g52880	R	S	S	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g53420	R	S	S	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g54350	R	S	R	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g54515	R	S	R	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g55050	R	S	R	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g56040	R	S	R	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g57230	R	S	R	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os01g57900	R	S	R	R	R	S	R	S	S	R	R	S	S	S	R	R	R	R	R	S	S	R
LOC_Os02g02650	R	S	S	R	S	R	S	R	S	R	R	S	R	S	S	S	R	R	R	R	S	R
LOC_Os02g09820	R	S	R	R	S	R	R	R	S	R	R	S	S	S	R	S	R	R	R	S	S	R
LOC_Os02g10120	R	S	R	R	S	R	R	R	S	R	R	S	S	S	R	S	R	R	R	S	S	R
LOC_Os02g10900	R	S	R	R	S	R	R	R	S	S	R	S	S	S	R	S	R	R	R	S	S	R
LOC_Os02g11820	R	S	R	S	S	R	R	R	S	S	R	S	S	S	R	S	R	R	R	S	S	R
LOC_Os02g34490	R	R	S	R	S	R	R	S	S	R	R	S	S	S	R	S	S	S	S	S	S	S
LOC_Os02g34850	R	R	S	R	S	R	R	S	S	R	R	S	S	S	R	S	S	S	S	S	S	S
LOC_Os02g35210	R	R	S	R	S	R	R	S	S	R	R	S	S	S	R	S	S	S	S	S	S	S
LOC_Os02g39590	R	R	S	R	S	R	S	S	S	R	R	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g42412	R	S	S	R	S	R	R	S	R	R	R	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g43460	R	S	S	R	S	R	S	S	R	R	S	S	S	S	S	R	S	S	S	S	S	S
LOC_Os02g44730	R	S	S	R	R	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
LOC_Os02g45160	R	S	S	R	R	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
LOC_Os02g45980	R	S	S	R	R	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
LOC_Os02g48210	R	S	S	R	R	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
LOC_Os02g49986	R	S	S	R	R	R	S	S	R	S	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g51900	R	S	S	R	R	R	S	R	R	S	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g52060	R	S	S	R	R	R	S	R	R	S	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g53970	R	S	S	S	R	R	S	R	R	R	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g54330	R	S	S	S	R	R	S	R	R	R	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g54500	R	S	S	S	R	R	S	R	R	R	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g55180	R	S	S	S	R	R	S	R	R	R	S	S	S	S	R	R	S	S	S	S	S	S
LOC_Os02g56380	R	S	S	S	R	R	S	R	R	R	S	S	S	S	R	R	R	S	R	S	S	R
LOC_Os02g56480	R	S	S	S	R	R	S	R	R	R	S	S	S	S	R	R	R	S	R	S	S	R
LOC_Os02g57960	R	S	S	S	R	R	S	R	R	R	S	S	S	S	H	R	R	S	R	S	S	R
LOC_Os02g58540	R	S	S	S	R	R	S	R	R	R	S	S	R	R	H	R	R	S	R	S	S	R
LOC_Os03g30130	R	R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R
LOC_Os03g37720	R	R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R
LOC_Os03g39150	R	R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R
LOC_Os03g40250	R	R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R
LOC_Os03g43684	R	R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	S
LOC_Os03g53220	R	S	S	S	S	R	R	R	S	R	R	S	R	S	S	S	R	R	R	R	S	S
LOC_Os03g56400	R	S	S	S	S	R	R	R	S	R	R	S	R	S	S	S	R	R	R	R	S	S
LOC_Os03g57160	R	S	S	S	S	R	R	R	S	R	R	S	R	S	S	S	R	R	R	R	S	S
LOC_Os03g58390	R	S	S	S	S	R	R	R	S	R	R	S	R	S	S	S	R	R	R	R	S	S
LOC_Os03g63110	R	S	S	S	S	R	S	R	S	R	S	S	R	S	S	S	R	R	R	R	S	S
LOC_Os04g05030	R	S	R	R	R	S	R	R	R	S	R	S	S	S	R	R	S	R	S	R	S	R
LOC_Os04g10460	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g11640	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g11970	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g15650	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g20680	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g21890	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g23620	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g23890	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	S	R	S	R
LOC_Os04g55760	R	S	S	S	S	R	S	R	R	S	R	S	S	S	S	R	S	S	S	R	S	R

Table 3.1 Continued

SB2 POPULATION	MCR	SB2-03	SB2-109	SB2-134	SB2-158	SB2-161	SB2-174	SB2-259	SB2-206	SB2-225	SB2-272	CCDR	SB2-99	SB2-13	SB2-48	SB2-88	SB2-126	SB2-144	SB2-203	SB2-255	SB2-276	SB2-314
2011 SBR	3.5	4.7	5.5	5.5	5.0	5.7	4.5	5.7	6.0	6.0	6.0	7.5	7.5	7.5	8.0	8.0	8.0	8.0	7.5	8.0	8.0	7.5
LOC_Os04g56250	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os04g57670	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os04g58720	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os04g58820	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os04g58910	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os04g59060	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os04g59540	R	S	S	S	S	R	S	R	R	R	R	S	S	S	S	R	R	S	R	R	S	R
LOC_Os05g37040	R	R	S	R	R	R	S	R	S	R	S	S	R	S	S	R	R	R	R	R	S	R
LOC_Os05g39760	R	R	S	R	R	R	S	R	S	R	S	S	R	S	S	R	R	R	R	R	S	R
LOC_Os05g40790	R	R	S	R	R	R	S	R	S	R	S	S	R	S	S	R	R	R	R	R	S	S
LOC_Os05g41130	R	R	S	R	R	R	S	R	S	R	S	S	R	S	S	R	R	R	R	R	S	S
LOC_Os05g41290	R	R	S	R	R	R	S	R	S	R	S	S	R	S	S	R	R	R	R	R	S	S
LOC_Os05g50660	R	S	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	R	R	R	S	S
LOC_Os06g13040	R	R	S	S	R	R	R	R	S	R	S	S	S	S	R	S	S	S	S	S	S	S
LOC_Os06g15170	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	S	S	S
LOC_Os06g19110	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g22020	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g22460	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g23530	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g28124	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g28670	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g29700	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g29844	R	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g31070	R	R	R	S	R	R	R	S	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g32350	R	R	R	S	R	H	R	S	S	R	S	S	S	S	R	S	S	R	S	R	S	S
LOC_Os06g35850	R	R	R	S	R	S	R	S	S	R	S	S	S	S	R	S	R	R	R	R	S	S
LOC_Os06g37500	R	R	R	S	R	S	R	S	S	R	S	S	S	S	R	S	R	R	R	R	S	S
LOC_Os06g44820	R	R	R	S	R	S	R	S	R	R	S	S	S	S	R	S	R	R	R	R	S	S
LOC_Os08g10560	R	R	R	R	R	R	S	R	R	R	R	S	R	S	S	R	R	R	R	S	S	S
LOC_Os08g12800	R	R	R	R	R	R	S	R	R	R	R	S	R	S	S	R	R	R	R	S	S	S
LOC_Os08g13870	R	R	R	R	R	R	S	R	R	R	R	S	R	S	S	R	R	R	R	S	S	S
LOC_Os08g19694	R	R	R	R	R	R	S	R	R	R	R	S	R	S	S	R	S	R	S	S	S	S
LOC_Os08g20020	R	R	R	R	R	R	S	R	R	R	R	S	R	S	S	R	S	R	S	S	S	S
LOC_Os08g30850	R	R	R	R	R	R	S	R	R	S	R	S	R	S	S	R	S	R	S	S	S	S
LOC_Os08g30910	R	R	R	R	R	R	S	R	R	S	R	S	R	S	S	R	S	R	S	S	S	S
LOC_Os08g35310	R	R	S	R	R	R	S	R	S	S	R	S	R	S	S	R	S	R	S	S	S	R
LOC_Os08g36320	R	R	S	R	R	R	S	S	S	S	R	S	R	S	S	R	S	R	S	S	S	R
LOC_Os08g36760	R	R	S	R	R	R	S	S	S	S	R	S	R	S	S	R	S	R	S	S	S	R
LOC_Os08g42930	R	R	S	S	S	R	R	S	S	S	S	S	R	S	S	R	R	R	R	R	S	R
LOC_Os09g16540	R	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	R	S	R	S	R
LOC_Os09g17600	R	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	R	S	R	S	R
LOC_Os09g17630	R	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	R	S	R	S	R
LOC_Os09g25620	R	S	S	S	R	R	S	R	R	S	S	S	S	S	S	S	R	S	R	S	S	R
LOC_Os09g25890	R	S	S	S	R	R	S	R	R	S	S	S	S	S	S	S	R	S	R	S	S	R
LOC_Os09g26300	R	S	S	S	R	R	S	R	R	S	S	S	S	S	S	S	R	S	R	S	S	S
LOC_Os09g27570	R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	R	S	R	S	R	R
LOC_Os09g32020	R	R	S	R	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os09g32860	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os09g33710	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g34180	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g36900	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g37230	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g37240	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g37590	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g37800	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g37880	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g38700	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g38710	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g38850	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g38970	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
LOC_Os09g39620	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	R
LOC_Os11g13650	R	R	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	S	R
LOC_Os11g19700	R	S	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	S	R
LOC_Os11g24060	R	S	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	S	R
LOC_Os11g24180	R	S	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	S	R
LOC_Os11g24770	R	S	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	S	R
LOC_Os11g28950	R	S	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	S	R
LOC_Os12g03554	R	R	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	S	R	S	R

Table 3.1 Continued

SB2 POPULATION	MCR	SB2-03	SB2-109	SB2-134	SB2-158	SB2-161	SB2-174	SB2-259	SB2-206	SB2-225	SB2-272	CCDR	SB2-99	SB2-13	SB2-48	SB2-88	SB2-126	SB2-144	SB2-203	SB2-255	SB2-276	SB2-314
2011 SBR	3.5	4.7	5.5	5.5	5.0	5.7	4.5	5.7	6.0	6.0	6.0	7.5	7.5	7.5	8.0	8.0	8.0	8.0	7.5	8.0	8.0	7.5
LOC_Os12g04660	R	R	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	S	R	S	R
LOC_Os12g06740	R	R	R	S	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R
LOC_Os12g06980	R	R	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	S
LOC_Os12g07800	R	R	R	S	R	R	R	S	S	S	R	S	S	S	S	S	S	S	R	R	S	S
LOC_Os12g07950	R	R	R	S	R	R	R	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S
LOC_Os12g09000	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S
LOC_Os12g09710	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os12g10180	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os12g10330	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os12g10410	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os12g13100	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
LOC_Os12g15460	R	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S

resistance related genes including four kinases (LOC_Os09g37230, LOC_Os09g37800, LOC_Os09g37880, and LOC_Os09g38850) important for pathogen recognition (Afzal *et al*. 2008), and activation and signaling factors for the response to pathogens (LOC_Os09g33710, LOC_Os09g34180, LOC_Os09g36900, LOC_Os09g37590, LOC_Os09g38700, LOC_Os09g37240, LOC_Os09g38710, LOC_Os09g38970). The second most important group of markers for SB resistance with R^2=0.698, was located on the short arm of chromosome 12 in the region where QTLs have been reported previously (Nelson *et al*., 2011; Li *et al*., 1995). These markers were based on nsSNPs in six disease resistance related genes including four nucleotide-binding domains containing proteins (NBS-LRR and NB-ARC) (LOC_Os12g09710, LOC_Os12g10180, LOC_Os12g10330, LOC_Os12g10410), WW domain containing protein (LOC_Os12g13100) and pentatricopeptide repeat protein (LOC_Os12g15460). Additional nsSNP markers located in disease resistant-related genes on the short arm of chromosome 6, long arm of chromosome 2, and long arm of chromosome 8 with p values < 0.05 were also considered as candidate markers.

Table 3.2 Ranking of 136 genotyped SB markers in SB2 mapping population based on raw P, Hochberg, Bonferroni, False Discovery, and R-squared values.

Rank	Marker	F Value	Raw_P-Value	Hochberg p-value	Stepdown Bonferroni	False Discovery Rate p-value	R-Squared
1	LOC_Os09g32860	149.154	0	0	0	0	0.892315
2	LOC_Os09g33710	61.04	0	0.00004	0.00004	0	0.772268
3	LOC_Os09g34180	61.04	0	0.00004	0.00004	0	0.772268
4	LOC_Os09g36900	61.04	0	0.00004	0.00004	0	0.772268
5	LOC_Os09g37230	61.04	0	0.00004	0.00004	0	0.772268
6	LOC_Os09g37240	61.04	0	0.00004	0.00004	0	0.772268
7	LOC_Os09g37590	61.04	0	0.00004	0.00004	0	0.772268
8	LOC_Os09g37800	61.04	0	0.00004	0.00004	0	0.772268
9	LOC_Os09g37880	61.04	0	0.00004	0.00004	0	0.772268
10	LOC_Os09g38700	61.04	0	0.00004	0.00004	0	0.772268
11	LOC_Os09g38710	61.04	0	0.00004	0.00004	0	0.772268
12	LOC_Os09g38850	61.04	0	0.00004	0.00004	0	0.772268
13	LOC_Os09g38970	61.04	0	0.00004	0.00004	0	0.772268
14	LOC_Os12g10330	41.633	0	0.00052	0.00053	0.00004	0.698154
15	LOC_Os12g10410	41.633	0	0.00052	0.00053	0.00004	0.698154
16	LOC_Os12g13100	41.633	0	0.00052	0.00053	0.00004	0.698154
17	LOC_Os12g15460	41.633	0	0.00052	0.00053	0.00004	0.698154
18	LOC_Os09g39620	37.145	0.00001	0.00106	0.00106	0.00008	0.673587
19	LOC_Os12g09710	33.113	0.00002	0.00211	0.00211	0.00016	0.56581
20	LOC_Os12g10180	33.113	0.00002	0.00211	0.00211	0.00016	0.56581
21	LOC_Os09g32020	23.457	0.00013	0.01449	0.01462	0.00097	0.483682
22	LOC_Os12g06980	23.457	0.00013	0.01449	0.01462	0.00097	0.483585
23	LOC_Os12g09000	16.856	0.00066	0.07303	0.07303	0.00468	0.449908
24	LOC_Os12g07950	14.722	0.00121	0.13166	0.13166	0.00807	0.351999
25	LOC_Os06g13040	9.778	0.00583	0.62916	0.62916	0.03699	0.3322
26	LOC_Os06g15170	8.954	0.00781	0.83613	0.83613	0.04726	0.284212
27	LOC_Os12g03554	7.147	0.0155	0.9835	1	0.08949	0.263827
28	LOC_Os12g04660	7.147	0.0155	0.9835	1	0.08949	0.263827
29	LOC_Os02g34490	6.283	0.02201	0.9835	1	0.10351	0.258734
30	LOC_Os02g34850	6.283	0.02201	0.9835	1	0.10351	0.258734
31	LOC_Os02g35210	6.283	0.02201	0.9835	1	0.10351	0.258734
32	LOC_Os12g07800	6.451	0.02053	0.9835	1	0.10351	0.255983
33	LOC_Os12g06740	6.451	0.02053	0.9835	1	0.10351	0.242599
34	LOC_Os08g19694	6.193	0.02284	0.9835	1	0.10361	0.241195
35	LOC_Os08g20020	5.721	0.02788	0.9835	1	0.11423	0.241195
36	LOC_Os08g30850	5.721	0.02788	0.9835	1	0.11423	0.187053
37	LOC_Os08g30910	5.765	0.02736	0.9835	1	0.11423	0.187053
38	LOC_Os06g19110	3.852	0.06535	0.9835	1	0.20748	0.17626
39	LOC_Os06g22020	3.852	0.06535	0.9835	1	0.20748	0.17626
40	LOC_Os06g22460	3.852	0.06535	0.9835	1	0.20748	0.17626
41	LOC_Os06g23530	3.852	0.06535	0.9835	1	0.20748	0.17626
42	LOC_Os06g28124	3.852	0.06535	0.9835	1	0.20748	0.17626
43	LOC_Os06g28670	3.852	0.06535	0.9835	1	0.20748	0.17626
44	LOC_Os06g29700	3.852	0.06535	0.9835	1	0.20748	0.17626
45	LOC_Os06g29844	4.142	0.05684	0.9835	1	0.20748	0.17626
46	LOC_Os03g43684	4.142	0.05684	0.9835	1	0.20748	0.165702
47	LOC_Os06g31070	3.575	0.07486	0.9835	1	0.23189	0.120051
48	LOC_Os06g32350	2.204	0.15499	0.9835	1	0.33529	0.120051
49	LOC_Os04g10460	2.204	0.15499	0.9835	1	0.33529	0.117506
50	LOC_Os04g11640	2.204	0.15499	0.9835	1	0.33529	0.117506
51	LOC_Os04g11970	2.204	0.15499	0.9835	1	0.33529	0.117506
52	LOC_Os04g15650	2.397	0.13899	0.9835	1	0.33529	0.117506
53	LOC_Os04g20680	2.397	0.13899	0.9835	1	0.33529	0.117506
54	LOC_Os04g21890	2.397	0.13899	0.9835	1	0.33529	0.117506
55	LOC_Os04g23620	2.397	0.13899	0.9835	1	0.33529	0.117506
56	LOC_Os04g23890	2.397	0.13899	0.9835	1	0.33529	0.117506

Table 3.2 Continued

Rank	Marker	F Value	Raw_P	Hochberg p-value	Stepdown Bonferroni	False Discovery Rate p-value	R-Squared
57	LOC_Os09g16540	2.397	0.13899	0.9835	1	0.33529	0.109532
58	LOC_Os09g17600	2.397	0.13899	0.9835	1	0.33529	0.109532
59	LOC_Os09g17630	2.397	0.13899	0.9835	1	0.33529	0.109532
60	LOC_Os03g30130	2.456	0.13451	0.9835	1	0.33529	0.109069
61	LOC_Os03g37720	2.456	0.13451	0.9835	1	0.33529	0.109069
62	LOC_Os03g39150	2.166	0.1584	0.9835	1	0.33529	0.109069
63	LOC_Os03g40250	2.166	0.1584	0.9835	1	0.33529	0.109069
64	LOC_Os08g10560	2.214	0.15407	0.9835	1	0.33529	0.107387
65	LOC_Os08g12800	2.214	0.15407	0.9835	1	0.33529	0.107387
66	LOC_Os08g13870	2.214	0.15407	0.9835	1	0.33529	0.107387
67	LOC_Os03g63110	1.475	0.24022	0.9835	1	0.46294	0.076657
68	LOC_Os02g42412	1.473	0.24058	0.9835	1	0.46294	0.075747
69	LOC_Os02g44730	1.473	0.24058	0.9835	1	0.46294	0.075636
70	LOC_Os02g45160	1.473	0.24058	0.9835	1	0.46294	0.075636
71	LOC_Os02g45980	1.473	0.24058	0.9835	1	0.46294	0.075636
72	LOC_Os02g48210	1.494	0.2373	0.9835	1	0.46294	0.075636
73	LOC_Os08g42930	1.203	0.2872	0.9835	1	0.54439	0.062643
74	LOC_Os11g13650	1.043	0.32068	0.9835	1	0.59891	0.062643
75	LOC_Os02g58540	0.929	0.34792	0.9835	1	0.63123	0.054766
76	LOC_Os01g13300	0.929	0.34792	0.9835	1	0.63123	0.049072
77	LOC_Os04g56250	0.898	0.35586	0.9835	1	0.63654	0.049072
78	LOC_Os08g35310	0.851	0.36855	0.9835	1	0.64118	0.04752
79	LOC_Os02g39590	0.851	0.36855	0.9835	1	0.64118	0.045129
80	LOC_Os02g51900	0.753	0.39685	0.9835	1	0.68108	0.045129
81	LOC_Os02g52060	0.615	0.44303	0.9835	1	0.70947	0.045129
82	LOC_Os02g02650	0.536	0.47334	0.9835	1	0.70947	0.04017
83	LOC_Os02g43460	0.536	0.47334	0.9835	1	0.70947	0.033049
84	LOC_Os02g53970	0.536	0.47334	0.9835	1	0.70947	0.02894
85	LOC_Os02g54330	0.536	0.47334	0.9835	1	0.70947	0.02894
86	LOC_Os02g54500	0.533	0.47484	0.9835	1	0.70947	0.02894
87	LOC_Os02g55180	0.533	0.47484	0.9835	1	0.70947	0.02894
88	LOC_Os04g57670	0.533	0.47484	0.9835	1	0.70947	0.028746
89	LOC_Os04g58720	0.533	0.47484	0.9835	1	0.70947	0.028746
90	LOC_Os04g58820	0.533	0.47484	0.9835	1	0.70947	0.028746
91	LOC_Os04g58910	0.533	0.47484	0.9835	1	0.70947	0.028746
92	LOC_Os04g59060	0.508	0.48531	0.9835	1	0.71668	0.028746
93	LOC_Os04g59540	0.433	0.51884	0.9835	1	0.74688	0.028746
94	LOC_Os05g50660	0.424	0.5234	0.9835	1	0.74688	0.028746
95	LOC_Os09g26300	0.424	0.5234	0.9835	1	0.74688	0.027427
96	LOC_Os04g05030	0.343	0.56525	0.9835	1	0.74778	0.023491
97	LOC_Os05g37040	0.343	0.56525	0.9835	1	0.74778	0.022989
98	LOC_Os05g39760	0.356	0.55839	0.9835	1	0.74778	0.022989
99	LOC_Os08g36320	0.362	0.55499	0.9835	1	0.74778	0.020628
100	LOC_Os08g36760	0.379	0.54578	0.9835	1	0.74778	0.020628
101	LOC_Os09g27570	0.379	0.54578	0.9835	1	0.74778	0.020628
102	LOC_Os06g44820	0.379	0.54578	0.9835	1	0.74778	0.019706
103	LOC_Os02g49986	0.137	0.71588	0.9835	1	0.85611	0.019372
104	LOC_Os02g09820	0.137	0.71588	0.9835	1	0.85611	0.018711
105	LOC_Os02g10120	0.137	0.71588	0.9835	1	0.85611	0.018711
106	LOC_Os02g56380	0.127	0.7261	0.9835	1	0.85611	0.007887
107	LOC_Os02g56480	0.143	0.70964	0.9835	1	0.85611	0.007887
108	LOC_Os02g57960	0.143	0.70964	0.9835	1	0.85611	0.007887
109	LOC_Os06g35850	0.143	0.70964	0.9835	1	0.85611	0.007887
110	LOC_Os06g37500	0.143	0.70964	0.9835	1	0.85611	0.007887
111	LOC_Os01g52330	0.112	0.74152	0.9835	1	0.85611	0.007538
112	LOC_Os01g52880	0.112	0.74152	0.9835	1	0.85611	0.007538
113	LOC_Os01g53420	0.112	0.74152	0.9835	1	0.85611	0.007538

Table 3.2 Continued

Rank	Marker	F Value	Raw_P	Hochberg p-value	Stepdown Bonferroni	False Discovery Rate p-value	R-Squared
114	LOC_Os09g25620	0.143	0.70964	0.9835	1	0.85611	0.007307
115	LOC_Os09g25890	0.143	0.70964	0.9835	1	0.85611	0.007307
116	LOC_Os02g10900	0.139	0.71698	0.9835	1	0.85611	0.006985
117	LOC_Os05g40790	0.132	0.7201	0.9835	1	0.85611	0.006195
118	LOC_Os05g41130	0.132	0.7201	0.9835	1	0.85611	0.006195
119	LOC_Os05g41290	0.132	0.7201	0.9835	1	0.85611	0.006195
120	LOC_Os11g19700	0.042	0.83928	0.9835	1	0.89571	0.002629
121	LOC_Os11g24060	0.042	0.83928	0.9835	1	0.89571	0.002629
122	LOC_Os11g24180	0.042	0.83928	0.9835	1	0.89571	0.002629
123	LOC_Os11g24770	0.042	0.83928	0.9835	1	0.89571	0.002629
124	LOC_Os11g28950	0.047	0.83001	0.9835	1	0.89571	0.002629
125	LOC_Os03g53220	0.047	0.83001	0.9835	1	0.89571	0.002347
126	LOC_Os03g56400	0.047	0.83001	0.9835	1	0.89571	0.002347
127	LOC_Os03g57160	0.047	0.83001	0.9835	1	0.89571	0.002347
128	LOC_Os03g58390	0.047	0.83001	0.9835	1	0.89571	0.002347
129	LOC_Os04g55760	0.031	0.86218	0.9835	1	0.91248	0.00172
130	LOC_Os02g11820	0	0.9835	0.9835	1	0.9835	0.000065
131	LOC_Os01g54350	0	0.9835	0.9835	1	0.9835	0.000024
132	LOC_Os01g54515	0	0.9835	0.9835	1	0.9835	0.000024
133	LOC_Os01g55050	0	0.9835	0.9835	1	0.9835	0.000024
134	LOC_Os01g56040	0	0.9835	0.9835	1	0.9835	0.000024
135	LOC_Os01g57230	0	0.9835	0.9835	1	0.9835	0.000024
136	LOC_Os01g57900	0.001	0.97305	0.9835	1	0.9835	0.000024

3.4 Discussion

SB2 produced by the RiceCAP project is a doubled-haploid (DH) population from the Cocodrie x MCR10277 cross, where MCR10277 is the resistance donor. This population was selected for this study because it has been well characterized and studied in multiple environments for SB (Silva *et al*., 2011; Nelson *et al*., 2012). The objective of this study was to identify the most important genomic regions involved in SB resistance based on SNP-based markers using the selective genotyping (SG) approach with the most susceptible and the most resistant lines from SB2. Thus, the top ranked markers were located at the bottom of the long arm of chromosome 9, confirming the importance of this region for the SB2 population reported by Nelson *et al*. (2012). This QTL on chromosome 9 has been reported in others studies using different populations with different sources of resistance including Teqing (Zuo *et al*., 2014), Jasmine 85 (Liu *et al*., 2009), Jarjan (Taguchi-Shiobara *et al*., 2013), Minghui 63 (Han *et al*.,

2003), and Pecos (Sharma *et al*., 2009). Meanwhile, the region in the middle of short arm of chromosome 12 ranging from the locus LOC_Os12g10330 to LOC_Os12g15460 is the second most important in the ranking, consistent with the multi-environment interval mapping analysis of SB2 reported by Nelson *et al*. (2012). This region was also identified as important in other studies (Li *et al*., 1995, Sato *et al*., 2004, Wang *et al*., 2012) These two regions contain genes related to disease resistance in plants including kinases, NBS-LRR, NB-ARC, and signaling and activation factors (See Table 2.2, Chapter 2).

R^2 values for 9 markers located in chromosomes 2, 6, and 8 were identified, based on p values <0.05, as being associated with QTLs in these regions reported by Nelson *et al*. (2012) for the SB2 population. QTLs in this region of chromosome 2 have been also reported by Sharma *et al.* (2009), Liu *et al.* (2009), Pinson *et al.* (2005), Zou *et al.* (2000), and Kunihiro *et al.* (2002). In chromosome 6, the selected markers were located in QTLs previously described by Liu *et al.* (2009), Pinson *et al.*(2005), and Xie *et al*. (2008). Finally, the region on chromosome 8 identified in this study was associated with QTLs reported by Pinson *et al*. (2005), Channamallikarjuna *et al*. (2009), and Xie *et al*. (2008).

QTL discovery typically has required intense efforts in genotyping of hundreds of individuals from segregating populations (Bernardo, 2008), and use of molecular markers such as SSRs that sometimes lack polymorphism in certain genomic regions that reduce resolution and accuracy of mapping. Selective genotyping (SG) has been shown to be an effective strategy for QTL identification (Sun *et al*. 2010, Lambel *et al*. 2014, Navabi *et al*. 2009). Results obtained in this study demonstrated that regions identified by Nelson *et al*. (2012) using the whole SB2 mapping population could be identified using 20 individuals from extreme phenotypes of the same population. All top-ranked markers mentioned above were detected in exons of genes

reported to be involved in disease resistance including kinases, nucleotide binding proteins and various regulatory factors. Therefore, the generation of allele-specific markers based on nsSNP plus SG may accelerate and reduce cost of gene discovery research in rice and other crop plants.

3.5 References

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CHAPTER 4. EVALUATION OF RESISTANCE OF DOUBLED-HAPLOID LINES CONTAINING SELECTED SNPS UNDER FIELD AND GREENHOUSE CONDITIONS.

4.1 Introduction

Sheath blight disease in rice (SB) is caused by the necrotrophic fungus *Rhizoctonia solani* Kuhn, anastomosis group 1 IA (AG-1 IA). Under favorable conditions of high humidity, temperature and planting density, the disease can cause 50% yield loss across different rice growing regions (Lee and Rush, 1983). In Louisiana the most popular rice varieties are rated as very susceptible to moderately susceptible with reductions in grain yield ranging from 5 to 35% (LSU AgCenter, 2014). To develop varieties resistant to SB, researchers have used traditional breeding methods with encouraging results. Rush *et al*. (2011) registered 25 resistant and moderately resistant lines using modified recurrent selection and backcrossing methods over a period of some 25 years. Ongoing challenges to develop resistant commercial varieties are due to relatively few resistant sources and to the quantitative nature of this host-pathogen interaction (Yadav *et al*., 2015).

Certain wild *Oryza* species have been reported as sources of SB resistance (Prasad and Eizenga, 2008), but high levels of incompatibility have been routinely encountered in interspecific crosses such as indica x japonica. Moreover, wild species as well as certain indica and japonica accessions contain undesirable traits that may be linked to desirable traits adapted to a specific region or location (Ouyang *et al*., 2010). Most sources of SB resistance are derived from indica accessions while in Louisiana the commercial inbred varieties are typically tropical japonica. Combining desirable genes such as SB resistance with acceptable agronomic traits in new lines is a major challenge for breeders. Therefore, strategies such as marker assisted selection combined with cell culture techniques may be required to overcome these challenges.

Mapping quantitative trait loci (QTLs) has been a promising approach to identify genomic regions involved in certain traits and to quantify their effects. Molecular markers associated with QTLs can be used for marker assisted selection to accelerate the breeding process (Collard *et al*., 2005). During the last 20 years, plant scientists have identified more than 1200 QTLs for important crops such as rice, wheat, maize, etc. (Bernardo, 2008). Some 50 QTLs have been reported for SB alone using different populations (Yadav *et al*., 2015). However, few markers associated with these QTLs have been applied to reduce SB levels in elite breeding materials. In most cases, where QTL-associated markers have been used to assist the breeding process, decreases in SB severity by introgression approaches have been modest (Chen *et al*., 2014; Zuo *et al*., 2014; Zuo *et al*., 2011; Wang *et al*., 2012). In these works, only one (Zuo *et al.*, 2011) or two (Chen *et al.*, 2014; Zou *et al*., 2014; Wang *et al*., 2012) QTLs were introgressed to increase levels of resistance. However, the maximum disease reduction was only 1.7 on a 0-9 scale by introgression of QTLs *qSB-7* and *qSB-9* (Chen *et al*., 2014). Approximately ten QTLs were detected in most studies focused on sheath blight resistance (Tagushi-siobara *et al*., 2013; Yadav *et al*., 2015; Liu *et al.*, 2009; Jia *et al*., 2012; Nelson *et al*., 2012). Therefore, low reduction of disease rates after introgression of one or two QTLs in susceptible materials is not surprising, assuming that interactions between various QTLs are required to produce significant change in the response to SB as suggested by Liu *et al*. (2014).

QTL mapping results tend to be inconsistent due to variable environments across locations that generate strong QTL x E effects (Wang *et al*., 2014). Reported QTLs effects for SB resistance depend not only on susceptibility of the host lines, but also favorable conditions for successful and consistent infection levels by *R. solani* (Park *et al*., 2008). For instance, Zeng *et al.* (2015) mapped QTLs for SB resistance in a doubled-haploid population in three different

environments, but major QTLs displayed different effects among different environments. These environmental effects resulted in reduced accuracy for selection of SB resistance. Alternative methods in more controlled environments have been tested in SB resistance research programs to increase accuracy and reproducibility of results. Mist chamber, micro-chamber, detached leaf, parafilm sachet and aluminum foil methods were described for Yia *et al*. (2013) for evaluation of sheath blight disease infection severity.

Combining the alleles required for complex traits is difficult using traditional breeding methods. SB resistance is not maintained through selection cycles due to segregation of the favorable alleles. To avoid the losses of alleles by segregation it is necessary to stabilize the genotypes producing homozygous lines. Anther culture method allows production of doubled-haploid pure lines in only one generation (Reiffers and Freire, 1989). Thus, individuals containing desired allele combinations can be obtained and propagated in an efficient manner. For instance, Mia *et al*. (1996) obtained homozygous salt tolerant lines of rice by anther culture from two different crosses between salt tolerant and salt sensitive lines. This technique has been also important for the development of rice varieties for the southern U.S. (Sha *et al*., 2006).

From the information and published literature described above, it is clear that breeding for SB resistance requires strategies that combine different approaches. The main objective of research described in this chapter is to evaluate the potential of combining genomic and standard breeding approaches to develop SB resistant lines. The combined approach involves development of populations using different SB resistance donors crossed with different susceptible varieties, marker assisted backcrossing using the nsSNP-based markers selected in Chapter 3, visual selection for agronomic traits, anther culture, and evaluation under field and mist chamber environments.

4.2 Materials and Methods

4.2.1 Plant Material and Population Development

As described in Chapter 3, a total of 136 SNP-based markers were evaluated in selected individuals with extreme phenotypes for SB resistant from the DH SB2 population derived from the cross Cocodrie x MCR10277. To extend marker analysis to other populations with different resistant sources, backcross populations were initiated in 2011 using the following lines with known high levels of resistance to SB: MCR010277 (GSOR 200327), YD4 (Chinese line from unknown source), Jasmine 85 (PI 595927), Araure 3 (F. Correa, unpublished), Oryzica Llanos 5 (GSOR 301111), line SB-3 from the SB2 population (see Chapter 3), PI 658335 (Rush *et al*., 2011), and known susceptible Louisiana varieties Cocodrie (PI 606331), Catahoula (PI 654462), CL151 (PI 654463) and Cypress (PI 561734) (Figure 4.1) Twenty three crosses between resistant and susceptible lines were made (Table 4.1), and 76 F_1 progeny were backcrossed to the four susceptible parents. A total of 422 BC_1F_1 individuals derived from 76 different crosses were screened with eight selected SB markers (two per each of the four most important regions identified in Chapter 3). Individuals containing the greatest number of the resistant alleles (between 4 and 8) were selected for an additional backcross to the respective recurrent parent. A total of 7062 BC_2F_1 plants were also screened with the eight selected SB markers. Twenty eight plants containing different combinations of resistant alleles and with acceptable agronomic traits, as well as four individuals containing no resistant alleles were selected for production of doubled- haploids by anther culture using the method described by Chu *et al*. (1998) with the assistance of Ms. Mona Meche in the anther culture lab in the LSU AgCenter Rice Research

Table 4.1 List of crosses and number of F_1, BC_1F_1, and BC_2F_1 and DH lines produced from each cross.

Susceptible parent	Resistant parent	Number of seeds produced	Number of F_1 backcrossed	BC_1F_1 produced	BC_2F_1 produced	DH lines produced
Cocodrie	MCR	30	7	32	631	12
Cocodrie	Jasmine 85	3	0	0	0	0
Cocodrie	Araure 3	7	4	6	97	0
Cocodrie	Oryzica Llanos 5	26	4	13	327	0
Cocodrie	YD4	15	3	18	415	2
Cocodrie	SB2-3	10	3	9	290	7
Cocodrie	PI 658335	13	6	34	457	1
Catahoula	MCR	17	7	71	805	0
Catahoula	Araure 3	7	4	15	336	0
Catahoula	Oryzica Llanos 5	24	6	25	601	8
Catahoula	YD4	13	2	6	339	0
Catahoula	SB2-3	32	8	27	372	14
Catahoula	PI 658335	21	6	31	362	0
Cypress	MCR	5	1	3	39	0
Cypress	Araure 3	6	2	16	324	1
Cypress	Oryzica Llanos 5	1	0	0	0	0
Cypress	YD4	15	5	26	621	0
Cypress	SB2-3	2	0	0	0	0
Cypress	PI 658335	4	0	0	0	0
CL151	MCR	9	2	33	472	0
CL151	Araure 3	16	5	51	487	0
CL151	Oryzica Llanos 5	8	0	0	0	0
CL151	SB2-3	14	1	6	87	0
Total		298	76	422	7062	45

Station at Crowley, LA. Regenerated plants from calli were planted in the greenhouse to select only "true" doubled haploids based on morphological characteristics. Thus, very small and weak plants were considered haploids, and individuals with very long, wide leaves were considered tetraploids. Seeds from the 45 DH plants derived from seven original crosses with six different donor parents were collected and planted for seed multiplication to evaluate for SB under field plot and greenhouse conditions.

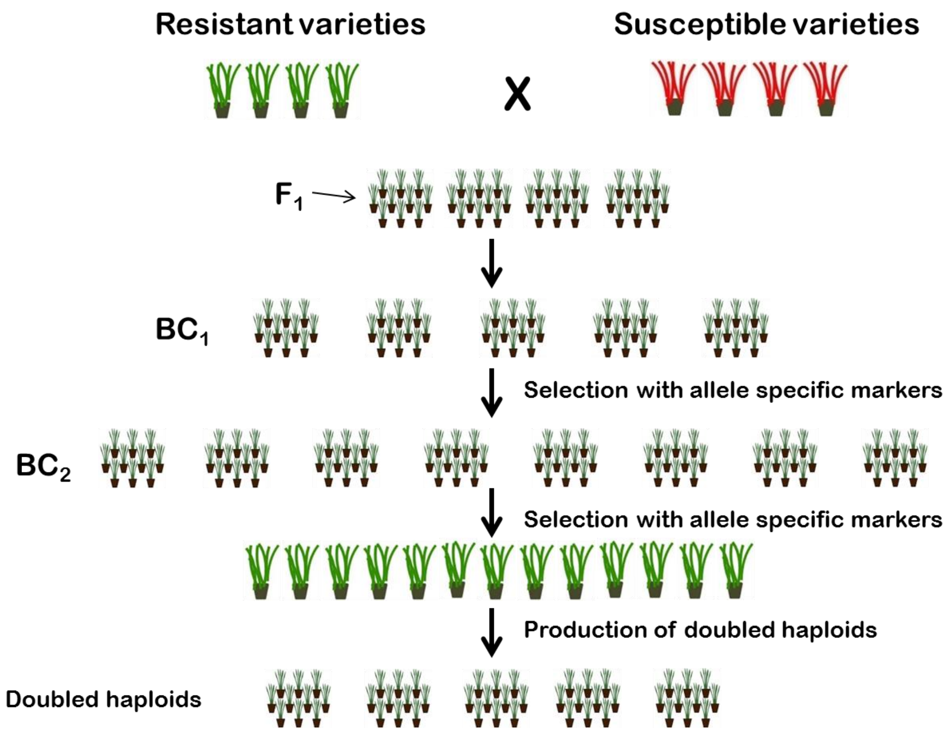

Figure 4.1 Schematic representation of the backcrossing and doubled-haploid development. 23 susceptible x resistant crosses were made using susceptible varieties Cocodrie, Catahoula, CL151 and Cypress, and the resistant lines MCR010277, YD4, Jasmine85, Araure 3, Oryzica Llanos 5, line SB-3 from the RiceCAP SB2 population and PI 658335. F_1 progenies were backcrossed to their respective susceptible parents. BC_1F_1 progenies were genotyped with eight SB resistance markers. Individuals containing the greatest number of selected SB resistance markers were backcrossed again to create the BC_2 F_1. Selection was based on nsSNP markers, plant height and overall plant type. Panicles from selected plants in booting stage were collected for production of DH by anther culture. Fertile, regenerated lines were subsequently evaluated under field and greenhouse conditions for SB resistance.

4.2.2 Marker-assisted Selection

DNA was extracted from BC_1F_1 and BC_2F_2 plants one week after transplanting in the field using the MATAB method described by Romero *et al*. (2014). Individuals were genotyped with eight of the top-ranked nsSNP-based markers located on chromosome 6, 8, 9 and 12 present in candidate genes for SB resistance (see Chapter 3). Only two markers per selected genomic region in four chromosomes were used for selection to reduce genotyping efforts:

LOC_Os09g32860 and LOC_Os09g38710 located at the bottom of the long arm on chromosome 9, LOC_Os12g06980 and LOC_Os12g15460 located in the middle of the short arm on chromosome 12, LOC_Os06g13040 and LOC_Os06g28670 located in the middle of short arm on chromosome 6, and LOC_Os08g10560 and LOC_Os08g20020 located on the short arm of chromosome 8. It is important to note that genetic material developed during the marker-assisted selection phase was not inoculated with *R. solani* to identify SB resistant backcrossed or DH lines.

4.2.3 Mist Chamber Evaluations

Mist chambers assays were performed during the month of October, 2014 in a greenhouse located on the LSU campus in Baton Rouge, LA with temperature inside the chamber ranging from minimum 27 ºC in the night to maximum 37 ºC in the day. Natural daylight was used with day length was approximately 11 hours 30 minutes. Humidity was maintained 80-90% using a cool mist humidifier of 1.2 gallons capacity (Vicks) that was programmed to function for a two hour period every six hours. The chamber frame was constructed with ¾ inch PVC pipe (Charlotte Pipe ®) covered by extra light plastic (0.31 mm) (Painter's Plastic – Poly America). Dimensions of the chamber were: 1.32 m wide, 2.70 m length, and 1.42 m height, (Figure 4.2) for a total capacity of 48 pots per chamber, each pot containing three plants.

A total of 48 lines including 45 selected DH lines plus MCR10277 and Oryzica Llanos 5 as resistant controls and Cocodrie parent as susceptible check, were planted in August 2014 under greenhouse conditions. Each pot contained three plants per line with the same lines replicated in a second mist chamber. Plants were inoculated 50 days after germination with a PDA medium disc (0.8 cm diameter) containing *Rhizoctonia solani* (LR172) mycelia placed at the base of the

Figure 4.2 Mist chambers with capacity for 48 plants per chamber. Dimensions: 1.32 m wide, 2.70 m length, and 1.42 m height.

stem and other disc placed between leaf blade and leaf sheath in the primary tiller of each plant. Inoculated plants were placed in the mist chambers for ten days, removed for 5 days, and placed again in the chamber for ten additional days as is described by Jia *et al*. (2013). After the incubation period was complete, effect of the fungus was evaluated by visually scoring disease on a 0-9 scale where 0 = not disease present and 9 = dead plant (Figure 4.3). Plant height (PH) and heading data (HD) was also recorded to determine the correlation between PH and HD with the incidence of SB disease. Pearson's coefficient of correlation was evaluated using the PROC CORR procedure in SAS 9.4 (SAS Institute, Cary, NC).

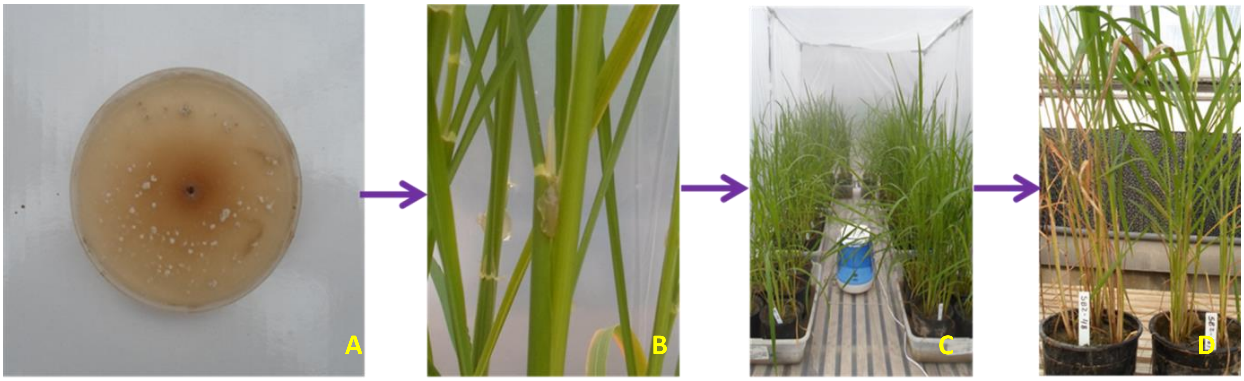

Figure 4.3 Inoculation of rice plants with *R. solani* in mist chamber. (A) Mycelia growing in PDA medium. (B) Agar discs containing mycelia are located between sheath and culm. (C) Plants placed in trays inside mist chamber (27 – 37 °C, 80-90 % humidity). (D) Lesions and necrotic tissue formed 21 days after inoculation, susceptible plant (left), resistant plant (right).

4.2.4 Field Evaluations

The 45 DH lines evaluated in the mist chamber described above plus resistant and susceptible reference varieties were evaluated under field conditions in the LSU AgCenter Rice Research Station in Crowley, LA during the summers of 2014 and 2015. Seeds were planted in two, one meter rows per line with two replications. The rows were inoculated with a moist grain/grain hull mixture (1:2) containing *Rhizoctonia solani* (LR172) mycelia as described by Groth (2005). The inoculum was uniformly applied by hand at the late-tillering stage. Disease incidence was scored using the 0-9 scale at the soft-dough stage of plant maturity (~90 days after planting).

4.2.5 Genotyping of DH Lines and Registered SB Resistant Lines Previously Registered

The 45 DH lines generated in this study and the 25 SB resistant lines reported by Rush *et al*. (2011) were genotyped using 30 of the SB resistance SNP-based markers (see Chapter 3) in the principal genomic regions containing QTLs. Thus, LOC_Os02g34490 and LOC_Os02g34850 from chromosome 2, LOC_Os04g10460 and LOC_Os04g20680 from

chromosome 4, LOC_Os06g13040, LOC_Os06g15170, LOC_Os06g22020, LOC_Os06g28124 from chromosome 6, LOC_Os08g19694 and LOC_Os08g20020 from chromosome 8, LOC_Os09g32860, LOC_Os09g34180, LOC_Os09g36900, LOC_Os09g37230, LOC_Os09g37590, LOC_Os09g37800, LOC_Os09g37880, LOC_Os09g38700, LOC_Os09g38710, LOC_Os09g38850, LOC_Os09g38970 and LOC_Os09g39620 from chromosome 9, and LOC_Os12g06980, LOC_Os12g07950, LOC_Os12g09710, LOC_Os12g10180, LOC_Os12g10330, LOC_Os12g10410, LOC_Os12g13100 and LOC_Os12g15460 from chromosome 12 were screened to identify resistant alleles introgressed into these lines.

4.3 Results

4.3.1 Marker-Assisted Backcrossing and Doubled-Haploids Production

Twenty three different susceptible x resistant crosses were made (Table 4.1). A total of 76 F_1 plants from all crosses were backcrossed to the four susceptible parents (Cocodrie, CL151, Catahoula and Cypress). A total of 422 BC_1F_1 and 7,062 BC_2F_1 individuals were obtained. From the 422 BC_1F_1 individuals, 178 plants containing a range of 4 to 8 resistant alleles were used for backcrossing to susceptible varieties to generate 535 BC_2F_1 populations for a total of 7,062 individuals. Some 326 BC_2F_1 individuals containing resistant alleles were identified from which 28 contained the greatest number of resistant alleles (between 4 and 8), in the regions located on chromosome 6, 8, 9 and 12. Selection of the 28 plants was also based on improved height and overall plant type. Panicles from the 28 BC_2F_1 containing the resistant alleles, and from four individuals containing only susceptible alleles, were collected in booting stage for anther culture and DH production.

Table 4.2 Pedigree and SB rating of the 45 selected DH lines BC $_2F_1$.

DH Line	Recurrent susceptible	Resistant Donor	Backcross	Average rating[a]
533-7-1	CCDR	PI 658335	BC2	3.67
129-4-3-25	CPRS	Oryzica Llanos 5	BC2	4.33
256-11-1	CTHL	SB2-3	BC2	4.4
256-5-11-13	CTHL	SB2-3	BC2	4.47
256-5-11-20	CTHL	SB2-3	BC2	4.47
129-4-3-10	CPRS	Oryzica Llanos 5	BC2	4.65
129-4-3-1	CPRS	Oryzica Llanos 5	BC2	4.67
129-4-3-2	CPRS	Oryzica Llanos 5	BC2	4.67
256-5-11-3	CTHL	SB2-3	BC2	4.73
129-4-11	CPRS	Oryzica Llanos 5	BC2	4.83
129-4-3-26	CPRS	Oryzica Llanos 5	BC2	4.92
129-4-3-6	CPRS	Oryzica Llanos 5	BC2	4.92
256-5-11-19	CTHL	SB2-3	BC2	5.00
539-7-3	CCDR	SB2-3	BC2	5.00
124-4-3-24	CCDR	MCR	BC2	5.07
193-10-11-1	CPRS	Araure 3	BC2	5.15
539-7-2	CCDR	SB2-3	BC2	5.17
129-4-3-14	CCDR	Oryzica Llanos 5	BC2	5.25
256-11-13	CTHL	SB2-3	BC2	5.57
539-7-7	CCDR	SB2-3	BC2	5.67
256-5-11-6	CTHL	SB2-3	BC2	5.73
539-7-1	CCDR	SB2-3	BC2	5.83
256-5-11-2	CTHL	SB2-3	BC2	5.92
12-11-004	CCDR	MCR	BC2	6.17
256-5-11-4	CTHL	SB2-3	BC2	6.17
112-11-1	CCDR	MCR	BC2	6.50
112-11-32	CCDR	MCR	BC2	6.50
112-11-33	CCDR	MCR	BC2	6.50
112-11-8	CCDR	MCR	BC2	6.50
539-9-6	CCDR	SB2-3	BC2	6.58
539-9-13	CCDR	SB2-3	BC2	6.67
539-9-2	CCDR	SB2-3	BC2	6.67
152-2-3	CTHL	SB2-3	BC2	6.73
112-11-30	CCDR	MCR	BC2	6.75
112-11-7	CCDR	MCR	BC2	6.83
175-6-3	CTHL	SB2-3	BC2	6.83
98-1-1	CCDR	YD4	BC2	6.83
98-1-2	CCDR	YD4	BC2	6.92
12-11-002	CCDR	MCR	BC2	7.00
175-6-2	CTHL	SB2-3	BC2	7.08
12-11-005	CCDR	MCR	BC2	7.25
152-2-15	CTHL	SB2-3	BC2	7.25
175-6-1	CTHL	SB2-3	BC2	7.25
12-11-006	CCDR	MCR	BC2	7.50
112-11-6	CCDR	MCR	BC2	7.57

[a] Rating based in a 0-9 visual scale under field and mist chamber conditions, where 0 indicates absence of disease and 9 indicates dead plant.

production. A total of 442 plants were regenerated by anther culture and transplanted into the greenhouse, from which ~ 41 % were considered haploids because they were very small and weak, ~18% died before flowering, and 30 % did not produce seed. Finally, 45 DH regenerated plants produced seeds that were planted for multiplication. Pedigree of the 45 DH lines is shown in Table 4.2.

4.3.2 Evaluation of SB-DH lines Under Field and Mist Chamber Environments

Ratings for incidence of SB disease in the SB-DH lines under field and mist chamber conditions, and plant height (PH) and heading date (HD) are shown in Table 4.3. Analysis of variance indicated that there was not a significant difference between incidence ratings among the 2014 and 2015 field studies and the mist chamber environment (p value = 0.083). Inoculations were successful in both field and mist chamber conditions with lesions on susceptible material detected 5-7 days after inoculation. Lesions produced by *R. solani* in DH lines under mist chamber conditions are shown in Figure 4.4. Consistency between field and mist chamber results shows the practical utility of the mist chamber essays for SB studies. Pearson correlation coefficients indicate that there was no correlation between PH and SB rates in any of the three environments (field 2014 = -0.1351, field 2015= -0.36331 and mist chamber= 0.02546, $p > 0.05$). However, negative correlations were significant between HD and SB rates in all the environments (field 2014= -0.55555, field 2015= -0.60259, and mist chamber = -0.69122, $p<0.05$). Therefore, resistance to SB was associated with late heading in the selected DH lines, although heading date for 3 of the DH lines with SB rates <5 fell within acceptable maturity range (between 70 – 75 days) for southern U.S. conditions. Fourteen of the DH lines produced average SB scores < 5 (on a 0-9 scale), which indicates a gain in the resistance superior to 2 points on the 0-9 scale compared with the susceptible variety Cocodrie that was rated 7.07 on

average. The most resistant line was 533-7-1, which originated from a Cocodrie x PI658335 (LSBR5/LMNT// TQNG/4/LSBR5/ LMNT /3/ H4CODF //NTAI(03-10993-11019) containing three sources of resistance, LSBR-5, TQNG, and H4CODF in the resistant donor. DH 533-7-1 produced similar scores to the resistant lines MCR10277 and Oryzica Llanos 5 in all three environments (Figure 4.5). The eight lines from the family 129-4-3, originated from a Cypress x Oryzica Llanos 5, also produced low average scores ranging from 4.33 to 5.25. Five lines from the family 256, from a Catahoula x RiceCAP SB2-3, generated "resistant" rates ranging from 4.4

Figure 4.4 Lesions produce by *R. solani* infection of six DH lines 21 days after inoculation in mist chamber. Susceptible lines (upper: Cocodrie, 539-9-6, 112-11-6 and 175-6-2) showing extensive leaf and sheath necrosis, rated between 6.5 and 7.25, 0-9 scale. Resistant lines (lower: MCR, 533-7-1, 129-4-3-2, 256-11-13) moderate lesion formation, rated between 3.25 and 4.35, 0-9 scale.

Table 4.3 SB rating (0-9 scale), plant height (PH), and heading date (HD) for 45 DH-lines, susceptible Cocodrie (CCDR), resistant MCR10277, and resistant Oryzica Llanos 5. Data obtained from field evaluations, Summer 2014, 2015, mist chamber (MC) in Fall 2014.

Line	2014 Field SB Rating	PH Field 2014	HD Field 2014	2015 Field SB Rating	PH Field 2015	HD Field 2015	SB Rating GH	PH MC	HD GH	Average SB rating[a]
OL5	3	117	82	3.5	108	87	3	96	100	3.17
533-7-1	3.5	80	75	4.0	98.5	80	3.5	78	80	3.67
MCR	3.5	93	70	4.5	102.5	75	3	80	75	3.67
129-4-3-25	5.25	95	80	4.3	117	80	3.5	98	82	4.33
256-11-13	3.7	83	69	5.5	90.16	74	4	78	72	4.40
256-5-11-13	3.7	82	80	4.7	99	78	5	80	85	4.47
256-5-11-20	3.7	80	82	5.2	105.5	82	4.5	82	85	4.47
129-4-3-10	4.75	107	80	4.7	123.5	83	4.5	101	79	4.65
129-4-3-1	5	83	72	5.5	99	76	3.5	90	75	4.67
129-4-3-2	5	86	78	6.0	102	81	3	90	82	4.67
256-5-11-3	4.2	91	84	5.5	88.5	86	4.5	90	86	4.73
129-4-11	5	100	76	5.0	111.5	80	4.5	102	80	4.83
129-4-3-26	5.25	90	83	5.5	103.6	82	4	96	79	4.92
129-4-3-6	5.25	100	82	5.5	104.7	80	4	94	81	4.92
256-5-11-19	4.5	83	83	5.5	90	84	5	82	87	5.00
539-7-3	5.5	90	72	5.0	112	74	4.5	94	70	5.00
124-4-3-24	6	97	83	4.7	110	84	4.5	93	81	5.07
193-10-11-1	4.25	82	71	6.2	98	74	5	88	75	5.15
539-7-2	6	96	71	5.0	109	78	4.5	90	75	5.17
129-4-3-14	5.8	96	77	6.0	109	80	4.0	100	76	5.25
256-5-11-1	4.7	93	75	6.5	70	80	5.5	75	83	5.57
539-7-7	6.5	89	72	5.0	109	76	5.5	98	70	5.67
256-5-11-6	4.7	90	69	6.5	93.5	74	6	88	75	5.73
539-7-1	6.5	93	79	5.0	108.5	82	6	90	75	5.83
256-5-11-2	5.25	67	81	6.5	67	84	6	75	85	5.92
12-11-004	6	89	70	6.5	101	69	6	102	72	6.17
256-5-11-4	6	76	81	7.0	68.5	84	5.5	65	83	6.17
112-11-1	6.5	84	72	6.5	101	74	6.5	103	70	6.50
112-11-32	5.5	93	68	7.0	112	73	7	102	68	6.50
112-11-33	6	103	75	7.0	115	80	6.5	78	73	6.50
112-11-8	6.25	88	68	6.8	98.5	71	6.5	80	70	6.50
539-9-6	7	92	68	6.8	92.5	72	6	97	70	6.58
539-9-13	7	85	67	6.8	98	72	6.25	88	70	6.67
539-9-2	6.5	88	67	7.0	96	70	6.5	83	70	6.67
152-2-3	6.7	87	70	7.0	97.7	73	6.5	94	69	6.73
112-11-30	6.75	99	72	7.0	115	76	6.5	92	71	6.75
112-11-7	7.25	92	68	6.5	98.6	70	6.75	92	69	6.83
175-6-3	6.25	83	69	6.8	101	72	7.5	94	68	6.83
98-1-1	6.5	83	68	7.0	99	70	7	88	67	6.83
98-1-2	6.75	87	67	7.0	100	69	7	90	66	6.92
12-11-002	7	94	71	6.5	111	69	7.5	98	70	7.00
CCDR	7.2	88	68	7.0	99	72	7	87	70	7.07
175-6-1	7.25	88	70	7.0	98.5	76	7	89	72	7.08
12-11-005	7.25	80	69	8.0	91	73	6.5	85	72	7.25
152-2-15	7	86	70	7.3	96.5	74	7.5	92	69	7.25
175-6-002	7	82	71	7.3	104.8	74	7.5	92	70	7.25
12-11-006	7.5	79	67	8.0	91.5	70	7	85	69	7.50
112-11-6	7	89	68	8.2	96	74	7.5	89	70	7.57

* SB and agronomic data represent average of two replications from each environment.

to 5. Other materials with acceptable gain in the scores compared with the susceptible control, were DH line 124-4-3-24 (score 5.07) from Cocodrie x MCR10277, DH line 193-10-11-1 (score 5.15) from Cypress x Araure 3, and DH family 539-7 (5.17 to 5.83) from a Cocodrie x SB2-3

cross. Lines originating from the families 12 and 112 (Cocodrie x MCR10277 originated crosses), which contained only susceptible alleles, obtained high scores for SB resistance ranging from 6.17 to 7.57. Figure 4.4 shows disease response after infection by *R. solani* in resistant and susceptible DH lines.

4.3.3 Genotyping of DH Lines and Other SB Resistant Inbred Lines

Genotypes of the 45 DH plus resistant and susceptible controls are shown in Table 4.4 with lines sorted by SB ratings resistant to susceptible. Accumulation of resistant alleles in the best lines for SB resistance is evident. The best DH line 533-7-1, rated 3.67 in average, originated from the Cocodrie x PI 658335 cross, contained sixteen resistant alleles including 10 out of 12 from chromosome 9, two from chromosome 2, two out of four evaluated on chromosome 4 and the two from chromosome 8 (Figure 4.5). Resistant alleles from chromosome 12 are the most abundant among the resistant DH lines. Except for 533-7-1 and 256-5-11-20 all the DH lines with scores < 5 contains groups of resistant alleles from chromosome12. In these lines there was always an association of the presence of chromosome 12 resistant alleles with resistant alleles on chromosome 2, 8 or 9. Among some of the most susceptible lines, there was also a group of the families, 98-1 (Cocodrie x YD4) and 175-6 (Catahoula x SB2-3) that contained resistant alleles in the region on chromosome 12. However, these appear to be associated with resistant alleles on chromosome 8 and in the region on chromosome 9 ranging from LOC_Os09g32860 to the locus LOC_Os09g37590. On the contrary, resistant DH lines with resistant allele introgressions from chromosome 12 were associated with the region on chromosome 9 containing the resistant allele of LOC_Os09g39620 and with the resistant allele

Table 4.4 Genotypes and average SB scores from three environments for DH lines derived from backcrossed lines selected by candidate resistant markers. Resistant alleles (green), susceptible alleles (red). Resistant source of DH line highlighted in yellow = PI 658335. Resistant source of DH lines highlighted in green = Oryzica llanos 5. Resistant source of DH line highlighted in orange = SB2-3. Resistant source of DH line highlighted in purple = Araure 3. Resistant source of DH line highlighted in blue = MCR. Resistant source of DH line highlighted in red = YD4. OL5 = Oryzica Llanos 5, MCR = MCR10277, CCDR = Cocodrie.

DH Line/ Variety	Avg.	LOC_Os02g34490	LOC_Os02g34850	LOC_Os04g10460	LOC_Os04g20680	LOC_Os06g13040	LOC_Os06g15170	LOC_Os06g22020	LOC_Os06g28124	LOC_Os08g19694	LOC_Os08g20020	LOC_Os09g32860	LOC_Os09g34180	LOC_Os09g36900	LOC_Os09g37230	LOC_Os09g37590	LOC_Os09g37800	LOC_Os09g37880	LOC_Os09g38700	LOC_Os09g38710	LOC_Os09g38850	LOC_Os09g38970	LOC_Os09g39620	LOC_Os12g06980	LOC_Os12g07950	LOC_Os12g09710	LOC_Os12g10180	LOC_Os12g10330	LOC_Os12g10410	LOC_Os12g13100	LOC_Os12g15460
OL5	3.17	R	R	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
533-7-1	3.67	R	R	S	S	S	S	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S
MCR	3.67	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
129-4-3-25	4.33	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	S	R
256-11-13	4.40	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R
256-5-11-13	4.47	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R
256-5-11-20	4.47	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S
129-4-3-10	4.65	S	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	S	R
129-4-3-1	4.67	S	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
129-4-3-2	4.67	S	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
256-5-11-3	4.73	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R
129-4-11	4.83	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
129-4-3-26	4.92	S	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	S	R
129-4-3-6	4.92	S	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
256-5-11-19	5.00	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R
539-7-3	5.00	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
124-4-3-24	5.07	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	S	R
193-10-11-1	5.15	R	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S
539-7-2	5.17	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
129-4-3-14	5.25	R	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	S	R
256-5-11-1	5.57	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
539-7-7	5.67	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
256-5-11-6	5.73	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
539-7-1	5.83	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
256-5-11-2	5.92	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
12-11-004	6.17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
256-5-11-4	6.17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
112-11-1	6.50	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
112-11-32	6.50	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
112-11-33	6.50	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
112-11-8	6.50	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
539-9-6	6.58	R	R	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
539-9-13	6.67	R	R	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
539-9-2	6.67	R	R	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
152-2-3	6.73	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
112-11-30	6.75	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
112-11-7	6.83	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
175-6-3	6.83	S	S	S	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
98-1-1	6.83	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R
98-1-2	6.92	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R
12-11-002	7.00	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CCDR	7.07	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
175-6-1	7.08	S	S	S	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
12-11-005	7.25	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
152-2-15	7.25	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
175-6-002	7.25	S	S	S	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
12-11-006	7.50	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
112-11-6	7.57	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

of LOC_Os02g34850. DH line 124-4-3-24, DH line 193-10-11-1, and individuals from family 129-4-3, carried the resistant introgression from chromosome 12 plus resistant allele of LOC_Os02g34850. Individuals from family 256 contained at least the resistant allele of LOC_Os09g39620 for the region detected on chromosome 9. Meanwhile, the DH family 539-7 contained the resistant alleles from chromosome 2, 8, 9 and 12. These results suggest that interaction between resistant alleles from chromosome 12 with LOC_Os02g34850 or LOC_Os09g39620 is important for increasing the resistance to SB.

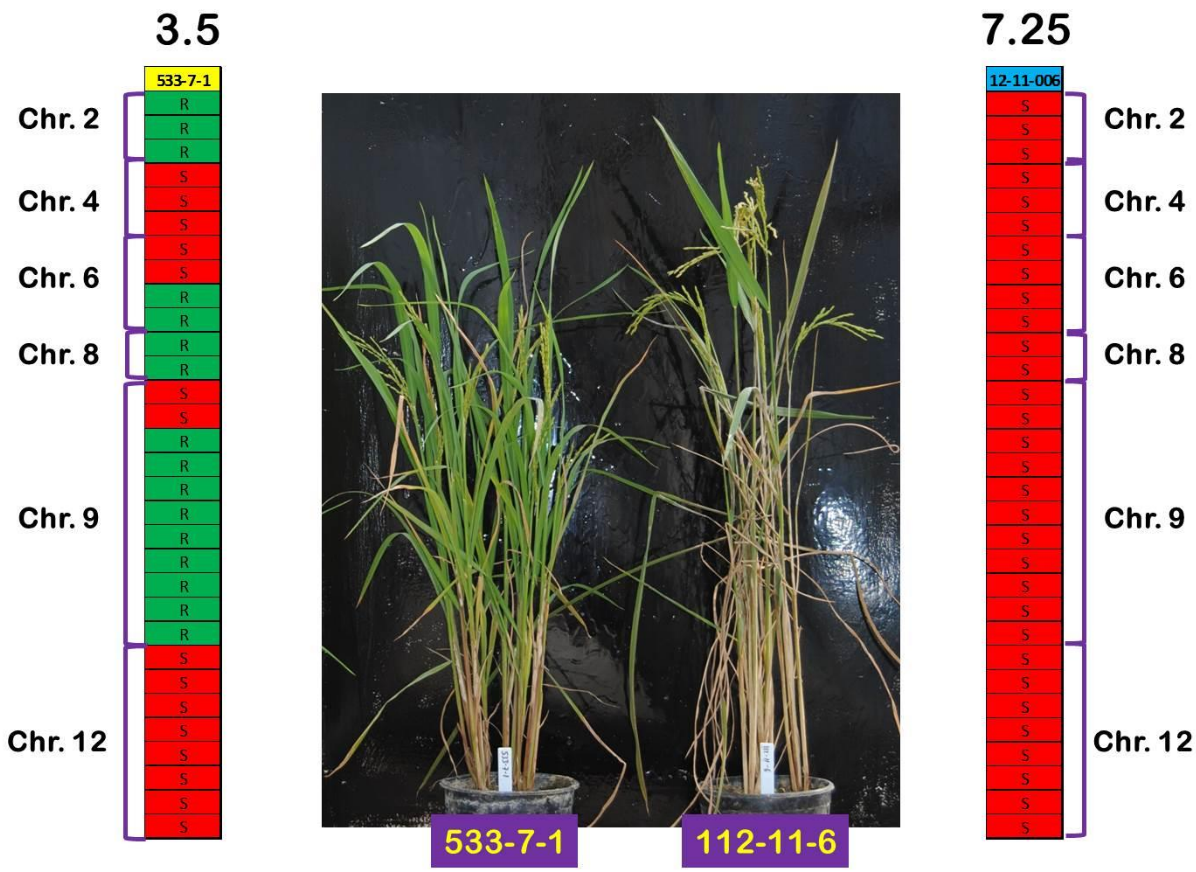

Figure 4.5 SB ratings and chromosomal locations of selected markers for two resistant and susceptible DH lines 21 days after inoculation. Resistant line on left (533-7-1, rated 3.5 in a 0-9 scale under mist chamber conditions) containing resistant alleles for sheath blight in chromosomes 2, 6, 8 and 9 (green cells). Susceptible line on right (112-11-6, rated 7.25 in a 0-9 scale under mist chamber conditions) containing only susceptible alleles (red cells).

Defense mechanisms present in 533-7-1 may be different from the other resistant DH lines as it did not contain any resistant allele from the region in chromosome 12. DH Lines originating from the families 12 and 112 containing only susceptible alleles did not have significant gain in SB resistance. R^2 results showed that LOC_09g39620 (R^2 = 0.32) had the largest effect on the resistance among the markers screened in the 45 DH lines. But the effect was very close to LOC_Os02g34850 (R^2= 0.32), followed by a group of markers in chromosome 12 with R^2 ranging from 0.25 to 0.30 (LOC_12g06980, LOC_12g07950, LOC_12g09710, LOC_12g10180, LOC_12g10330, LOC_12g13100). R^2 for all the 30 markers screened in DH lines are shown in Table 4.5.

Table 4.5 R^2 for the 30 SB markers screened in 45 DH lines (left columns) compared with R^2 for the same markers in SB population (Right columns) (Chapter 3).

SB Marker	DH R-squared		SB Marker	SB2 R-squared
LOC_Os09g39620	0.324952		LOC_Os09g32860	0.892315
LOC_Os02g34850	0.324612		LOC_Os09g34180	0.772268
LOC_Os12g06980	0.296715		LOC_Os09g36900	0.772268
LOC_Os12g10330	0.277838		LOC_Os09g37230	0.772268
LOC_Os12g10410	0.277838		LOC_Os09g37590	0.772268
LOC_Os12g07950	0.253048		LOC_Os09g37800	0.772268
LOC_Os12g09710	0.253048		LOC_Os09g37880	0.772268
LOC_Os12g10180	0.253048		LOC_Os09g38700	0.772268
LOC_Os12g15460	0.253048		LOC_Os09g38710	0.772268
LOC_Os09g38700	0.228112		LOC_Os09g38850	0.772268
LOC_Os09g38710	0.228112		LOC_Os09g38970	0.772268
LOC_Os09g38850	0.228112		LOC_Os12g10330	0.698154
LOC_Os09g38970	0.228112		LOC_Os12g10410	0.698154
LOC_Os06g13040	0.190222		LOC_Os12g13100	0.698154
LOC_Os06g15170	0.190222		LOC_Os12g15460	0.698154
LOC_Os09g37800	0.189654		LOC_Os09g39620	0.673587
LOC_Os09g37880	0.189654		LOC_Os12g09710	0.56581
LOC_Os08g19694	0.175398		LOC_Os12g10180	0.56581
LOC_Os08g20020	0.175398		LOC_Os12g06980	0.483585
LOC_Os12g13100	0.12774		LOC_Os12g07950	0.351999
LOC_Os02g34490	0.088463		LOC_Os04g10460	0.33529
LOC_Os09g37230	0.085229		LOC_Os04g20680	0.33529
LOC_Os09g37590	0.085229		LOC_Os06g13040	0.284212
LOC_Os04g10460	0.074505		LOC_Os06g15170	0.284212
LOC_Os04g20680	0.074505		LOC_Os02g34490	0.258734
LOC_Os06g22020	0.05772		LOC_Os02g34850	0.258734
LOC_Os06g28124	0.05772		LOC_Os08g19694	0.241195
LOC_Os09g36900	0.010557		LOC_Os08g20020	0.241195
LOC_Os09g32860	0.003165		LOC_Os06g22020	0.17626
LOC_Os09g34180	0.000172		LOC_Os06g28124	0.17626

In addition to the DH lines generated in this study, 25 SB resistant inbred lines described by Rush *et al*. (2011) were also genotyped using the same 30 selected SNP-based markers. Results are shown in Table 4.6. Similar to the DH lines results, interaction(s) may occur for the region on chromosome 12 with resistant alleles in other chromosomes from these lines. Eight of the nine lines with resistant alleles introgressed from chromosome 12 contained at least one additional introgression from chromosome 2, 4, 6, 8 or 9.

A majority of the lines (18 out of 25) reported by Rush *et al*. (2011) contained resistant alleles. The seven lines that did not presented resistant alleles of the 30 markers evaluated initially were screened with eight additional nsSNP-based markers located on chromosomes 1, 3, 5, 11, to identify other possible resistant alleles introgressed (Table 4.7). Thus, two resistant alleles on chromosome 11 in three of the lines (PI658326, PI658327, and PI658328) were identified. Two of the donor parents for these lines were Teqing and LSBR-5 and were included in the additional screening. As expected, the indica variety Teqing carried all the resistant alleles as it was one of the varieties used to identify the nsSNPs (see Chapter 2). According to Xia *et al*. (1992) LSBR-5 is a somaclonal mutant derived from the susceptible *japonica* variety Labelle. However, LSBR-5 carried eight resistant alleles of the fourteen markers evaluated suggesting that the origin of LSBR-5 may be from an indica source rather than from the japonica Labelle as reported by Nelson *et al*. (2012). No resistant alleles were found in the lines PI658325, PI658329, PI658330, and PI658334 using the SNP-based markers presented in Table 4.6 and 4.7. Therefore, these four lines presumably carry resistant alleles from other genomic regions not considered in this study.

Table 4.6 Genotypes for the 10 most resistant lines from SB2 population (Chapter 3) and 25 SB resistant inbreed lines (Rush *et al*, 2011) plus MCR10277 (MCR) and Cocodrie (CCDR). Green cells indicate resistant alleles and red cells indicate susceptible alleles.

Lines	SB rating	LOC_Os02 g34490	LOC_Os02 g34850	LOC_Os04 g10460	LOC_Os04 g20680	LOC_Os06 g13040	LOC_Os06 g15170	LOC_Os06 g22020	LOC_Os06 g28124	LOC_Os08 g19694	LOC_Os08 g20020	LOC_Os09 g32860	LOC_Os09 g34180	LOC_Os09 g36900	LOC_Os09 g37230	LOC_Os09 g37590	LOC_Os09 g37800	LOC_Os09 g37880	LOC_Os09 g38700	LOC_Os09 g38710	LOC_Os09 g38850	LOC_Os09 g38970	LOC_Os09 g39620	LOC_Os12 g06980	LOC_Os12 g07950	LOC_Os12 g09710	LOC_Os12 g10180	LOC_Os12 g10330	LOC_Os12 g10410	LOC_Os12 g13100	LOC_Os12 g15480
SB2-3	4.7	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
MCR	3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-109	5.5	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-134	5.5	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-158	5	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-161	5.7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-174	4.5	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-206	6	S	S	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SB2-225	6	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S
SB2-259	5.7	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S
SB2-272	6	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CCDR	7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658312	4.3	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658313	4.5	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658314	4.3	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658315	4.9	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658316	4.6	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658317	4.1	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658318	4.5	R	R	S	S	S	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S
PI658319	5.2	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658320	4.1	S	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658321	4.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658322	3.8	R	R	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658323	4.6	R	R	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S
PI658324	4.3	S	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658325	4.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658326	4.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658327	3.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658328	3.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658329	4.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658330	4.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658331	4.3	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658332	4.5	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658333	5.1	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
PI658334	4.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658335	4.3	R	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PI658336	4.3	S	S	R	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 4.7 Additional markers from chromosomes 1, 3, 5, and 11. Markers from chromosome 8, 9 and12 that were evaluated initially were screened again as controls and to evaluate the genotype of the donor parent LSBR-5 and its assumed origin from the variety Labelle. Susceptible alleles (red) resistant alleles (green).

Rush *et al.* 2011 Lines	LOC_Os01 g52880	LOC_Os01 g54515	LOC_Os03 g40250	LOC_Os03 g43684	LOC_Os05 g41290	LOC_Os05 g50660	LOC_Os08 g19694	LOC_Os08 g20020	LOC_Os09 g37230	LOC_Os09 g34180	LOC_Os11 g24060	LOC_Os11 g24770	LOC_Os12 g10180	LOC_Os12 g13100
Teqing	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Lemont	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Labelle	S	S	S	S	S	S	S	S	S	S	S	S	S	S
LSBR-5	S	S	R	R	R	R	R	R	S	S	S	S	R	R
LSBR-33	S	S	R	R	R	R	R	R	S	S	S	S	R	R
PI658325	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658326	S	S	S	S	S	S	S	S	S	S	R	R	S	S
PI658327	S	S	S	S	S	S	S	S	S	S	R	R	S	S
PI658328	S	S	S	S	S	S	S	S	S	S	R	R	S	S
PI658329	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658330	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI658334	S	S	S	S	S	S	S	S	S	S	S	S	S	S

4.4 Discussion

Approximately 50 SB resistance-related QTLs with different effects have been reported (Yadav *et al*. 2015). However, gain in SB resistance with the introgression of these QTLs in susceptible materials has been moderate (Chen *et al*, 2014, Zuo *et al*, 2014a, Zuo *et al*. 2011, Wang *et al*. 2012). Using the approaches described in this study, I obtained a maximum gain of 3.4, comparing the best DH line 533-7-1 (SB score = 3.67) vs. the susceptible parent Cocodrie (SB score = 7.07), which is double the gain achieved by introgressing *qSB-7* and *qSB-9* from the resistant variety Teqing to the susceptible variety WLJ1 (Chen *et al*. 2014). The donor of resistance for 533-7-1 is the SB line PI 658335 originated from crosses using Teqing, LSBR-5, and H4/CODF as resistant donors that produced a SB score = 4.3 (Rush *et al*. 2011). This DH line contains introgressed resistant alleles in chromosome 9, but also from chromosome 2, 6 and 8. It suggest that the major effect of that region in chromosome 9, reported previously (Nelson *et*

al. 2012, Pinson *et al.* 2005, Tan *et al*. 2005, Liu *et al.* 2009, Sharma *et al.* 2009 and Tagushi-Shiobara *et al*. 2013) combined with the effect of chromosome 2, 6 and 8 presumable contributed to the observed increase in SB resistance compared with susceptible parents.

The QTL at the bottom of the long arm of chromosome 9 have been confirmed to exert a relatively large effect on resistance to SB among different sources of resistance including Teqing, Jasmine 85, Mighui 63 (Zuo *et al*. 2014b), and MCR10277 (Nelson *et al*. 2012). The effect of introgressions of QTL on chromosome 9 from MCR10277 was found most frequently in DH family 256 where a unique protein kinase resistant allele (LOC_Os09g39620) was introgressed. This type of protein acts as receptors that recognize the presence of specific pathogens and triggers plant defense mechanisms (Martin *et al*. 2003). A protein kinase in maize was associated with the quantitative resistance to head smut caused by the basidiomycete fungus *Sporisorium reilianum* (Zuo *et al*. 2015). In rice, protein kinases have been associated with resistance to blast (Chen *et al*. 2006) and bacterial blight disease (Sun *et al*. 2004). Therefore, LOC_Os09g39620 may play a role in the host response to *R. solani*. Fine mapping of the QTL $qSB\text{-}9^{TQ}$ on chromosome 9 identified 18 candidate genes for the resistance (Zuo *et al*. 2014c). However, LOC_Os09g39620 was not identified in the fine mapped QTL, although another protein kinase (LOC_Os09g37230) was found in that region. It is important to note that the sources of resistance for the family 256, and the mapping population used for fine mapping are different. Family 98-1, originating from Cocodrie x YD4, carried the resistant allele for the locus LOC_Os09g37230 reported in the fine-mapped QTL, but it was susceptible as the control Cocodrie. Thus, the fine-mapped QTL containing the LOC_Os09g37230 might work for the Lemont x Teqing population used by Zuo *et al.* (2014c), but is not critical in the lines generated in my study or in those lines described by Rush *et al.* (2011).

Seventeen resistant DH lines from this study, including individuals from the families 124-4-3, 129-4-3, 256 and 539-3, and nine resistant inbred lines from Rush *et al*. (2011) contained the introgression of resistant alleles from chromosome 12. This region contains genes that express nucleotide binding protein (NBS-LRR and NB-ARC). These R genes are involved in the mediation of recognition of the elicitor produced by the pathogen, activating the immune response in plants (Moffett *et al*. 2002, van Ooijen *et al*. 2008). However, as mentioned above, positive interaction with resistant alleles from other chromosomes may be required to increase resistance levels against SB. Fourteen of the resistant DH lines and four of the lines reported by Rush *et al*. (2011), containing resistant alleles from chromosome 12, also carry the resistant allele of LOC_Os02g34850 from chromosome 2. This gene produces a histone methyltransferase domain. Proteins containing this type of domain are important in the regulation of the response to necrotrophic fungal pathogens (Berr *et al*. 2010). Lines from the family 539-9 contained the introgression of the resistant allele of LOC_Os02g34850, but did not contain any resistant alleles from other chromosomes, resulting in a susceptible response. Only one of the resistant lines containing the LOC_Os02g34850 resistant allele, from all DH and Rush lines, had no detected introgression from other chromosomes that were studied. However additional research will be required to identify additional makers not covered in this study to identify other possible resistant alleles involved in the resistance. Identifying the specific combination of resistant alleles from specific sources of resistant is necessary to understand the mechanisms of resistance to SB and increase efficiency of markers-assisted selection.

Seven of the Rush *et al*. (2011) lines did not contain any resistant allele of the thirty SNP-based markers screened initially. For that reason, additional markers from different chromosomes were screened in the seven lines, two donor parents of these lines (Teqing and

LSBR-5), the susceptible parent Lemont, and the susceptible variety Labelle. LSBR-5 was reported as a somaclonal mutant that originated from the susceptible variety Labelle (Xie *et al*. 1992). However, Nelson *et al*. (2012) suggested that LSBR-5 was not derived from Labelle, but rather originated from an indica accession of unknown origin. The results shown in Table 4.7 indicate that LSBR-5 carried more indica alleles that japonica alleles reinforcing the assumption by Nelson *et al*. 2012. Thus, three of the seven lines that did not contain selected resistant alleles in the first screening with the 30 selected markers (Table 4.6) were subsequently found to carry resistant alleles from chromosome 11. The remaining four lines that did not contain any of the selected alleles presumably carry resistant alleles from other genomic regions not considered in this study.

Li *et al.* (1995) and Sharma *et al*. (2009) reported that most QTLs for SB were associated with plant height (PH) and heading date (HD). Moreover, Pinson *et al*. 2005 identified six SB resistance QTLs (*qSB3-1*, *qSB8-1*, *qSB-1*, *qSB-2*, *qSB-6-1*, *qSB-12*) also associated with HD. Correlation analysis of SB ratings vs HD and PH on the DH lines showed that there were negative correlations between HD and SB resistance, confirming the results from previous studies. However, no correlation was detected between SB ratings and PH. This can be explained because selections in BC_2F_1 to produce DH lines were based not only on presence of resistant alleles, but also on morphological characteristics like PH. On the contrary, HD was not considered in BC_2F_1 selection. Han *et al*. (2003) concluded that morphological traits like HD and PH can have some indirect effect on the SB rating because it change the microclimate where the fungus grows, but these traits are not critical for the direct expression of the response to SB. Therefore, increasing selection pressure, taking in account HD, and using the SNP markers it may be possible to reduce the correlation of SB ratings with HD in future studies.

It is not clear yet what exactly are the mechanisms involved in SB resistance. However, the SNP-based markers, DH lines and breeding strategies used in this study resulted in considerable gains in SB resistance, and represent a valuable source of information to direct future applied research on resistance to *R. solani* in rice. The genetic material and marker information produced from this study may also facilitate future studies to investigate mechanisms of rice-*R. solani* interactions.

4.5 References

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CHAPTER 5 SUMMARY AND CONCLUSIONS

5.1 Development of nsSNP-based markers

Sheath blight (SB) disease is the second most important disease in rice around the world causing important losses in Louisiana where environmental conditions and use of susceptible varieties favor infection produced by the fungus *Rhizoctonia solani*. Sources of partial resistance exist in the rice germplasm database, but they are not adapted to the southern U.S. Therefore, it is necessary to introgress the reistance in an efficient manner avoiding the introgression of undiserable traits. Variation in phenotyping results and the quantitative nature of the resistance to SB make it difficult to select and maintain the desirable alleles responsible for the resistance. Silva *et al*.(2012) identified ~200 nsSNP between resistant and susceptible lines in genes related to disease resistance by next generation sequencing (NGS). Based on this information, I developed 136 SNP based markers for a standard agarose-based platform that were validated on the susceptible varieties Cocodrie, Cypress, and Lemont, and the resistant materials MCR10277, Jasmine 85 and Teqing. Four different approaches were considered for marker design, but a modified approach based on the Drenkard *et al*. (2000) method was the most common in my study. Twelve of the nsSNP were validated by Sanger sequencing. The overall results showed the efficiency of the allele-specific nsSNP-based markers for discrimination of resistant and susceptible materials used in this study. Thus, these markers constitute an important tool for marker-assisted selection.

5.2 Selective genotyping for identification of candidate markers for SB resistance

Many QTLs for SB resistance have been identified in several populations. It has involved genotyping of populations with hundreds of individuals, and the use of different types of markers

with levels of polymorphism that do not allow a precise identification of chromosomal regions involved in the resistance. Selective genotyping (SG) can reduce the number of individuals that have to be genotyped by selecting only the extreme phenotypes. In my study, the 10 most resistant and the 10 most susceptible individuals from the RiceCap doubled-haploid SB2 population were genotyped with the 136 nsSNP-based markers developed in Chapter 2 which are located within QTLs reported in the literature. A total of 37 SB candidate nsSNP-based markers were identified on chromosomes 6, 8, 9, and 12 located within QTLs reported in a previous study using the same SB2 population (Nelson et al. 2012). It confirms the efficiency of SG for identification of candidate markers in a mapping population. These markers may be used efficiently in a marker-assisted selection strategy for development of SB resistant lines.

5.3 Production and evaluation of doubled-haploid lines for SB resistance

There is currently no SB resistant or partially resistant rice varieties adapted to Louisiana or southern U.S. conditions. Various QTLs have been identified for SB resistance, but efficient use of these markers for varietal development has not not reported. Eight of the selected markers described in Chapter 3 located on chromosomes 6, 8, 9, and 12, were used in a marker-assisted backcross approach. The crosses were made from seven resistant lines of different sources (MCR10277, Jasmine 85, YD4, Araure 3, Oryzica Llanos 5, SB2-3, and PI 658335) and four susceptible Louisiana varieties (Cocodrie, Cypress, Catahoula, and CL151). Seventy six F_1 individuals were backcrossed to the respective susceptible parents producing 422 BC_1F_1, which were genotyped with the eight selected nsSNP-based markers. Individuals containing between 4 and 8 resistant alleles were selected for a new backcross to the susceptible parents. BC_2F_1 consisted of 7062 progeny, which were genotyped and individuals containing 4-8 resistant alleles, but also producing acceptable agronomic traits were selected for production of doubled-

haploids by anther culture. A total of 45 DH lines originated from seven different crosses involving six different resistant parents, were obtained. These were evaluated for SB disease under field and mist chamber conditions. From these lines, 14 DH lines showed SB ratings <5. The DH line 533-7-1 produced values similar to the resistant line MCR10277 used as a control. All DH lines were genotyped with 30 nsSNP-based markers to identified resistant alleles introgressed from the resistant donors. All of the 14 most resistant DH lines carried SB resistant alleles, ranging from five to 24 alleles across lines. Twenty five resistant lines reported by Rush *et al*. (2011) were also genotyped using the 30 nsSNP markers used with the DH lines. From the 25 lines reported by Rush *et al*. (2011), 18 contained resistant alleles for the selected markers. The remaining seven were genotyped with eight additional markers that allows the identification of resistant allele introgression from chromosome 11.

The overall results indicate the efficacy of the strategy used in this study. The combination of next generation sequencing, SNP-based molecular markers, selective genotyping for candidate marker identification, marker-assisted backcrossing, anther culture and accurate methods of SB disease evaluation, resulted in rapid development of resistant SB lines with desirable agronomic traits. The germplasm, markers, and strategy generated in this study may be leveraged for future works directed to produce SB resistant varieties adapted to Louisiana. Moreover, this strategy may be applied to studies in other species for others quantitative traits. Additional studies are necessary to understand the genetic and molecular basis of the resistance to make the marker-assisted selection strategies even more efficient.

APPENDIX A. SEQUENCES CONFIRMING nsSNPs IN CANDIDATE GENES FOR SHEATH BLIGHT RESISTANCE

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1. LOCUS LOC_Os09g37590

Alignment Os09g37590_seqFOR  Variation C/T

LOC_Os09g37590_seqFOR_JAPONICA     GTAAGTGACTTCCACGACGCCTCCCAGTTCGACAGGTTCATGGACCACGT 437
LOC_Os09g37590_seqFOR_CCDR_R1      GTAAGTGACTTCCACGACGCCTCCCAGTTCGACAGGTTCATGGACCACGT 444
LOC_Os09g37590_seqFOR_CCDR_R2      GTAAGTGACTTCCACGACGCCTCCCAGTTCGACAGGTTCATGGACCACGT 447
LOC_Os09g37590_seqFOR_CCDR_R3      GTAAGTGACTTCCACGACGCCTCCCAGTTCGACAGGTTCATGGACCACGT 446
LOC_Os09g37590_seqFOR_ARA3_R1      GTAAGTGACTTCCACGACGCCTCCTAGTTCGACAGGTTCATGGACCACGC 445
LOC_Os09g37590_seqFOR_ARA3_R2      GTAAGTGACTTCCACGACGCCTCCTAGTTCGACAGGTTCATGGACCACGC 444
LOC_Os09g37590_seqFOR_ARA3_R3      GTAAGTGACTTCCACGACGCCTCCTAGTTCGACAGGTTCATGGACCACGC 448
LOC_Os09g37590_seqFOR_INDICA       GTAAGTGACTTCCACGACGCCTCCTAGTTCGACAGGTTCATGGACCACGC 441
                                   ************************ ************************

Alignment Os09g37590_seqREV  Variation G/A

LOC_Os09g37590_seqREV_JAPONICA     CCAAGAGATGCAACACGTGGTCCATGAACCTGTCGAACTGGGAGGCGTCG 199
LOC_Os09g37590_seqREV_CCDR_R1      CCAAGAGATGCAACACGTGGTCCATGAACCTGTCNAACTGGGAGGCGTCN 190
LOC_Os09g37590_seqREV_CCDR_R2      CCAAGAGATGCAACACGTGGTCCATGAACCTGTCGAACTGGGAGGCGTCG 190
LOC_Os09g37590_seqREV_CCDR_R3      CCAAGAGATGCAACACGTGGTCCATGAACCTGTCGAACTGGGAGGCGTCG 191
LOC_Os09g37590_seqREV_ARA3_R1      CCAAGAGATGCAACGCGTGGTCCATGAACCTGTCGAACTAGGAGGCGTCG 191
LOC_Os09g37590_seqREV_ARA3_R2      CCAAGAGATGCAACGCGTGGTCCATGAACCTGTCGAACTAGGAGGCGTCG 196
LOC_Os09g37590_seqREV_ARA3_R3      CCAAGAGATGCAACGCGTGGTCCATGAACCTGTCGAACTAGGAGGCGTCG 194
LOC_Os09g37590_seqREV_INDICA       CCAAGAGATGCAACGCGTGGTCCATGAACCTGTCGAACTAGGAGGCGTCG 199
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Figure A1. Comparison of sequences from the locus LOC_Os09g37590 confirming the presence of the nsSNP located in the position 21666818 on chromosome 9. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

```
2. LOCUS LOC_Os04g58910

Alignment Os04g58910_seqFOR  Variation T/C

LOC_Os04g58910_seqFOR_JAPONICA     TTCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_CCDR_R1      TTCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_CCDR_R2      TTCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_CCDR_R3      TTCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_ARA3_R1      TCCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_ARA3_R2      TCCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_ARA3_R3      TCCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
LOC_Os04g58910_seqFOR_INDICA       TCCACCATGACAATCTTGACCAAGTTGTCTGGGTCAGAATTCTCGCGGGG 300
                                   * ************************************************

Alignament Os04g58910_seqREV  Variation A/G

LOC_Os04g58910_seqREV_JAPONICA     GGTGAACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 350
LOC_Os04g58910_seqREV_CCDR_R1      GGTGAACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 321
LOC_Os04g58910_seqREV_CCDR_R2      GGTGAACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 321
LOC_Os04g58910_seqREV_CCDR_R3      GGTGAACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 321
LOC_Os04g58910_seqREV_ARA3_R1      GGTGGACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 321
LOC_Os04g58910_seqREV_ARA3_R2      GGTGGACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 322
LOC_Os04g58910_seqREV_ARA3_R3      GGTGGACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 322
LOC_Os04g58910_seqREV_INDICA       GGTGGACAATGATGGCAACAGTAGTTCGACCCAAGGCAATACACTTAGCG 350
                                   **** *********************************************
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Figure A2. Comparison of sequences from the locus LOC_Os04g58910 confirming the presence of the nsSNP located in the position 34856814 on chromosome 4. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

3. LOCUS LOC_Os02g54330

Alignment Os02g54330_seqFOR   Variation C/G

```
LOC_Os02g54330_seqFOR_JAPONICA     CCCTCCTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 299
LOC_Os02g54330_seqFOR_CCDR_R1      CCCTCCTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 297
LOC_Os02g54330_seqFOR_CCDR_R2      CCCTCCTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 297
LOC_Os02g54330_seqFOR_CCDR_R3      CCCTCCTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 297
LOC_Os02g54330_seqFOR_ARA3_R1      CCCTCGTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 288
LOC_Os02g54330_seqFOR_ARA3_R2      CCCTCGTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 288
LOC_Os02g54330_seqFOR_ARA3_R3      CCCTCGTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 287
LOC_Os02g54330_seqFOR_INDICA       CCCTCGTCGTCACGAACCGGAAGGCGCCATCCTGATAGATGACATCCTGT 287
                                   ***** ********************************************
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Alignment Os02g54330_seqREV   Variation G/C

```
LOC_Os02g54330_seqREV_JAPONICA     CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGAGGAGGGG 229
LOC_Os02g54330_seqREV_CCDR_R1      CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGAGGAGGGG 247
LOC_Os02g54330_seqREV_CCDR_R2      CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGAGGAGGGG 250
LOC_Os02g54330_seqREV_CCDR_R3      CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGAGGAGGGG 250
LOC_Os02g54330_seqREV_ARA3_R1      CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGACGAGGGG 246
LOC_Os02g54330_seqREV_ARA3_R2      CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGACGAGGGG 245
LOC_Os02g54330_seqREV_ARA3_R3      CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGACGAGGGG 245
LOC_Os02g54330_seqREV_INDICA       CAGGATGTCATCTATCAGGATGGCGCCTTCCGGTTCGTGACGACGAGGGG 229
                                   ******************************************* ******
```

Figure A3. Comparison of sequences from the locus LOC_Os02g54330 confirming the presence of the nsSNP located in the position 33307448 on chromosome 2. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

4. LOCUS LOC_Os01g52880

Alignment Os01g52880_seqFOR   Variation G/A

```
LOC_Os01g52880_seqFOR_JAPONICA     TCTCCGGCCTCCGAAACCTCCAGTGCTTGATCATGGACAACAACCCAATG 300
LOC_Os01g52880_seqFOR_CCDR_R1      TCTCCGGCCTCCGAAACCTCCAGTGCTTGATCATGGACAACAACCCAATG 281
LOC_Os01g52880_seqFOR_CCDR_R2      TCTCCGGCCTCCGAAACCTCCAGTGCTTGATCATGGACAACAACCCAATG 280
LOC_Os01g52880_seqFOR_CCDR_R3      TCTCCGGCCTCCGAAACCTCCAGTGCTTGATCATGGACAACAACCCAATG 280
LOC_Os01g52880_seqFOR_ARA3_R1      TCTCCGGCCTCCGAAACCTCCAGTACTTGATCATGGACAACAACCCAATG 285
LOC_Os01g52880_seqFOR_ARA3_R2      TCTCCGGCCTCCGAAACCTCCAGTACTTGATCATGGACAACAACCCAATG 287
LOC_Os01g52880_seqFOR_ARA3_R3      TCTCCGGCCTCCGAAACCTCCAGTACTTGATCATGGACAACAACCCAATG 284
LOC_Os01g52880_seqFOR_INDICA       TCTCCGGCCTCCGAAACCTCCAGTACTTGATCATGGACAACAACCCAATG 300
                                   ************************ *************************
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Alignment Os01g52880_seqREV   Variation C/T

```
LOC_Os01g52880_seqREV_JAPONICA     AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGCACTGGAGGTTTCG 150
LOC_Os01g52880_seqREV_CCDR_R1      AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGCACTGGAGGTTTCG 111
LOC_Os01g52880_seqREV_CCDR_R2      AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGCACTGGAGGTTTCG 109
LOC_Os01g52880_seqREV_CCDR_R3      AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGCACTGGAGGTTTCG 110
LOC_Os01g52880_seqREV_ARA3_R1      AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGTACTGGAGGTTTCG 134
LOC_Os01g52880_seqREV_ARA3_R2      AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGTACTGGAGGTTTCG 134
LOC_Os01g52880_seqREV_ARA3_R3      AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGTACTGGAGGTTTCG 134
LOC_Os01g52880_seqREV_INDICA       AAGGGGACATTCATTGGGTTGTTGTCCATGATCAAGTACTGGAGGTTTCG 150
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Figure A4 Comparison of sequences from the locus LOC_Os01g52880 confirming the presence of the nsSNP located in the position 30406859 on chromosome 1. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

```
5.  LOCUS LOC_Os03g37720

Alignment Os03g37720_seqFOR   Variation A/G

LOC_Os03g37720_seqFOR_JAPONICA      AGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
LOC_Os03g37720_seqFOR_CCDR_R1       AGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
LOC_Os03g37720_seqFOR_CCDR_R2       AGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
LOC_Os03g37720_seqFOR_CCDR_R3       AGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
LOC_Os03g37720_seqFOR_ARA3_R1       GGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
LOC_Os03g37720_seqFOR_ARA3_R2       GGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
LOC_Os03g37720_seqFOR_ARA3_R3       GGGCCGCCCATCTTCTTGGNTAGCTCCAATGCAACCTTCTCCATCGGTGA 200
LOC_Os03g37720_seqFOR_INDICA        GGGCCGCCCATCTTCTTGGCTAGCTCCAATGCAACCTTCTCCATCGGTGA 199
                                    ****************** ******************************

Alignment Os03g37720_seqFOR   Variation T/C

LOC_Os03g37720_seqREV_JAPONICA      AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCTACCATTACCA 349
LOC_Os03g37720_seqREV_CCDR_R1       AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCTACCATTACCA 349
LOC_Os03g37720_seqREV_CCDR_R2       AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCTACCATTACCA 349
LOC_Os03g37720_seqREV_CCDR_R3       AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCTACCATTACCA 350
LOC_Os03g37720_seqREV_ARA3_R1       AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCCACCATTACCA 349
LOC_Os03g37720_seqREV_ARA3_R2       AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCCACCATTACCA 349
LOC_Os03g37720_seqREV_ARA3_R3       AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCCACCATTACCA 349
LOC_Os03g37720_seqREV_INDICA        AGAAGGTTGCATTGGAGCTAGCCAAGAAGATGGGCGGCCCACCATTACCA 349
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Figure A5 Comparison of sequences from the locus LOC_Os03g37720 confirming the presence of the nsSNP located in the position 20914617 on chromosome 3. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

```
6. LOCUS LOC_Os04g59540

Alignment Os04g59540_seqFOR   Variation  C/G

LOC_Os04g59540_seqFOR_JAPONICA      AGTTAAATGATCTCCAGGAACAAGTATTCGCCAAAACCAACCAAAAAATG 300
LOC_Os04g59540_seqFOR_CCDR_R1       AGTTAAATGATCTCCAGGAACAAGTATTCGCCAAAACCAACCAAAAAATG 300
LOC_Os04g59540_seqFOR_CCDR_R2       AGTTAAATGATCTCCAGGAACAAGTATTCGCCAAAACCAACCAAAAAATG 300
LOC_Os04g59540_seqFOR_CCDR_R3       AGTTAAATGATCTCCAGGAACAAGTATTCGCCAAAACCAACCAAAAAATG 300
LOC_Os04g59540_seqFOR_ARA3_R1       AGTTAAGTGATCTCCAGGAACAAGTATTCGCCAAAACCAACGAAAAAATG 300
LOC_Os04g59540_seqFOR_ARA3_R2       AGTTAAGTGATCTCCAGGAACAAGTATTCGCCAAAACCAACGAAAAAATG 300
LOC_Os04g59540_seqFOR_ARA3_R3       AGTTAAGTGATCTCCAGGAACAAGTATTCGCCAAAACCAACGAAAAAATG 300
LOC_Os04g59540_seqFOR_INDICA        AGTTAAGTGATCTCCAGGAACAAGTATTCGCCAAAACCAACGAAAAAATG 300
                                    ****** ********************************** ********

Alignment Os04g59540_seqREV   Variation  G/C

LOC_Os04g59540_seqREV_JAPONICA      AGTACCGAAAGGATCAGGCTGTGACATTTTTTGGTTGGTTTTGGCGAATA 129
LOC_Os04g59540_seqREV_CCDR_R1       AGTACCGAAAGGATCAGGCTGTGACATTTTTTGGTTGGTTTTGGCGAATA 148
LOC_Os04g59540_seqREV_CCDR_R2       AGTACCGAAAGGATCAGGCTGTGACATTTTTTGGTTGGTTTTGGCGAATA 142
LOC_Os04g59540_seqREV_CCDR_R3       AGTACCGAAAGGATCAGGCTGTGACATTTTTTGGTTGGTTTTGGCGAATA 150
LOC_Os04g59540_seqREV_ARA3_R1       AGTACCGAAAGGATCAGGCTGTGACATTTTTTCGTTGGTTTTGGCGAATA 149
LOC_Os04g59540_seqREV_ARA3_R2       AGTACCGAAAGGATCAGGCTGTGACATTTTTTCGTTGGTTTTGGCGAATA 147
LOC_Os04g59540_seqREV_ARA3_R3       AGTACCGAAAGGATCAGGCTGTGACATTTTTTCGTTGGTTTTGGCGAATA 148
LOC_Os04g59540_seqREV_INDICA        AGTACCGAAAGGATCAGGCTGTGACATTTTTTCGTTGGTTTTGGCGAATA 129
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Figure A6 Comparison of sequences from the locus LOC_Os04g59540 confirming the presence of the nsSNP located in the position 35230058 on chromosome 4. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

```
7. LOCUS LOC_Os02g02650

Alignment Os02g02650_seqFOR   Variation  T/G

LOC_Os02g02650_seqFOR_JAPONICA      GACAACCTTGTTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_CCDR_R1       GACAACCTTGTTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_CCDR_R2       GACAACCTTGTTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_CCDR_R3       GACAACCTTGTTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_ARA3_R1       GACAACCTTGGTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_ARA3_R2       GACAACCTTGGTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_ARA3_R3       GACAACCTTGGTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
LOC_Os02g02650_seqFOR_INDICA        GACAACCTTGGTCTTGTTGGGGCTGCAGTCATCCATGCACGCAGATGAAC 200
                                    ********** ***************************************

Alignment Os02g02650_seqREV   Variation  A/C

LOC_Os02g02650_seqREV_JAPONICA      GCAGCCCCAACAAGAACAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_CCDR_R1       GCAGCCCCAACAAGAACAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_CCDR_R2       GCAGCCCCAACAAGAACAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_CCDR_R3       GCAGCCCCAACAAGAACAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_ARA3_R1       GCAGCCCCAACAAGACCAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_ARA3_R2       GCAGCCCCAACAAGACCAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_ARA3_R3       GCAGCCCCAACAAGACCAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
LOC_Os02g02650_seqREV_INDICA        GCAGCCCCAACAAGACCAAGGTTGTCGCCTGCGACTGCGCCACCTTTTGC 300
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Figure A7 Comparison of sequences from the locus LOC_Os02g02650 confirming the presence of the nsSNP located in the position 975892 on chromosome 2. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

```
8. LOCUS LOC_Os06g29700

Alignment Os06g29700_seqFOR   Variation  A/G

LOC_Os06g29700_seqFOR_JAPONICA      ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCA 150
LOC_Os06g29700_seqFOR_CCDR_R1       ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCA 150
LOC_Os06g29700_seqFOR_CCDR_R2       ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCA 150
LOC_Os06g29700_seqFOR_CCDR_R3       ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCA 150
LOC_Os06g29700_seqFOR_ARA3_R1       ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCG 150
LOC_Os06g29700_seqFOR_ARA3_R2       ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCG 150
LOC_Os06g29700_seqFOR_ARA3_R3       ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCG 150
LOC_Os06g29700_seqFOR_INDICA        ATTGTCATTGTGCTGGCACAATCGTATTCGTCTTCAGCTGATCGTCTGCG 150
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Alignment Os06g29700_seqREV   Variation  T/C

LOC_Os06g29700_seqREV_JAPONICA      CTGGAATGCAGACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_CCDR_R1       CTGGAATGCAGACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_CCDR_R2       CTGGAATGCAGACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_CCDR_R3       CTGGAATGCANACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_ARA3_R1       CTGGAACGCAGACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_ARA3_R2       CTGGAACGCAGACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_ARA3_R3       CTGGAACGCANACGATCANCTGAANACGAATACNATTGTGCCAGCACAAT 300
LOC_Os06g29700_seqREV_INDICA        CTGGAACGCAGACGATCAGCTGAAGACGAATACGATTGTGCCAGCACAAT 300
                                    ****** *** ******* ***** ******** ****************
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Figure A8 Comparison of sequences from the locus LOC_Os06g29700 confirming the presence of the nsSNP located in the position 17044919 on chromosome 6. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

9. LOCUS LOC_Os06g28124

```
Alignment Os06g28124_seqFOR   Variation  T/C

LOC_Os06g28124_seqFOR_JAPONICA      TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGTC 50
LOC_Os06g28124_seqFOR_CCDR_R1       TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGTC 50
LOC_Os06g28124_seqFOR_CCDR_R2       TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGTC 50
LOC_Os06g28124_seqFOR_CCDR_R3       TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGTC 50
LOC_Os06g28124_seqFOR_ARA3_R1       TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGCC 50
LOC_Os06g28124_seqFOR_ARA3_R2       TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGCC 50
LOC_Os06g28124_seqFOR_ARA3_R3       TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGCC 50
LOC_Os06g28124_seqFOR_INDICA        TCGCGGTGGCTGCGGAGTCCTACGGTGATTTTCGGGTAGCACCGGACGCC 50
                                    ************************************************ *

Alignment Os06g28124_seqREV   Variation  A/G

LOC_Os06g28124_seqREV_JAPONICA      ACTTCAACCAGGACAGCGACGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_CCDR_R1       ACTTCAACCAGGACAGCGACGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_CCDR_R2       ACTTCAACCAGGACAGCGACGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_CCDR_R3       ACTTCAACCAGGACAGCGACGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_ARA3_R1       ACTTCAACCAGGACAGCGGCGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_ARA3_R2       ACTTCAACCAGGACAGCGGCGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_ARA3_R3       ACTTCAACCAGGACAGCGGCGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
LOC_Os06g28124_seqREV_INDICA        ACTTCAACCAGGACAGCGGCGTCCGGTGCTACCCGAAAATCACCGTAGGA 250
                                    ****************** *******************************
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Figure A9 Comparison of sequences from the locus LOC_Os06g28124 confirming the presence of the nsSNP located in the position 15968674 on chromosome 6. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

10. Locus LOC_Os09g17630

```
Alignment Os09g17630_seqFOR   Variation T/C

LOC_Os09g17630_seqFOR_JAPONICA      TACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 300
LOC_Os09g17630_seqFOR_CCDR_R1       TACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 268
LOC_Os09g17630_seqFOR_CCDR_R2       TACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 268
LOC_Os09g17630_seqFOR_CCDR_R3       TACCACTTCAACCATTTCTGTCAGACCTCANCCTTGCTGCNNACAGCCTG 268
LOC_Os09g17630_seqFOR_ARA3_R1       CACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 265
LOC_Os09g17630_seqFOR_ARA3_R2       CACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 266
LOC_Os09g17630_seqFOR_ARA3_R3       CACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 266
LOC_Os09g17630_seqFOR_INDICA        CACCACTTCAACCATTTCTGTCAGACCTCAGCCTTGCTGCAAACAGCCTG 300
                                     ***************************** *********  ********

Alignment Os09g17630_seqREV   Variation A/G

LOC_Os09g17630_seqREV_JAPONICA      GAGGTCTGACAGAAATGGTTGAAGTGGTATGTGCCCCTCAAGCAGGCTCA 239
LOC_Os09g17630_seqFOR_CCDR_R1       GAGGTCTGACAGAAATGGTTGAAGTGGTATGTGCCCCTCAAGCAGGCTCA 239
LOC_Os09g17630_seqFOR_CCDR_R2       GAGGTCTGACAGAAATGGTTGAAGTGGTATGTGCCCCTCAAGCAGGCTCA 235
LOC_Os09g17630_seqFOR_CCDR_R3       GAGGTCTGACAGAAATGGTTGAAGTGGTATGTGCCCCTCAAGCAGGCTCA 234
LOC_Os09g17630_seqREV_ARA3_R1       GAGGTCTGACAGAAATGGTTGAAGTGGTGTGTGCCCCTCAAGCAGGCTCA 226
LOC_Os09g17630_seqREV_ARA3_R2       GAGGTCTGACAGAAATGGTTGAAGTGGTGTGTGCCCCTCAAGCAGGCTCA 231
LOC_Os09g17630_seqREV_ARA3_R3       GAGGTCTGACAGAAATGGTTGAAGTGGTGTGTGCCCCTCAAGCAGGCTCA 226
LOC_Os09g17630_seqREV_INDICA        GAGGTCTGACAGAAATGGTTGAAGTGGTGTGTGCCCCTCAAGCAGGCTCA 226
                                    **************************** *********************
```

Figure A10 Comparison of sequences from the locus LOC_Os09g17630 confirming the presence of the nsSNP located in the position 10792494 on chromosome 9. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison

11. Locus LOC_Os02g35210

Alignment Os02g35210_seqFOR   Variation G/A

```
LOC_Os02g35210_seqFOR_Japonica     TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCGACTTGC 298
LOC_Os02g35210_seqFOR_CCDR_R1      TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCGACTTGC 290
LOC_Os02g35210_seqFOR_CCDR_R2      TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCGACTTGC 289
LOC_Os02g35210_seqFOR_CCDR_R3      TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCGACTTGC 295
LOC_Os02g35210_seqFOR_ARA3_R1      TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCAACTTGC 292
LOC_Os02g35210_seqFOR_ARA3_R2      TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCAACTTGC 288
LOC_Os02g35210_seqFOR_ARA3_R3      TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCAACTTGC 287
LOC_Os02g35210_seqFOR_Indica       TGCTACTTTTGGTGCAATGGACTCTGTCCTCAGCAAGCTCACCAACTTGC 298
                                   ******************************************* ******
```

Alignment Os02g35210_seqREV   Variation C/T

```
LOC_Os02g35210_seqREV_JAPONICA     AGGTCAGCAAGTCGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 240
LOC_Os02g35210_seqREV_CCDR_R1      AGGTCAGCAAGTCGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 240
LOC_Os02g35210_seqREV_CCDR_R2      AGGTCAGCAAGTCGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 237
LOC_Os02g35210_seqREV_CCDR_R3      AGGTCAGCAAGTCGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 240
LOC_Os02g35210_seqREV_ARA3_R1      AGGTCAGCAAGTTGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 239
LOC_Os02g35210_seqREV_ARA3_R2      AGGTCAGCAAGTTGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 237
LOC_Os02g35210_seqREV_ARA3_R3      AGGTCAGCAAGTTGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 238
LOC_Os02g35210_seqREV_INDICA       AGGTCAGCAAGTTGGTGAGCTTGCTGAGGACAGAGTCCATTGCACCAAAA 240
                                   ************ *************************************
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Figure A11 Comparison of sequences from the locus LOC_Os02g35210 confirming the presence of the nsSNP located in the position 21160861 on chromosome 2. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

12. Locus LOC_Os12g10180

Alignment Os12g10180_seqFOR   Variation C/G

```
LOC_Os12g10180_seqFOR_JAPONICA     TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGCAGCCTGGATGA 292
LOC_Os12g10180_seqFOR_CCDR_R1      TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGCAGCCTGGATGA 296
LOC_Os12g10180_seqFOR_CCDR_R2      TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGCAGCCTGGATGA 292
LOC_Os12g10180_seqFOR_CCDR_R3      TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGCAGCCTGGATGA 290
LOC_Os12g10180_seqFOR_ARA3_R1      TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGGAGCCTGGATGA 295
LOC_Os12g10180_seqFOR_ARA3_R2      TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGGAGCCTGGATGA 299
LOC_Os12g10180_seQFOR_ARA3_R3      TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGGAGCCTGGATGA 282
LOC_Os12g10180_seqFOR_INDICA       TGAGTTCAGACATTTACATTCTTCGACAACTACGACCAGGAGCCTGGATGA 295
                                   *************************************** ***********
```

Alignment Os12g10180_seqREV   Variation G/C

```
LOC_Os12g10180_seqREV_Japonica     CATCCAGGCTGCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 299
LOC_Os12g10180_seqREV_CCDR_R1      CATCCAGGCTGCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 264
LOC_Os12g10180_seqREV_CCDR_R2      CATCCAGGCTGCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 265
LOC_Os12g10180_seqREV_CCDR_R3      CATCCAGGCTGCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 266
LOC_Os12g10180_seqREV_ARA3_R1      CATCCAGGCTCCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 267
LOC_Os12g10180_seqREV_ARA3_R2      CATCCAGGCTCCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 256
LOC_Os12g10180_seqREV_ARA3_R3      CATCCAGGCTCCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 265
LOC_Os12g10180_seqREV_INDICA       CATCCAGGCTCCTGGTCGTAGTTGTCGAAGAATGTAAATGTCTGAACTCAC 260
                                   ********** ****************************************
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Figure A12 Comparison of sequences from the locus LOC_Os12g10180 confirming the presence of the nsSNP located in the position 5378630 on chromosome 12. The variation is shown in green between the SB susceptible variety Cocodrie (CCDR) and the SB resistant variety Araure 3 (ARA3). Nipponbare (japonica) and 93-11 (indica) reference sequences were also included in the comparison.

APPENDIX B. DESIGN OF ALLELE SPECIFIC SNP-BASED MARKERS FOR FIVE IMPORTANT GENES IN RICE.

Markers for Sterility genes

Primer name	Primer sequence	Product size
pms3-3R_REF-F	GTGTTGATAAAAATTTTACTCTTGATGGATGGGAG	170
pms3-3R_REF-R	TGAGCAACATGAGAACTTCAGCTTGAGATATACATA	
pms3-2L_ALT-F	ATGGTGAAGCAAAGAAGTGCATTGTTTCTG	241
pms3-2L_ALT-R	CACATTTTCCTTCTGGACTAGGAGCAAGCTA	

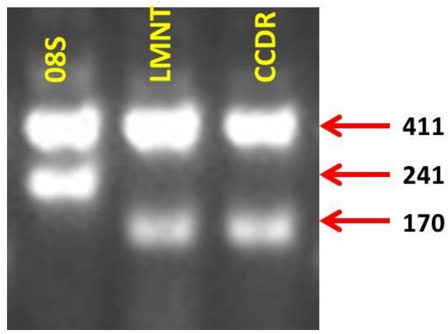

Figure B1. Primer sequences for the sterility genes pms3 (LOC_Os12g36030). Image below shows the polymorphism between the sterile line 08S and the fertile varieties Lemont and Cocodrie.

Primer name	Primer sequence	Product size
pms1-2L_REF-F	CTGTATCTTGCTATATTCCTTCGGTTATATGTGTTG	230
pms1-2L_REF-R	ATTAATGGCCCTAGCGAAGAAATTCCTACATTTAT	
pms1-1R_ALT-F	AAATTGCACAGAGAAAGAACTAGGATCCCTTACATA	174
pms1-1R_ALT-R	ATGGAGATCGCAAGTGGGCAGAGA	

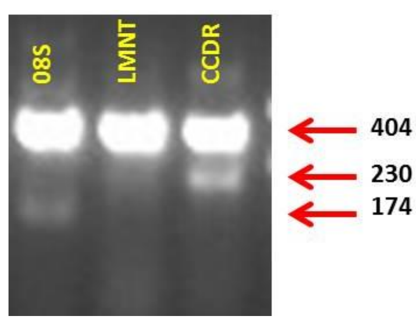

Figure B2. Primers sequences for the sterility gene pms1 (LOC_Os07g12130). Image below shows the polymorphism between the sterile line 08S and the fertile varieties Lemont and Cocodrie.

Primers for Clearfield (161-C)

Primer name	Primer sequence	Product size
ALS-1L_REF-F	GCATGTGCTGCCTATGATCCCACG	175
ALS-1L_REF-R	AGAGCACATACAAACATCATAGGCATACCACTCT	
ALS-2R_ALT-F	CATGTCCTTGAATGCGCCCCAAT	263
ALS-2R_ALT-R	AATGGGAGGATAGGTTTTACAAGGCAAATAGG	

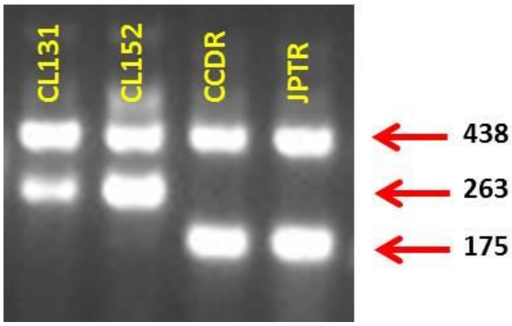

Figure B3. Primer sequences for the imidazolinone herbicide resistance gene ALS (BGIOSGA008288) Image below shows the polymorphism between the imidazolinone resistant varieties CL131 and CL152, and the imidazolinone susceptible varieties Cocodrie and Jupiter.

Primers for Amylose content

Primer name	Primer sequence	Product size
waxy-1L_REF-F	GTTGTTCATCAGGAAGAACATCTGCGAGT	151
waxy-1L_REF-R	GCCCAACACCTTACAGAAATTAGCATGTATGA	
waxy-2R_ALT-F	GAGGGGAAACAAAGAATTATAAACATATATGTACAC	259
waxy-2R_ALT-R	GGGAGGGAGAGGGGGAGAGAGAGAT	

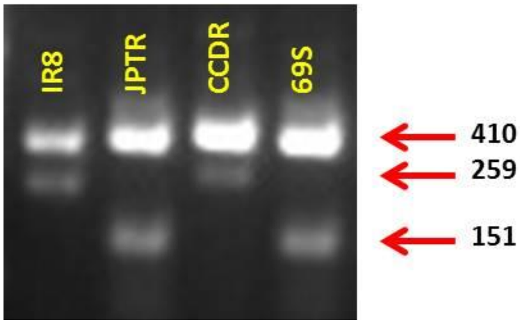

Figure B4. Primer sequences for the gene waxy (OS06G0133000). Image below shows the polymorphism between the high amylose content varieties IR8 and Cocodrie, and the low amylose content varieties Jupiter and 69S.

Primers for Herbicide Resistance (Provisia)

Primer name	Primer sequence	Product size
HT-1C-L-ALT1-F	CAAGGAAGATGGACTTGGTGTGGAGAACT	142
HT-1C-L-ALT1-R	AAGTCGAGCAAGATAAGCTCCTATTCCAACAG	
HT-2-R-REF1-F	CACTGGCAATAGCAGCACTTCCATGAAT	252
HT-2-R-REF1-R	GTGCTCGAATTGGCATAGCAGATGAAGT	

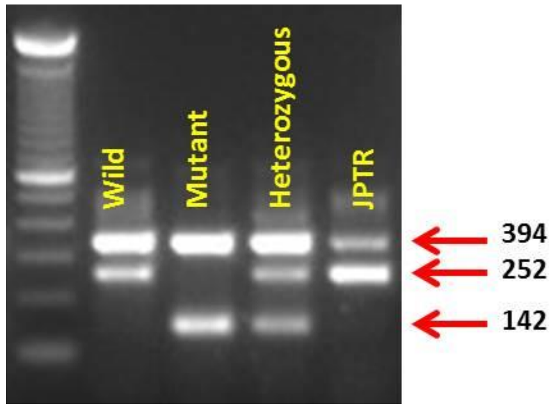

Figure B5. Primer sequences for resistance to herbicide (Provicia). Image below shows the polymorphism between the resistant mutant, an heterozygous an the susceptible wild and the susceptible variety Jupiter.

Primers for additional nsSNP-based markers for other traits:

Gelatinization temperature ALK (LOC_Os06g12450)

	Forward	Reverse	Product Size
ALK 3 Ref	TGCCGCGCACCTGGAGC	CGCCGAGCCGCACAAGC	~90
ALK 3 Alt	CATGCCGCGCACCTGGAAA	CGCCGAGCCGCACAAGC	~90

Aroma

	Forward	Reverse	Product size
Ref	CTGGTATATATTTCAGCTGATC	AAAGATTATGGCTTCAGCTGATC	237
Alt	CCAGTGAAACAGGCTGTCAA	AAAGATTATGGCTTCAGCTGATC	237

VITA

Yamid Sanabria Góngora, is the third of four children of Rosa Maria Góngora Góngora and José Adalberto Sanabria Amortegui. He was born and grew up in Ibagué, capital of Tolima in Colombia, where he attended Liceo Dios Niño elementary school and later begun high school in Colegio José Acevedo y Gómez. At 16 years of age, Yamid was admitted into the Universidad del Tolima and earned a Bachelor of Science in biology. Before graduation he moved to Cali, Colombia to work as intern in the International Center for Tropical Agriculture (CIAT) under Dr. Zaida Lentini working on gene flow in rice and later under Dr. César Martínez, leader of rice breeding program who influenced Yamid with the passion for rice breeding research encouraging him to continue working at CIAT as research assistant.

In 2010, after six enriching years working in rice research at CIAT and living in Cali, he was admitted as PhD student in Louisiana State University in Baton Rouge, Louisiana under Dr. James Oard, supported for a research assistantship by the School of Plant Environmental and Soil Sciences. In 2011 Yamid was awarded with the Global Rice Science Scholarship (GRiSS). After 5 years and 6 months participating in one of the most important rice research programs in the United States, he is earning his PhD in Agronomy in December 2015.

After an invaluable experience living in Louisiana he received an offer to lead the rice breeding program of the Latin American Fund for Irrigated Rice (FLAR) for the south of the continent. Now he is moving from the south of the United States to the south of the continent to the city of Treinta y Tres in Uruguay.