"QTL MAPPING FOR BACTERIAL LEAF BLIGHT, SHEATH BLIGHT, BLAST AND BROWN SPOT TOLERANCE USING RIL POPULATION OF RICE (*Oryza sativa* L.)."

Ph.D. THESIS

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CERTIFICATE -I

This is to certify that the thesis entitled **"QTL mapping for bacterial leaf blight, sheath blight, blast and brown spot tolerance using RIL population of Rice (*Oryza sativa* L.)."** submitted in the partial fulfillment of the requirements for the degree of **"Doctor of Philosophy in Plant Molecular Biology and Biotechnology"** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur is a record of the bonafide research work carried out by **Lincoln Mandal** under my guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published / published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Date: 01.10.2013

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CERTIFICATE -II

This is to certify that the thesis entitled **"QTL mapping for bacterial leaf blight, sheath blight, blast and brown spot tolerance using RIL population of Rice (*Oryza sativa* L.)."** submitted by **Lincoln Mandal** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in the **Department of Plant Molecular Biology and Biotechnology** has been approved by the External Examiner and Student's Advisory Committee after oral examination.

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LIST OF ABBREVIATIONS

Abbreviation	Details
ADW	Autoclave distilled water
APS	Ammonium persulphate
ANOVA	Analysis of Variance
BAC	Bacterial Artificial Chromosome
BLB	Bacterial leaf blight
BB	Bacterial blight
CIM	Composite Interval Mapping
CR	Complete resistance
CTAB	Cetyl trimethylammonium bromide
EDTA	Ethylene diaminetetraacetic acid
EtOH	Ethyl alcohol
HD	Heading date
IM	Interval Mapping
IRRI	International Rice Research Institute
LOD	Logarithm (base 10) of odds
MAS	Marker-Assisted Selection
MIM	Multiple Interval Mapping
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NILs	Near isogenic lines
PAGE	Polyacrylamide Agarose Gel Electophoresis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PR	Partial resistance
PVP	Poly (vinyl phenol)
QDR	Quantitative disease resistance
QRL	Quantitative resistance loci
QTL	Quantitative trait locus
RAPD	Random Amplified Polymorphic DNA
RBD	Randomized Block Design
RCBD	Randomized Complete Block Design

RFLP	Restriction Fragment Length Polymorphism
RGA	Resistance gene analog
RGH	Rice grain hull
RILs	Recombinant inbred lines
RLH	Relative lesion height
SD	Standard deviation
SDS	Sodium dodecyl sulfate
ShB	Sheath blight resistance
SMA	Single marker analysis
SSR	Simple sequence repeat
STS	Sequence tagged site
TBE	Tris/Borate/EDTA
TEMED	N-N-N-N-Tetramethylethylenediamine
Xoo	*Xanthomonas oryzae* pv. *Oryzae*
EST	Expressed sequence tags
DH	Double haploid
PVE	Phenotypic variation explained
PH	Plant height
RGH	Rice grain hull mixture
DAT	Days after transplanting
DAS	Days after sowing
RLH	Relative lesion height
DAI	Day after inoculation
SES	Standard Evaluation System

Introduction

CHAPTER -I

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of half of the world's human population (Khush, 2005). Rice is one of the oldest domesticated crops constitutes a major source of calories for urban and rural inhabitants as well as model monocot plant for genetic and genomic studies. Besides its economic importance, rice has a small genome (430 Mb) size as compared to sorghum (1000 Mb), maize (3000 Mb), barley (5000 Mb), wheat(16000 Mb) (Arumugnathan and Earle, 1991) and it is three times larger than *Arabidopsis* (*Arabidopsis* Genome Initiative (AGI), 2000). An international consortium including – Japan, the United States of America, United Kingdom, China and Korea, called the International Rice Genome Sequencing Project (IRGSP) is sequencing the entire rice genome. Recently, two research groups have sequenced more than 90% of the rice genome and estimated around 30,000 to 60,000 genes using computational gene prediction programs (Goff *et al.,* 2002; Yu *et al.,* 2002). More than 90% of the world's rice is produced and consumed in Asia, where 60% of the people live. In India, rice is grown on about 44.5 Mha and provides food for more than 70% of the population and serves as the principal energy source for most of the people. Rice environments in India are extremely diverse.

Bacterial blight caused by the pathogen *Xanthomonas oryzae* pv. *oryzae (Xoo)* is one of the most destructive diseases of rice throughout the world. In some areas of Asia, *Xoo* has the potential to reduce yield by more than 50%. In Japan, BB damage there was reported to range from 20 to 30% and as high as 50% (Ou, 1972). Rice and its bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) are considered a model system to study interactions between host plants and their pathogens. The presence of complete resistance and partial resistance (PR) to *Xoo* in rice has been

reported. The former is governed by many *R* genes in a gene-for-gene manner and the latter is controlled by numerous quantitative resistance loci (QRL).

The second most economically important disease of rice in the world (Lee and Rush, 1983) is Sheath blight (ShB), caused by *Rhizoctonia solani* Kuhn. Under conditions favoring disease, up to 50% of grain yield may be lost (Marchetti and Bollich, 1991). Yield losses of 5–10% have been estimated for tropical lowland rice in Asia (Savary *et al*., 2000a). Breeding for sheath blight resistance has been difficult, mainly because of the lack of identified resistant donors in cultivated varieties (Bonmann *et al*., 1992). To date, no rice variety has been found to be immune to *R. solani*, although cultivars with varying levels of resistance have been reported. Rice Sheath blight resistance is generally believed to be a typical quantitative trait controlled by several genes (Li *et al*., 1995). The identification of genes that affect complexly inherited trait is often difficult and best approached through developing a genetic linkage map to identify quantitative trait loci (QTLs) (Tanksley and McCouch, 1997). Consequently, a great progress has been made in identifying and mapping major quantitative trait locus (QTL) underlying Sheath blight resistance in rice and several major QTLs for resistance had been detected since the 1990s (Zhou *et al.*, 2000). Cloning and characterization of genes controlling quantitative trait is a major research frontier in terms of understanding important agronomic traits including disease resistance in crops (Ishimaru *et al*., 2004). Once genes present in the region where QTLs are present can be better characterized and exploited.

Rice blast caused by the fungus *Magnaporthe oryzae*, is the most severe disease of rice in many countries. This disease causes about 10 to 20% yield loss in regular seasons and as high as 100% yield loss in years with blast epidemics. To control the diseases, the use of resistant cultivars is an effective measure; thus, rice breeders have been developing resistant cultivars. The wide scale deployment of the

single gene in the rice growing areas has led to their breakdown due to the appearance of new virulent races. In contrast, partial resistance is more stable to different races of the pathogen and it is thought to be nonspecific; therefore it is promising for long-term blast control.

Brown spot disease caused by the fungus *Bipolaris oryzae*, is one of the most prevalent fungal diseases of rice and significantly reduces the yield and milling quality of grain (Datnoff *et al*., 1992). Brown spot has been reported in all rice-growing areas in the world. It is especially common in rainfed (Singh and Singh, 2000) and upland areas (Gupta and O'Toole, 1986). It affects the grain yield, both in wet and dry seasons, with heavy natural infection (Bakonyi *et al*., 1995). The use of resistant varieties would be the most economical means of controlling brown spot. No major genes with resistance to brown spot have yet been identified. However, varieties with partial resistance and three quantitative trait loci (QTL) for disease resistance have been identified (Sato *et al*., 2008).

Due to high variability and evaluation in pathogenicity of pathogen and short life of resistant cultivars there are need of identification of more resistant genes and QTLs. The concept of quantitative traits is fundamental in genetics and also encountered in many other areas of biological sciences. A modern type of study is to locate genes controlling a quantitative trait, or QTL mapping. The polygenic control underlying quantitative resistance has been presumed to be much more durable than qualitative resistance, because each gene involved has a small effect on host resistance. The accumulation of such small effects may process longer life span in crop production than the resistance conferred by a single *R* gene. The QTLs are valuable resources for durable and broad-spectrum resistance. DNA markers tightly

linked to quantitative resistance loci (QRLs) controlling QDR can be used for marker-assisted selection (MAS) to incorporate these valuable traits.

Identification and mapping of QTL is a valuable starting point for positional cloning of genes present in the QTL region of the genomes. The two general goals of QTL mapping in plants are to (a) increase our biological knowledge of the inheritance and genetic architecture of quantitative traits, both within a species and across related species and (b) identify markers that can be used as indirect selection tools in breeding (Bernardo, 2008). During the past two decades, the ability to transfer target genomic regions using molecular markers resulted in extensive QTL mapping experiments in most economically important crops, aiming at the development of molecular markers for marker assisted selection (Xu, 1998; Semagn *et al.,* 2006; Xu, 2010) and QTL cloning (Salvi and Tuberosa, 2005). The studies QTL mapping provides information on (a) the number and chromosomal location of QTLs affecting a trait; (b) the magnitude and direction of effect of each QTL (*i.e.*, whether a phenotypic trait is controlled by many genes or many independent loci of small effect or by a few genes of large effect); (c) the mode of gene action at each QTL (dominant or additive); (d) the parental sources of beneficial QTL alleles and (e) whether there is interaction between different QTLs (epistasis, *i.e.,* interactions between two QTLs that result in an effect on the trait that would not be predicted from the sum of the individual QTL effects) or between genotypes and environment (Bradshaw, 1996).

In recent years, much has been learned about the genes and pathways involved in the plant defense response and many studies have been done to identify chromosomal regions conditioning QDR (quantitative trait loci for disease, or disease QTL). Insights into quantitative disease resistance (QDR) have implications both for understanding host-pathogen interactions and for improving crop production. Cloning

of genes controlling quantitative traits is now a major research frontier trait of agronomic importance in crops defense (Ishimaru *et al.,* 2004). Once genes conditioning QTL are identified for crop plants, natural variation can be better characterized and exploited. Identification of positional candidate genes is a step toward isolation of the genetic factors controlling quantitative traits (Remington and Purugganan, 2003). Much effort has thus been made to identify positional procandidates for a number of traits, including QDR (Ramalingam *et al.,* 2003; Wen *et al.,* 2003). For practical purposes, positional candidates for QDR may be considered as all putative genes underlying a small QTL region, or defense-related genes in a broad QTL region. *R* genes and defense-associated transcription factors are attractive classes of candidate genes for investigation of QDR. Once the tightly linked markers have been identified, the quantitative trait loci (QTLs) can be selected for in breeding programs using marker-assisted selection (MAS) strategy. The present study entitled as **"QTL mapping for bacterial leaf blight, sheath blight, blast and brown spot tolerance using RIL population of Rice (*Oryza sativa* L.)."** was undertaken with the following major objectives:

1. Phenotyping of RIL population for bacterial leaf blight, sheath blight, blast and brown spot reactions
2. Genotyping of RIL population and construction of linkage map
3. Mapping of QTL for bacterial leaf blight, sheath blight, blast and brown spot tolerance
4. *In silico* analysis of the identified QTL

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

Rice is a worldwide staple as well as a model for cereal biology (Bennetzen and Ma, 2003; Ronald and Leung, 2002; Shimamoto and Kyozuka, 2002). In the developing world as a whole, rice provides 27 percent of dietary energy supply and 20 percent of dietary protein intake. Rice began cultured in Asia and now is cultivated in 113 countries and on all continents except Antarctica. Two of 23 species from the genus *Oryza* are cultivated: *Oryza sativa*, which originated in the humid tropics of Asia and *Oryza glaberrima* from West Africa. The two main strains of *O. sativa* are japonica and indica. Rice is a model monocot because it has the smallest genome size (390 Mb) among the major cereals, its genome is syntenic with the genomes of the other cereals and it can be transformed easily. The small genome of rice includes a large percentage (ca. 75%) of single-copy DNA (McCouch *et al.,* 1988). Rice has proven to be the most readily transformable cereal crop (Hiei *et al*., 1994). In the last ten years, two high-density molecular linkage maps of rice containing about 3000 markers have been developed in the US and Japan, making the marker density in the rice genome, on average, one marker per cM (200-300 kb) (Causse *et al*., 1994; Harushima *et al.,* 1998). Over 300,000 expressed sequence tags (EST) have been deposited in the public database (Sasaki *et al.,* 2005). With the completed sequence available from the International Rice Genome Sequencing Project, it was expected that the genome sequence will facilitate pioneering research in functional and applied genomics.

2.1 Impact of diseases on rice production

Among the biotic stresses, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is an important disease that results in significant yield reduction worldwide. The disease, in its severe form, is known to cause yield losses ranging from 74 to 81% in susceptible cultivars (Srinivasan and Gnanamanickam, 2005). The sheath blight caused by the fungus *Rhizoctonia solani* Kuhn, the second most important diseases of rice (*Oryza sativa* L.) causing severe loss in grain yield and quality worldwide (Lee and Rush, 1983). Various estimates of crop losses due to sheath blight have been made; losses generally vary from negligible to 50% depending on the severity of the disease and the stage at which the crop is infected and environmental conditions. According to Lee and Rush (1983) losses occur between 20 to 50% when all the sheaths are infected. Rice blast caused by *Pyricularia oryzae* Cavara [synonym *Pyricularia grisea* Sacc. the anamorph of *Magnaporthe grisea* (Herbert) Yaegashi and Udagawa], is the most destructive and wide spread diseases (Jia *et al*., 2000). This disease has caused significant yield losses in many rice growing countries *e.g.* 75% losses of grains in India (Padmanabhan, 1965), 50% loss in Philippines (Awodera and Esuruoso, 1975) and 40% loss in Nigeria (Ou, 1985). The brown spot causes both quantitative and qualitative losses. Surveys show that brown spot causes a 5% yield loss across all lowland rice production situations in South and Southeast Asia (Savary *et al*., 2000b).

2.2 Plant disease resistance

Plants resist pathogen attacks both with preformed defenses such as antimicrobial compounds and by induced defense responses (Hwang *et al.,* 2005).

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Disease resistance in plants can be classified into two major categories such as

vertical versus horizontal resistance (Van der Plank, 1968), qualitative versus quantitative resistance (Ou *et al*., 1975) and complete versus partial resistance (Parlevliet, 1979). In most cases, qualitative resistance is modulated by direct or indirect interaction between the products of a major disease resistance (*R*) gene and an avirulence gene; this type of resistance is specific to pathogen race and is life time limited in a particular cultivar due to the strong selection pressure against and the rapid evolution of the pathogen (McDonald and Linde, 2002). The pathogen proteins, designated effectors proteins are recognized by plant disease resistance (*R*) proteins in a specific manner first described genetically as the gene-for-gene interaction (Flor, 1971). Physical interactions between R proteins and effectors have been demonstrated only for *PTO* with *AvrPto* or *AvrPtoB* (Kim *et al.,* 2002), *Pi-ta* with *AVR-Pita* (Jia *et al.,* 2000) and *RPS2* with *AvrRpt2* and the noncognate effector *AvrB* (Leister and Katagiri, 2000). The major class of *R* genes encodes proteins that contain a nucleotide-binding site plus leucine-rich repeat domains (NBS–LRR proteins) (Hulbert *et al.,* 2001; Howles *et al*., 2005*)*. These NBS-LRR genes represent a superfamily of *R* genes in both monocot and dicot species.

2.2.1 Quantitative resistance

Quantitative resistance (QR) is defined as a resistance that varies in a continuous way between the various phenotypes of the host population, from almost imperceptible (only a slight reduction in the growth of the pathogen) to quite strong (little growth of the pathogen). Quantitative resistance, in contrast with qualitative resistance, is generally considered as partial resistance in a particular cultivar (Parlevliet, 1979). Genetic and molecular evidence have suggested that quantitative resistance can be pathogen race-nonspecific and even pathogen species-nonspecific,

that is, broad-spectrum resistance (Kou and Wang, 2010). QDR has been described as host plant resistance that leads to a reduction in disease, rather than the absence of disease (Poland *et al*., 2008). QDR in plants including partial, complex, polygenic, oligogenic, horizontal, field and durable. This type of resistance can also remain effective in cultivars grown for prolonged periods in environments favourable to the spread of disease (Krattinger *et al*., 2009). This type of disease resistance is controlled by multiple loci, referred to as QTLs, and does not comply with simple Mendelian inheritance. Thus, selecting for these QTLs is difficult. Mapping quantitative trait loci (QTLs) is a powerful tool for genetic dissection of QDR. DNA markers tightly linked to quantitative resistance loci (QRLs) controlling QDR can be used for marker-assisted selection (MAS) to incorporate these valuable traits.

Polygenic resistance involves quantitative trait loci (QTL) and some of them may be race-specific and others race-nonspecific (Fukuoka and Okuno, 2001). An approach for studying complex and polygenic forms of disease resistance is known as QTL mapping, which is based on the use of DNA markers (Tanksley, 1993). Resistance to the bacterial blight pathogen, *Xoo*, has been reported to have both qualitative and quantitative components (Li *et al.,* 2001). QTL mapping and high resolution mapping offers an entry point for the most ambitious goal of all, cloning genes known only by their small effects, in order to elucidate the genetic and molecular basis of quantitative trait variation. Examples include the cloned tomato-fruit-weight QTL, *fw2.2* (Frary *et al.,* 2000) and a salt tolerance QTL in rice (Ren *et al.,* 2005). The study included physical mapping of rolled leaf QTLs (Shao *et al.,* 2005) and a grain-weight QTL, *gw3.1* in rice (Li *et al.,* 2004).

2.2.2 Marker-assisted selection for quantitative trait loci

MAS has been used extensively for transferring and pyramiding major-effect qualitative genes for traits with high heritability into elite breeding lines or cultivars but less so for QTLs (Bernardo, 2008**,** Collard and Mackill, 2008**;** Xu and Crouch, 2008). MAS for quantitative traits controlled by one or a few major-effect QTLs and traits controlled by many minor-effect QTLs require different strategies for trait improvement (Bernardo, 2008; Eathington, *et al*., 2007**)**. MAS for major-effect QTLs can be similar to MAS for qualitative genes in that the process involves the direct transfer of favourable alleles with a relatively large phenotypic effect to a recurrent parent *via* MAS backcrossing or via MAS-assisted transfer between breeding lines or populations to replace unfavourable alleles with favourable alleles. In contrast, multiple minor-effect QTLs are more appropriately targets for population-based improvement methods such as marker-assisted recurrent selection (MARS), in which the frequency of favourable QTL alleles in the population can be increased through cycles of MAS for multiple QTLs and intermating of the selected individuals in the population in a recurrent selection scheme (Bernardo, 2008).

2.3 Bacterial blight of Rice

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Ishiyama, 1922; Swing *et al*., 1990) is one of the most destructive diseases of rice throughout the world (Mew, 1987). The disease was initially observed by farmers in Japan during 1884-85 and it is widespread throughout Asia but has also been reported to occur in Australia, the United States and several rice growing countries of Latin America and Africa. The reduction in yield in case of severe infection could be as high as 50% (Mew *et al.*, 1993) where as 10- 12% yield reduction has been recorded

in case of mild infection (Ou, 1985). Rice yield losses caused by Bacterial blight in some areas of Asia can be as high as 50% (Adhikari *et al.*, 1995). In tropical countries, Bacterial blight is even more destructive. Reports from the Philippines, Indonesia and India estimate that losses due to the kresek syndrome of Bacterial blight, which affects recently transplanted seedlings, have reached 60–75%, depending on weather, location and rice variety (Reddy *et al*., 1979). In addition to reducing yield, Bacterial blight may also affect grain quality by interfering with maturation (Goto, 1992; Ou, 1985). Agarwal *et al*., 2005 reported that in the Basmati rice, yield loss can reach up to 100%.

Bacterial blight occurs at all growth stages of rice and is manifested by either leaf blight or "Kresek" symptoms. The causal organism invades plants through water pores and wounds (Mizukami, 1956; Tabei & Mukoo, 1960). Bacteria multiply in the intercellular spaces of the underlying epitheme, then enter and spread into the plant through the xylem (Noda and Kaku, 1999). The *Xanthomonas oryzae* pv. *oryzae* may also gain access to the xylem through wounds or openings caused by emerging roots at the base of the leaf sheath (Ou,1985). Within the xylem, *Xoo* presumably interacts with xylem parenchyma cells (Hilaire *et al*., 2001). Since the water pores are located at the margins of upper parts of the leaf, the lesion starts from the leaf margins near its tip. As the disease progresses, the tiny water soaked lesions turns yellow, enlarges in size progressively and develop into an elongated irregular lesion with wavy margins. Bacterial ooze, which consists of small, yellowish, spherical masses, may sometimes be seen on the margins or veins of the freshly infected leaf under moist conditions. The disease is also characterized by a systemic infection phase, which is manifested

by acute wilting of young plants. This is commonly referred to as “Kresek” phase (Reitsma and Schure, 1950).

2.3.1 Genetic variation in pathogen of Bacterial blight

There were two major pathotypes of the bacterium have been identified in India (Reddy and Reddy, 1992). Each race has specific virulence to varieties with different resistance genes, showing a gene for gene relationship in the host pathogen interaction (Mew, 1987; Veracruz and Mew, 1989). Adhikari *et al.*, 1995 tried to group the pathotypes of Asia. They analyzed 308 strains of *Xoo* from several rice growing regions in Asia using RFLPs and virulence typing and grouped all the pathotypes into five major clusters. Of the five clusters, three consisted of strains from a single country. Finckh and Nelson (1999) reported the presence of eight races of *Xoo* which are widely used to represent the diversity of the pathogen in IRRI, Philippines. Such studies are useful for resistance breeding against the Philippines isolates.

2.3.2 Control of disease for Bacterial blight

The use of resistant cultivars is the most economical and effective method to control this disease (Ogawa, 1993). There are numerous donors for resistances to *Xoo* have been identified (Lin *et al*., 1996). Breeding and deployment of resistant cultivars carrying major resistance (*R*) genes has been the most effective approach to controlling Bacterial blight. The *R* genes to Bacterial blight have been identified (Table 2.1), mostly from *O. sativa ssp. indica* cultivars but some also from *japonica* varieties and from related wild species including *O. longistaminata, O. rufipogon, O. minuta* and *O. officinalis* (Brar and Khush, 1997; Lee *et al*., 2003). In addition, several resistance genes or alleles have been produced by mutating cultivated rice lines e.g. by treatment with N-methyl-N-nitrosourea or thermal neutron irradiation or

by somaclonal mutagenesis (Gao *et al*., 2001; Lee *et al*., 2003). Some *R* genes are effective only in adult plants (e.g. *Xa21*) whereas most do not seem to be developmentally regulated (e.g. *Xa23, Xa26*). Curiously, *Xa3* is typically effective only in adult plants but against at least one race, it is effective at all stages of growth. Some genes condition resistance to a wide spectrum of *Xoo* races (e.g. *Xa21*, *Xa23*), whereas others are effective against only one or a few races that may be limited to a particular geographical location (e.g. *Xa1*). Most *R* genes to Bacterial blight are dominant but some are recessive (e.g. *xa5, xa13*) and some display semidominance (e.g. *Xa27*). Most *R* genes to Bacterial blight have been introgressed into the background of the susceptible indica cultivar IR24 to develop a set of near-isogenic lines (NILs) and some have been pyramided, either through classical breeding and marker-assisted selection or through genetic engineering, to develop new plant types and NILs (Narayanan *et al*., 2002; Sanchez *et al*., 2000; Singh *et al*., 2001). Pyramid lines have displayed higher levels and/or wider spectra of resistance to Bacterial blight than the parental NILs with single *R* genes, suggesting synergism and complementation among *R* genes (Adhikari *et al*., 1999; Huang *et al*., 1997; Narayanan *et al*., 2002).

2.3.3 Genetic diversity of host plant resistance for Bacterial blight

Rice and its bacterial blight pathogen are considered a model system to study interactions between host plants and their pathogens. Large number of studies conducted in several countries has identified the presence of many major genes conferring resistance to various races of the pathogen. These genes are designated as *Xa-1* to *Xa-29* (Table 2.1).

Table 2.1 Genes conferring resistance to different races of Bacterial blight pathogen

Gene	Chr	Source	References
Xa1	4	Kogyoku	(Yoshimura *et al*., 1998)
Xa2	4	Tetep	(He *et al*., 2006; Oryzabase, 2006)
Xa3	11	Wase Aikoku 3	(Kaku and Ogawa, 2000; Ogawa *et al*., 1986; Qi and Mew, 1985; Sun *et al*., 2004)
Xa4	11	TKM6	(Wang *et al*., 2001)
xa 5	5	Aus Boro lines (e.g. DZ192)	(Iyer and McCouch, 2004)
Xa 7	6	DV85	(Lee and Khush, 2000; Porter *et al*., 2003)
xa8	7	PI231129	(Sidhu *et al*., 1978; Singh *et al*., 2002)
Xa10	11	Cas 209	(Oryzabase, 2006; Yoshimura *et al*., 1983)
Xa11		IR8, IR944	(Mew, 1987; Oryzabase, 2006)
Xa12	4	Kogyoku	(Mew, 1987; Oryzabase, 2006)
xa13	8	BJ1 (Aus Boro)	(Chu *et al*., 2006)
Xa14	4	TN1	(Oryzabase, 2006)
xa15		M41, a Harebare mutant line	(Gnanamanickam *et al*., 1999; Nakai *et al*., 1988)
Xa16		Tetep	(Oryzabase, 2006)
Xa17		Asominori	(Oryzabase, 2006)
Xa18		IR24, Toyonishiki	(Liu *et al*., 2004; Oryzabase, 2006)
xa19		XM5	(Lee *et al*., 2003; Oryzabase, 2006)
xa20		XM6	(Lee *et al*., 2003; Oryzabase, 2006)
Xa21	11	*O. longistaminata*	(Song *et al*., 1995)
Xa22	11	Zhachanglong	(Oryzabase, 2006; Sun *et al*., 2004; Wang *et al*., 2003)
Xa23	11	*O. rufipogon*	(Zhang *et al*., 1998, 2001)
xa24		DV86, DV85, Aus 295	(Khush and Angeles, 1999; Lee *et al*., 2000)
Xa25(a)	4	HX-3, a somaclonal mutant of Minghui 63	(Gao *et al*., 2001, 2005)
Xa25(b)	12	Minghui 63	(Chen *et al*., 2002)
Xa26	11	Minghui 63	(Sun *et al*., 2004; Yang *et al*., 2003)
*Xa27**	6	*O. minuta*	(Gu *et al*., 2004, 2005; Lee *et al*., 2003)
xa28		Lota Sail	(Lee *et al*., 2003)
Xa29(t)	1	*O. officinalis*	(Tan *et al*., 2004)

*- semidominant.

2.3.4 The major genes cloned and sequenced for Bacterial blight

Only 10 genes cloned out of 29 genes have been tagged and mapped to different chromosomes (Table 2.2).These genes are both dominant and recessive in nature. Majority of genes conferring resistance to *X. oryzae* pv. *oryzae* are dominant in nature. The resistance gene *Xa-1* conferring resistance to the Japanese *Xoo* race I was first reported by Sakaguchi (1967). The *Xa-1* gene was extensively studied and tagged with a RFLP marker XNpb235 and mapped to chromosome 4 (Yoshimura *et al*., 1996). The broad spectrum bacterial blight resistance gene *Xa-21* was introgressed

from a wild species *O. longistaminata* into *O. sativa* background (Khush *et al*., 1989). Ronald et al. 1992 tagged the *Xa-21* gene with RAPD marker RAPD 248. Ronald (1997) adopted a map-based cloning strategy for the first time to clone *Xa-21* in rice.

2.3.5 Major recessive genes–mapped and cloned for Bacterial blight

The recessive resistance gene *xa-5* was tagged with RFLP markers RG556 and RZ390 and microsatellite markers RM122 and RM390. It was mapped on chromosome 5 based on the segregation data for 207 F_2 individuals of two populations, IR 24 × IRBB5 and Chinsurah BoroII × IR64 (Blair and McCouch, 1997). The *xa-13* confers resistance to the Philippines race 6 of *Xoo*. The gene was tagged with the RAPD marker OPAC 05-900 and RFLP marker RG136 and mapped on chromosome 8 using the doubled haploid mapping population of IR64 and Azucena (Zhang *et al*., 1996a).

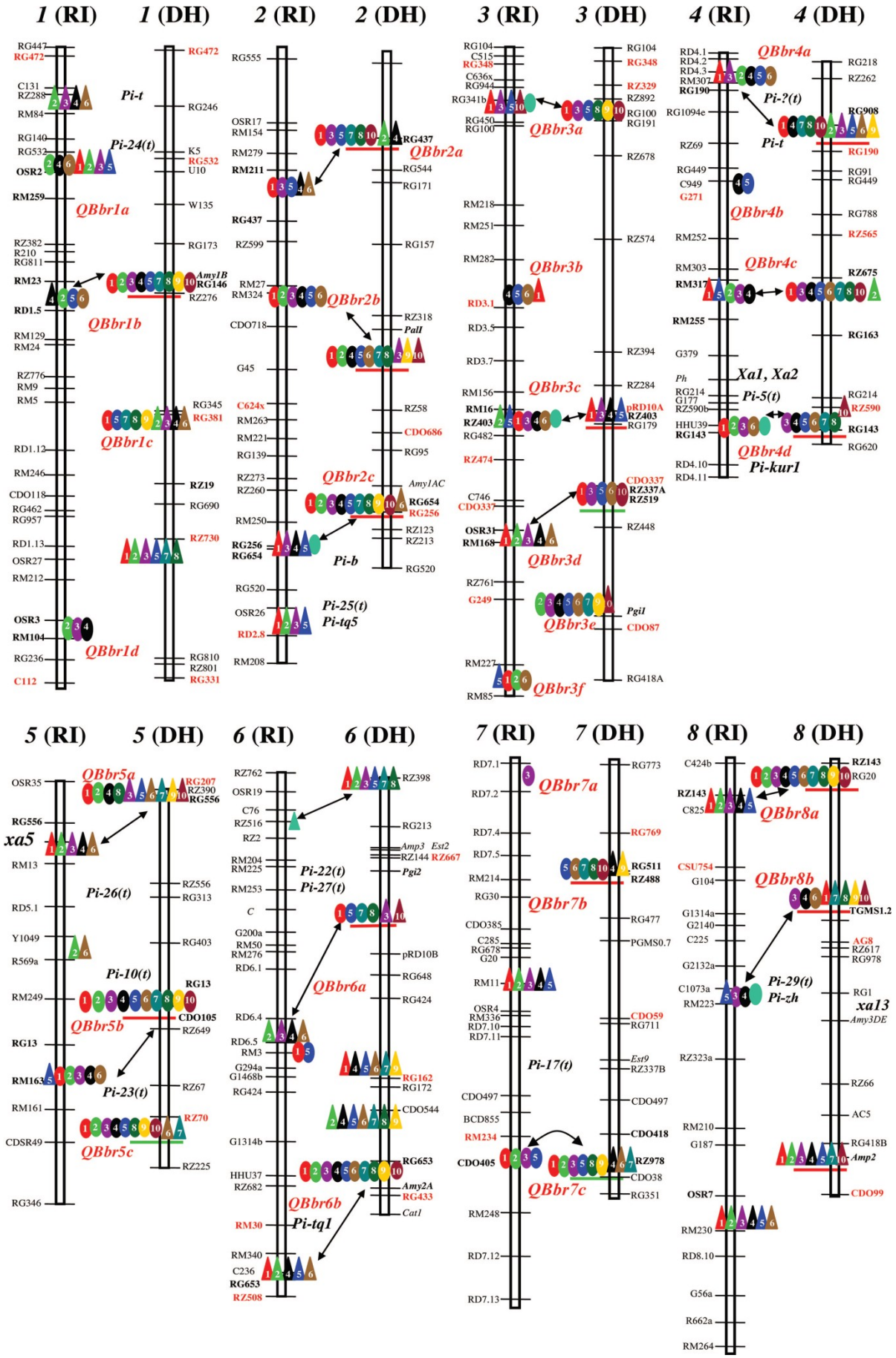
Table 2.2 Bacterial blight resistance genes tagged and cloned

S. N.	BB resistance gene	Donor/cross	Type of marker	References
1	*Xa-1*	IRBB1 Kogyoku/IR24	RFLP	Yoshimura *et al.,* 1996
2	*Xa-3*	IRBB3 (Chugoku45)/IR24	RFLP	Yoshimura *et al*., 1992
3	*Xa-4*	IRBB4 (IR1545-339)/IR24	RFLP	Yoshimura *et al*.,1995 Wang *et al.,*2001
4	*xa-5*	IRBB5/IR24 (Chinsurah Boroll)/IR64	RFLP SSR	McCouch *et al.,*1992, Blair and McCouch, 1997
5	*Xa-7*	No information	RFLP	Borines *et al*., 2000
6	*Xa-10*	Cas209	RFLP	Yoshimura *et al.,* 1995
7	*xa-13*	IR66699-55-42/IR24	RAPD RFLP RFLP	Zhang *et al.,* 1996a Sanchez *et al.,*1999
8	*Xa-14*	Japonica/Zhengzhuai (indica)	RFLP	Tan *et al.,* 1999
9	*Xa-21*	*O. longistaminata*/IR24	RFLP RAPD	Ronald *et al.,*1992 Williams *et al*., 1996
10	*Xa-22(t)*	Zhachanglong/ Zhonchu Ali	RFLP	Lin *et al.,* 1996

2.3.6 The QTLs for Bacterial blight resistance

The presence of complete resistance and partial resistance to *Xoo* in rice has been reported (Zhang and Mew, 1985; Parlevliet and Zadoks, 1977). Resistance of rice to specific *Xoo* races is governed by both major *R* genes with a qualitative effect that condition complete resistance (CR) and polygenes with a quantitative effect (quantitative trait loci, QTL) that condition partial resistance (PR) (Figure 2.1) (Koch and Parlevliet, 1991b; Li *et al.*, 2006). A recent study of the epistatic effects between *R* genes and QTL for resistance in rice revealed a complex genetic network in which the interactions between alleles at the rice *R* locus and alleles at the corresponding avirulence loci in *Xoo* lead to complete resistance and interactions between rice QTL for resistance and corresponding aggressiveness loci in *Xoo* lead to partial resistance. The race specificity of the QTLs during partial resistance and strong genetic overlap between complete resistance and partial resistance suggested that PR is essentially a 'weaker' CR (Li *et al*., 2006).

The distinction between complete resistance and partial resistance may be masked by the fact that some QTLs for Bacterial blight may in fact be 'defeated' dominant *R* genes, or, in a sense, *R* genes that have lost their qualitative nature and adopted new, intermediate phenotypes (Koch and Parlevliet, 1991a; Li *et al*., 1999). An example is *Xa4*, a single dominant gene for resistance to bacterial blight widely used in Asian rice breeding programmes. The gene *Xa4* conferred durable resistance in cultivars IR20 and IR64, among others developed at IRRI, before being overcome by the emergence of two new Chinese races in the early 1970s (Mew *et al*., 1992). The breakdown of *Xa4* mediated resistance was manifested by significant changes in the qualitative action of *Xa4* (i.e. loss of dominance) and by a quantitative reduction of 50% in the magnitude of the effect of the *Xa4* gene (Li *et al*., 1999). However, the defeated *Xa4* can still act as a recessive QTL and show quantitative complementation when pyramided with other resistance genes in elite cultivar breeding.


1 (RI)
1 (DH)
2 (RI)
2 (DH)
3 (RI)
3 (DH)
4 (RI)
4 (DH)
5 (RI)
5 (DH)
6 (RI)
6 (DH)
7 (RI)
7 (DH)
8 (RI)
8 (DH)
Pi-t
Pi-24(t)
QBbr1a
QBbr1b
QBbr1c
QBbr1d
QBbr2a
QBbr2b
QBbr2c
Pi-b
Pi-25(t)
Pi-tq5
QBbr3a
QBbr3b
QBbr3c
QBbr3d
QBbr3e
QBbr3f
QBbr4a
Pi-?(t)
Pi-t
QBbr4b
QBbr4c
Xa1, Xa2
Pi-5(t)
QBbr4d
Pi-kur1
QBbr5a
xa5
Pi-26(t)
Pi-10(t)
QBbr5b
Pi-23(t)
QBbr5c
Pi-22(t)
Pi-27(t)
QBbr6a
QBbr6b
Pi-tq1
QBbr7a
QBbr7b
Pi-17(t)
QBbr7c
QBbr8a
QBbr8b
Pi-29(t)
Pi-zh
xa13

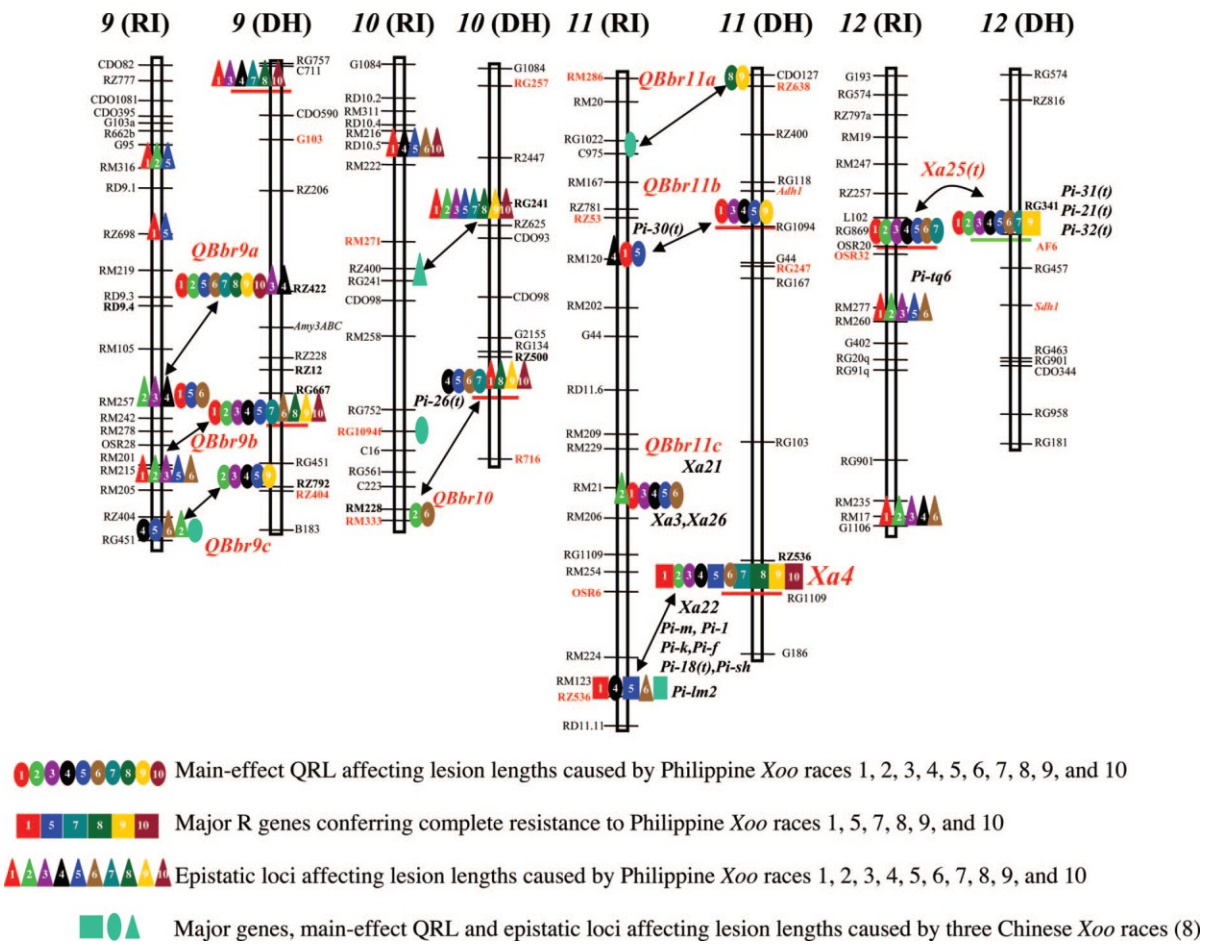


Figure 2.1 Genomic locations of *Xa4*, *Xa25*(*t*), QRL (in red italics), and epistatic loci (triangles) associated with complete and partial resistance to 10 Philippine and 3 Chinese *Xoo* races detected in the Lemont × Teqing RILs (RI) and IR64 × Azucena DHLs (DH) population

2.4 Sheath blight of Rice

Sheath blight, caused by the fungus *Rhizoctonia solani* Kuhn, is one of the major foliar diseases of rice worldwide that severely impairs both grain yield and quality (Ou, 1985; Savary *et al.*, 2006). The disease was first reported in Japan in 1910 and subsequently reported to be widespread (Rush *et al*., 1992). The *Rhizoctonia solani* is a semisaprophytic fungus with a broad host range, affecting many crops including rice, maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), sorghum [*Sorghum bicolor* (L.) Moench], common bean (*Phaseolus* spp.), and soybean [*Glycine max* (L.) Merr.] (Zhao *et al*., 2006). The yield losses of 5–10% have been estimated for tropical

lowland rice in Asia (Savary *et al.*, 2000a). At the late tillering or early internode elongation growth stage, a disease lesion often is observed in the leaf sheath near the water line from germinating sclerotia, the primary source of inoculum in the field (Ou, 1985). Disease lesions may coalesce to form bigger lesions and the disease can spread to adjacent plants in the field.

Resistance to rice ShB is a complex, quantitative trait controlled by polygenes (Sha and Zhu 1990; Li *et al.*, 1995; Pinson *et al.,* 2005). On the other hand, a few studies (Xie *et al.*, 1992) proposed that ShB in some rice varieties are controlled by a few major genes. Breeding for ShB has been difficult, mainly because of the lack of identified resistant donors in cultivated varieties (Bonmann *et al.*, 1992). The identification of genes that affect complexly inherited trait is often difficult and best approached through developing a genetic linkage map to identify quantitative trait loci (QTLs) (Tanksley and McCouch, 1997). The accurate measurement of ShB under field conditions (Yuen and Forbes, 2009) depends on a range of environmental factors (Ou 1985; Castilla *et al.*, 1996; Eizenga *et al.,* 2002) and plant morphological traits, such as plant height (Li *et al.,* 1995; Zou *et al.,* 2000; Pinson *et al.,* 2005) which interacts, resulting in the observed variation in resistant (susceptible) phenotypes. There were many sheath blight QTL have been reported to be associated with plant morphological traits and heading date (HD).

2.4.1 Epidemiological features of Sheath blight

The source of the inoculum is mainly soil-borne sclerotia (Roy 1986; Kim and Kim 1987; Mgonja *et al.,* 1987; Damicone *et al,.* 1993; Fan *et al.,* 1993) or infected plant debris (Kobayashi *et al.*, 2006). Another aspect of the disease is that its secondary spread depends almost exclusively on running hyphae that progress out from the initial lesions, from the lower part of the crop canopy towards its upper part

along tillers and leaves and across adjacent plant units (individual plants or hills). This has been commonly referred to as the 'vertical' and 'horizontal' spread process (Hashiba *et al.,* 1982). The main spread mechanism of the disease explains why sheath blight is an extremely aggregated disease when compared to other plant diseases (Madden *et al.,* 2007). This aggregation of sheath blight is a central feature of rice sheath blight epidemics (Gou *et al.,* 1983; Savary *et al.,* 1997, Gong and Zhang, 2002; Tan and Wang, 2002) and a critical element to assess resistance (Zadoks and Schein, 1979).

2.4.2 Methods used for assessing resistance to Sheath blight of rice

Methodology choices also have major consequences on the accuracy, precision, repeatability and ultimately usefulness of the results (Madden *et al*., 2007; Yuen and Forbes, 2009). These methodological components are described in (Table 2.3). The bulk of screening for resistance to sheath blight within *O. sativa* species has been conducted at the plot scale. One of the more common inoculation method is that of inserting a rice grain hull (RGH) mixture colonized by the fungus among the tillers at the base of the hill (IRRI, 1992). Inoculation is mostly performed on all plants or hills (IRRI, 1992), but sometimes by spreading inoculum between rows (Marchetti and Bollich, 1991; Li *et al.,* 1995). The relative lesion height (RLH) which is the height of the highest Sheath blight lesion divided by PH (Hashiba, 1984; Ahn *et al.,* 1986) or related variables (Marchetti and Bollich, 1991; Savary and Mew, 1996) are standard measurements used to reflect disease intensity. A scale based on RLH, ranging from highly resistant to highly susceptible genotypes, has been developed (IRRI, 1987). Inoculation of potted plants in the greenhouse, by placing a rice grain hull mixture at the base of plants when they are at the maximum tillering stage, has been used to screen accessions of *Oryza spp*. (IRRI, 1987, 1992, 1993). One

inoculation method recently used is the inoculation of young potted plants with infected PDA disks, followed by incubation in plastic bottles ('micro-chambers') (Jia *et al.*, 2007). This method has also been used to screen wild rice species (Prasad and Eizenga, 2008) and assess recombinant inbred lines (RILs) (Liu *et al.*, 2009).

Table 2.3 The methodologies for Sheath blight (ShB) screening and phenotyping

Biological hierarchy level	Inoculation method	Incubation conditions	Assessment method	References
Plant	Colonized RGH, at maximum tillering	Greenhouse	7 DAI: lesion height; RLH	IRRI (1992) IRRI (l993)
	Colonized PDA block, on 35-day-old seedlings	Greenhouse, plants under plastic bottles	7—10 DAI: index of lesion length	Prasad and Eizenga (2008)
	Colonized PDA block, on 21-day-old seedlings		8—10 DAI: index of lesion length	Liu *et al.*, 2009
	Colonized PDA plug, at 50 DAS	Greenhouse, high humidity environment	15 DAI: severity index scale	Liu *et al.*, 2009
Tiller	Colonized toothpick in leaf sheath, at heading	Controlled Conditions	7 DAI: severity on leaf; lesion length 7-10 DAI: 0-9 scale	Eizenga *et. al.*, 2002; Prasad and Eizenga (2008) Eizenga *et al.*, 2009
	Colonized toothpick in leaf sheath, at stem elongation	Field	30 days after heading: 0—9 scale	Zou *et al.*, 2000 Zuo *et al.*, 2008 Yin *et al.*,2009
	Sclerotia placed beneath the sheath		10 DAI: lesion length on the sheath	Chnnamallikaju na *et al.,* 2010
	Injection of mycelium on the third leaf sheath from leaf flag		28 DAI: severity on the sheath of the second leaf 3 DAI: lesion length	Sato *et al.*, 2004 IRRl, l987
Leaf	Colonized PDA disc	Controlled conditions	3 DAI: severity on leaf; lesion length	Pasad and Eizenga, 2008

RGH = rice grain hull mixture, DAT= days after transplanting, DAS = days after sowing, PDA= potato dextrose agar, RLH = relative lesion height, DAI = day after inoculation, SES= Standard Evaluation System for rice

[a] = SS *Oryza sativa* germplasm screening, P = phenotyping, WS = rice wild relative (*Oryza spp.*) screen

2.4.3 The sources of resistance to Sheath blight of rice

Several groups have attempted to identify sources of Sheath blight resistance by screening local accessions, cultivars, landraces and advanced breeding lines. The most promising genotypes are shown in (Table 2.4). These studies resulted in the identification of genotypes with moderate to high levels of resistance (Ram and Ansari, 1990; Borah *et al.*, 1994; Biswas, 2001; Hua *et al.,* 2000; Meena *et al.,* 2000; Singha and Borah, 2000; Mohanta *et al.,* 2002; Goel and Lore, 2004; Mew *et al.,* 2004; Mosaddeque *et al.,* 2008).

Table 2.4 The important ShB resistance sources reported in the literature

S.N.	References	Promising genotypes
1	Das (1970)	NC 678, Dudsor, Bhasamanik
2	Wu (1971)	Chin-kou-tsan, Zenith, CO.17, Dinominga, Puang Nahk 16, Baok, Toma-112, R.T.S.31, Kele Kala
3	Roy (1977)	Lalsatkara
4	Bhaktavatsalam *et al.,* 1978	ARC15762, ARC 18119, ARC 18275, ARC 18545
5	Rajan and Nair (1979)	IR24, IR26, IR29, Jaya, Jaganath, Mashoori, Pankaj, Rajeshwari, Supriya, Sabari, TKM6
6	Manian and Rao (1979)	Nizersail, Rajasail, Tabend, Ta-poo-cho-z, Kattachambha, DA 29, ARC 5925, ARC 5943, ARC 14529, ARC 10572, ARC 10618, ARC 10836
7	Crill *et al*., 1982	Tapoochoz, Bahagia, Laka
8	Borthakur and Addy (1988)	Taraboli 1, Dholamula, Supkheru, Chidon
9	Gokulapulan and Nair (1983)	Bharati, Rohini
10	Ansari *et al.*, 1989	Bog II, Aduthurni, Chinese galendopuram, Arkavati, Saket-4, Neela, MTU-3, MTU-7, MTU-13, MTU-3642, BPT-6
11	Sha and Zhu (1990)	Tetep, Tapoo-cho-z, Guyanal
12	Xie *et al.*, 1992	LSBR-5, LSBR-33
13	Marchetti *et al.,*1995	RU8703196, B82-761
14	Singh and Dodan (1995)	KK2, Dodan, IR40 and Camor
15	Singha and Borah (2000)	Chingdar, As 93-1, Mairan, N–22, Panjasali, Up-52, Upland-2
16	Pinson *et al.,* 2008	TIL:455, TIL:514, TIL:642

2.4.4 The QTLs for Sheath blight resistance in rice

Srinivasachary *et al*., 2011 reviewed sheath blight resistance QTL (ShB-QTL) that have been published in different articles. Among 12 ShB-QTL studies reviewed, 11 studies were conducted with indica rice cultivars and only one study was carried out with a tropical japonica cultivar (Sharma *et al.,* 2009). A detailed list of mapping populations, detected QTL is given in (Table 2.5). A total of 33 ShB-QTL were identified; these were located in all 12 rice chromosomes. Most of these individual QTL only explained about 10–15% of phenotypic variance. Of these 33 QTL, only 16 (on chromosomes 1, 2, 3, 4, 7, 8, 9, and 11, respectively) were consistent. They used the rice genetic map published by (Temnykh *et al.,* 2001). The maps presented here were drawn using MapChart (Voorrips, 2002). In most cases, SSR markers were used as anchor points to indicate ShB-QTL. A total of six PH-QTL (chromosomes 1, 2, 3, 7, 8, and 9) from different studies were mapped on the same regions as ShB-QTL (Table 2.5 and Figure 2.2). In many cases, the peak LOD score (a logarithmic index of statistical significance) of PH-QTL co-localized with the peak LOD for ShB-QTL. Similarly, seven of the HD-QTL, one each on chromosomes 1, 3, 6, 7, 8, 9, and 12, co-localized with ShB-QTL.

Table 2.5 Details of rice ShB-QTLs identified in different mapping population

S.N.	QTL name	Peak marker locus	Genetic material	References
1	*qSB-2* *qSB-3* *qSB-4* *qSB-8* *qSB-9* *qSB-12*	RG654-RZ260 R0348-RG944 RG143-RG214 RG2O-RG1034 RG9IOb-RZ777 RG214a-RZ397	255 F_4	Li *et al*.,1995
2	*qSB-2* *qSB-3* *qSB-7*	G243-RM29 R250-C746 RG30-RG447	F_2 clonal families	Pan *et al*.,1999
3	*qSB-2* *qSB-3* *qSB-7*	RM29 R250-C746 RG3O-RG477	128 F_2 clonal families	Zou *et al*., 2000

	qSB-9-1 *qSB-9-2* *qSB-11*	C397-G103 RG570-C356 G44-RG118		
4	*qSBR-2* *qSBR-3* *qSBR-7* *qSBR-l1*	RG171-G243A G249-G164 RG511-TCT122 CT224-CT44	DH, 127	Kunihiro *et al.,* 2002
5	*qSB-5* *qSB-9*	C624-RM26 RM242-C472	RILs 240 lines	Han *et al*., 2002
6	*Rsb 1*	RM173	1032 F_2	Che *et al*.,2003
7	*qSB-3* *qSB-12*	RM13856 RM1880	60 BC_1F_1	Sato *et al.*, 2004
8	*qSB-9* *qSB-11*	RM205-RM201 RM167-Y529	115 F_2 clonal population	Tan *et al.,* 2005
9	*qSB-1* *qSB-2* *qSB-3-1* *qSB-3-2* *qSB-4-1* *qSB-4-2* *qSB-5* *qSB-6-1* *qSB-6-2* *qSB-7* *qSB-8-l* *qSB-8-2* *qSB-9* *qSB-l0* *qSB-12*	RG532x C624x RG348x RZ474 RGIO94e RZ59Ox Y1049 C RZ508 C285 G104 R662 RZ404 RG561 G1106	300 RILs F_{10} and F_{11}	Pinson *et al*., 2005
10		RM1339 RM3685 RM7072 RM3823	279 $F_{2:3}$	Sharma *et al*., 2009
11	*qSB-1* *qSB-2-1* *qSB-2-2* *qSB-3-1* *qSB-3-3* *qSB-5* *qSB-6* *qSB-9-1* *qSB-9-2*	RM104 RM341 RM250 RM5626/RM426 RM85 RM13 RM190 RM434 RM245	250 F_5 RILs	Liu *et al*., 2009
12	*qSBRI-1* *qSBR3-1* *qSBR7-1* *qSBR8-1* *qSBR9-1* *qSBR3-11-1* *qSBR3-1 1-2* *qSBR3-11-3*	HVSSR1-68 RM251 RM336/IRM3691 RM210 RM257 RM224 RM209 RM202	127 RIL (F2:10)	Channamallikarjuna *et al.*, 2010

42

Vertical bars denoted R1–R12 represent rice chromosomes 1–12. Linkage map has been adapted from (Temnykh *et al.,* 2001)

Figure 2.2 Chromosomal location of quantitative trait loci (QTL) reported for Sheath blight resistance (ShB) and associated traits in rice

2.4.5 The relationship between ShB-QTL and QTLs for other plant traits

In addition to ShB resistance, eight mapping studies also accounted for other morpho-developmental traits, including PH (PH-QTL) and HD (HD-QTL). Many of these studies found a strong association between resistance to ShB and tall or late-maturity. This association was indicated by the co-localization of QTL and by a partial to high correlation between these morpho-developmental traits and ShB resistance.

A total of six PH-QTL (chromosomes 1, 2, 3, 7, 8 and 9) from different studies were mapped on the same regions as ShB-QTL. In many cases, the peak LOD score (a logarithmic index of statistical significance) of PH-QTL co-localized with the peak LOD for ShB-QTL. Similarly, seven of the HD-QTL, one each on chromosomes 1, 3, 6, 7, 8, 9, and 12, co-localized with ShB-QTL (Figure 2.2). Thus, about 34.5% of the total QTL reported for ShB resistance co-localized with either PH-QTL or HD-QTL, possibly indicating pleiotropic effects or tight linkages between the genes controlling morpho-developmental and ShB resistance traits. In a Teqing/Lemont F_4 population, Li *et al.,* 1995 reported that a large proportion of the phenotypic variation in ShB resistance was explained by the morpho-developmental traits (mainly HD, 42%, and PH, 4%). Similarly, PH and HD explained 43% of the ShB reaction in a mapping population derived from Pecos, a tropical *japonica* reported to be ShB resistant (Sharma *et al.,* 2009).

2.4.6 The QTL validation and use in marker-assisted selection

In rice variety Tetep, identified and mapped the QTL *qSBR11-1* on chromosome 11, in the genomic region spanning RM1233 to RM224 (Channamallikarjuna *et al.*, 2010). A dominant ShB-QTL, *qSB-9TQ* has been well characterized from an indica cultivar, Teqing (Pinson *et al.*, 2005; Tan *et al.,* 2005;

Yin *et al.,* 2009). Pinson *et al.*, 2008 using the markers tightly linked to ShB-QTL, performed marker-assisted selection to introgress resistant alleles from Teqing (PI 536047) into three rice germplasm lines that have been released as varieties in the USA.

2.5 Leaf blast of Rice

Blast disease of rice caused by the filamentous fungus *Magnaporthe oryzae* has been one of the most damaging diseases of rice and remains one of the most difficult crop diseases to manage (Khush and Jena, 2007). The fungus *Magnaporthe grisea* is a haploid filamentous *Ascomycete* with a relatively small genome of ~40 Mb divided into seven chromosomes (Dean *et al.,* 2005). The *M. grisea* is becoming an excellent model organism for studying fungal phytopathogenicity and host-parasite interactions. *M. grisea* is one of the most devastating threats to food security worldwide. Conservatively, each year enough rice is destroyed by rice blast disease to feed 60 million people (Zeigler *et al.,* 1994). The blast pathosystem consists of two major interrelated phases: leaf blast and panicle blast, with the former providing inoculum for the later (Ou, 1985). Blast resistance in rice has been generally classified into two types: complete (qualitative) or true and partial (quantitative) or Wild resistance (Ezuka, 1972). The deployment of resistant cultivars is the most effective and economical way of controlling blast disease, so breeding for resistant cultivars continues to be a priority in rice improvement (Ikehashi & Khush, 1979).

Rice blast is a well-studied disease and several recent reviews focused on the biology of the fungal infection (Caracuel-Rios and Talbot, 2007; Ebbole, 2007; Veneault-Fourrey and Talbot, 2005) and rice resistance (Dai *et al.,* 2007). Moreover, with the completion of the rice (Goff *et al.*, 2002) and *M. oryzae* (Dean *et al.*, 2005) genome sequences, rice blast disease has strength its position as a model for plant–

pathogen interactions in monocotyledons (Ribot *et al.*, 2007; Valent 1990; Veneault-Fourrey and Talbot, 2005).

Despite the relatively long history of genetic studies on rice resistance to blast, examples of cultivars with durable resistance are very few (Ou, 1985) and resistance breakdown shortly after cultivation of newly released cultivars is the rule. This phenomenon is due, in part, to pathogen evolution toward virulence. Reviewing breeding for resistance to blast, Ou (1985) concludes: "Some cultivars show breakdown in resistance after a few years and others become susceptible in other geographic areas. The problem arises because of the variability in pathogenicity of the fungus, a factor which in the past has been underestimated by workers in this field". Different strategies to breed durable resistance have been proposed to counter blast evolution. Some strategies, such as pyramiding (Bonman *et al.*, 1992), lineage exclusion (Zeigler *et al.*, 1994), multilines (Abe, 2004) and mixtures (Zhu *et al.*, 2000) are based on the use of complete and specific resistance genes. Others are based on the accumulation of partial resistance (Bonman *et al.*, 1992), a strategy thought to be more durable because it is assumed to be more general.

2.5.1 The overview of blast disease resistance in rice

Most of the qualitative genes are dominant. The first report of the inheritance of host resistance to rice blast (Sasaki, 1923). The first *Pi* gene in rice was named by Kiyosawa, 1966. Many reports mention that the genes affecting blast resistance are colocalized on chromosomes 6, 11 and 12 (Wu, *et al.*, 2005). Ballini *et al.*, 2008 also reported that 80% of the complete resistance genes for rice blast colocalize with nucleotide-binding site leucine-rich repeat (NBS-LRR) candidates.

Table 2.6 The genes responsible for the rice blast resistance are listed chromosome wise

Gene	Chromosome	references
Pit	1	Háyashi *et al.*, 2006
Pi27(t)	1	Zhu *et al.*, 2004
Pi24(t)	1	Sallaud *et al.*, 2003
Pitp(t)	1	Barman *et al.*, 2004
Pi35(t)	1	Nguyen *et al.*, 2006
Pi37	1	Lin *et al.*, 2007
Pish	1	Fukuta *et al.*, 2004
Pidl(t)	2	Chen *et al.*, 2004
Pig(t)	2	Zhou *et al.*, 2004
Pitq5	2	Tabien *et al.*, 2000
Piyl(t)	2	Lei *et al.*, 2005
Piy2(t)	2	Lei *et al.*, 2005
Pib	2	Fjellstrom *et al.,* 2004
Pi25(t)	2	Sallaud *et al*., 2003
Pi14(t)	2	Pan *et al*., 1996
pi21	4	Fukuoka and Okuno, 2001
Pikur1	4	Goto, 1988
Pi39(t)	4	Terashima *et al.,* 2008
Pi(1)	4	Causse *et al.,* 1994
Pi26(t)	5	Sallaud *et al.,* 2003
Pi23(t)	5	Naqbi *et al.,* 1995
Pi10	5	Naqbi and Chatto, 1996
Pi26(t)	6	Wu *et al.,* 2005
Pi27(t)	6	Sallaud *et al.,* 2003
Pi40(t)	6	Jeung *et al*., 2007
Piz-5	6	Zhou *et al.,* 2006
Piz	6	Goto, 1976; Hashimoto *et al*., 1998; Hayashi *et al.,* 2006
Piz-t	6	Zhou *et al.,* 2006
Pi9	6	Qu *et al.,* 2006
Pi25(t)	6	Wu *et al*., 2005
Pid2	6	Chen *et al.,* 2006
Pitq1	6	Tâbien *et al.,* 2000
Pi8	6	Pan *et al.,* 1995; Pan *et al.,*1996
Pi13(t)	6	Pan *et al.,*1996
Pi13	6	Ballini *et al.,* 2008
Pi17(t)	7	Pan *et.al.,* 1995, Iwata, 1996
Pi36	8	Liu *et al.,* 2007b
Pizh	8	Causse *et al.,* 1994
Pi29(t)	8	Sállaud *et al.,* 2003
PiGD-1(t)	8	Liu *et al.,* 2004
Pii2(t)	9	Kinoshita and Kiyosawa, 1997

Pi5(t)	9	Jeon *et al.,* 2003
Pi3(t)	9	Inükai *et al.,* 1996
Pi15	9	Pan *et al.*, 1996
Pii	9	Ise, 1991
PI28(t)	10	Sallaud *et al.,* 2003
PiGD2(t)	10	Liu *et al.,* 2004.
Pia	11	Goto *et.al.,* 1981
PiCO39(t)	11	Chauhan *et al.,* 2002
Pilm2	11	Tabien *et.al.,* 2000
Pi30(t)	11	Sallaud *et al.,* 2003
Pi7(t)	11	Wang *et al.,* 1994
Pi34	11	Zenbayashi *et al.,* 2002
Pi38	11	Gowda *et al.,* 2006
PBR	11	Fujii *et al.,* 1995
Pbl	11	Fujii *et al.,* 2000
Pi44(t)	11	Chen *et al.,* 1999
Pik-h	11	Sharma *et al.,* 2005
Pil	11	Hittalmani *et al.,* 2000
Pik-m	11	Kaji and Ogawa,1996
Pik	11	Hayashi *et al.,* 2006
Pik-p	11	Hayashi et al., 2006
Pik-s	11	Fjellstrom *et al.,* 2004
Pik-g	11	Pan *et al.,* 1996
Pise1	11	Goto, 1970
Pif	11	Shinoda *et al.,* 1971
Mpiz	11	Goto, 1976
Pikur2	11	Goto, 1988
Piisi	11	Goto, 1970
Pi24(t)	12	Koizumi, 2007
Pi62(t)	12	Wu *et al.,* 1996
Pitq6	12	Tabien *et al.,* 2000
Pi6(t)	12	McCouch *et al.,*1994
pi31 (t)	12	Sallaud *et al.,* 2003
pi31 (t)	12	Sallaud *et al.,* 2003
Ipi(t)	12	Causse *et al.,*1994
Ipi3(t)	12	Causse *et al.,*1994
Pi157	12	Naqvi and Chattoo, 1996
Pita	12	Bryan *et al.,* 2000
Pita-2	12	Nakamura *et al.,19*97, Hayashi *et al.,* 2006
Pi19(t)	12	Hayashi *et al.,*1996, Iwata, 1997
Pi39(t)	12	Liu *et al.,* 2007a
Pi20(t)	12	Li *et al.,* 2008
PiGD-3(t)	12	Liu *et al.,* 2005

2.5.2 Isolated and cloned complete resistance genes of blast

As of 2013, eight complete resistance genes to *Pib* (Wang *et al*., 1999), *Pita* (Bryan *et al*., 2000), *Pikh* (Sharma *et al*., 2005), *Pi9* (Qu *et al*., 2006), *Pi2*/*Piz*t (Zhou *et al*., 2006), *Pid2* (Chen *et al*., 2006), *Pi36* (Liu *et al*., 2007b) and *Pi37* (Lin *et al*., 2007) have been isolated and cloned using map-based cloning strategies are summarized in (Table 2.7).

Table 2.7 The information on cloned resistance genes of blast

S.N.	Gene symbol	Chr.	Locus structure	References
1	*Pib*	2	2 genes	Wang *et al.,* 1999
2	*Pita*	12	2 genes	Bryan *et al.,* 2000
3	*Pikh*	11	6 genes	Sharma *et al.,* 2005
4	*Pi9*	6	9 genes	Qu *et al.,* 2006
5	*Pi2/Pizt*	6	9 genes	Zhou *et al.,* 2006
6	*Pid2*	6	simple	Chen *et al.,* 2006
7	*Pi36*	8	simple	Liu *et al.,* 2007b
8	*Pi37*	1	4 genes	Lin *et al.,* 2007

2.5.3 The quantitative resistance to *M. grisea* the rice blast fungus

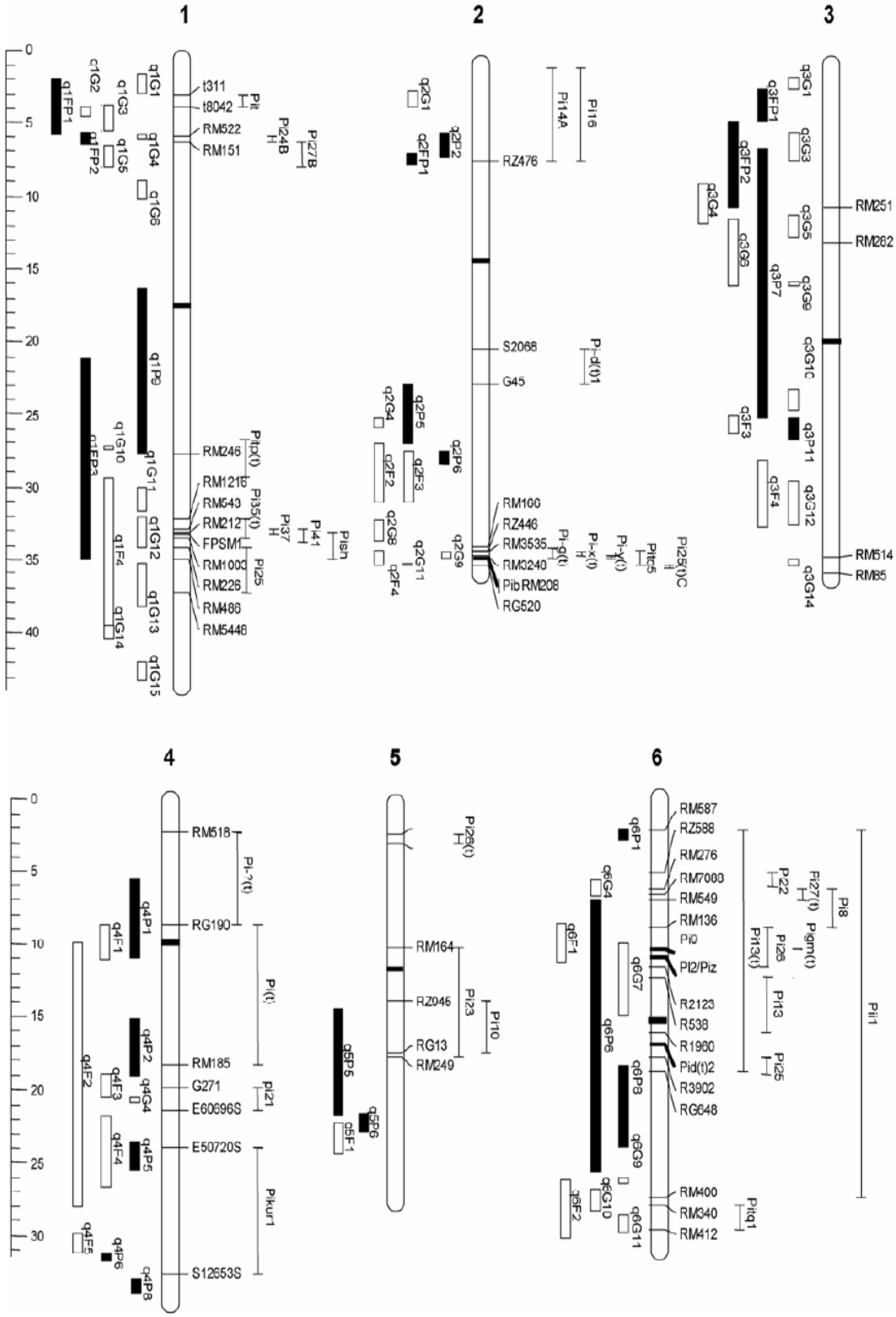
The quantitative resistance system that has been especially well characterized in rice is resistance to the blast fungus (Wang *et al.,* 1994; Sallaud *et al.,* 2003; Talukder *et al.,* 2005). In rice blast QTL study, Wang et al. 1994 was analyzed a durable source of resistance known as Moroberekan for both *R* genes and quantitative (partial) resistance loci. In the QTL mapping study by Sallaud *et al*., 2003, five new blast resistance loci named *Pi-24*(t) to *Pi-28*(t) were identified using a QTL mapping approach. Another study tested the specificity of QTL for partial resistance to blast disease by using isolates for which no major *R* gene segregated in a mapping population (Talukder *et al.,* 2004). Since the first publication of a QTL analysis of rice resistance to blast (Wang *et al*., 1994), many such studies have been published in (Table 2.8). The meta-analysis allowed detection of 165 metaQTL, thus reducing

significantly the initial dataset of 347 QTL. A graphical overview of the position of these metaQTL is presented in (Figure 2.2). The average size of a metaQTL is 3.3 Mbp. In all, 11% of the metaQTL are finely mapped (<500 kb) and 5% are large (>12 Mbp).

Table 2.8 The literature sources used in the meta-analysis of QTL for blast resistance

References	Population	Environment	Mapping	Partial resistance	Total no. of QTL detected
Sirithunya *et al.,* 2002	CT9993-5-10-1-m × KDML105	G and F	Yes	Nd	6
Bagali *et al.*, 2000	IR64 × Azucena	G and F	Yes	No	13
Fukuoka and Okuno, 2001	Nipponbare × Owarihatamochi	F	Yes	No	4
Miyamoto *et al.*, 2001, 2003	Kahei × Koshihikari	F	Yes	No	2
Sallaud *et al.*, 2003	IR64 × Azucena	G	Yes	No	9
Chen *et al.,* 2003	Zhenshan 97 × Minghui 63	G	Yes	No	12
Huang *et al.*, 2004	Tainung 69 × Koshihikari	G and F	Yes	No	2
Xu *et al.,* 2004	ZYQ8 × JX17	G	Yes	No	77
Sato *et al.,* 2006	URN12 × Koshihikari	F	No	No	2
Wang *et al.,* 1994	Moroberekan × Co39	G and F	Yes	Yes	22
Tabien *et al*., 2002	Lemont × Teqing	G and F	Yes	No	11
Zenbayashi *et al*., 2002.	Norin29 × Chubu32	F	No	Yes	1
Loan *et al*., 2003	Lemont × Teqing	G	Yes	Yes	14
Talukder *et al*., 2004, 2005.	Bala × Azucena	G	No	Yes	41
Wu *et al.,* 2005	Zhong 156 × Gumei 2	G and F	Yes	Yes	21
Lopez-Gerena, 2006	Oryzica Llanos 5 × Fanny	G	Yes	Yes	21

G = greenhouse and F = field, Nd=type of resistance not determined


1
2
3
4
5
6
t311
t8042
RM522
RM151
RM246
RM1216
RM543
RM212
FPSM1
RM1000
RM226
RM486
RM5446
RZ476
S2066
G45
RM100
RZ446
RM3535
RM3240
Pib RM208
RG520
RM251
RM262
RM514
RM85
RM518
RG190
RM185
G271
E60696S
E50720S
S12653S
RM164
RZ945
RG13
RM249
RM587
RZ588
RM276
RM7000
RM549
RM136
Pi9
Pi2/Piz
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R1960
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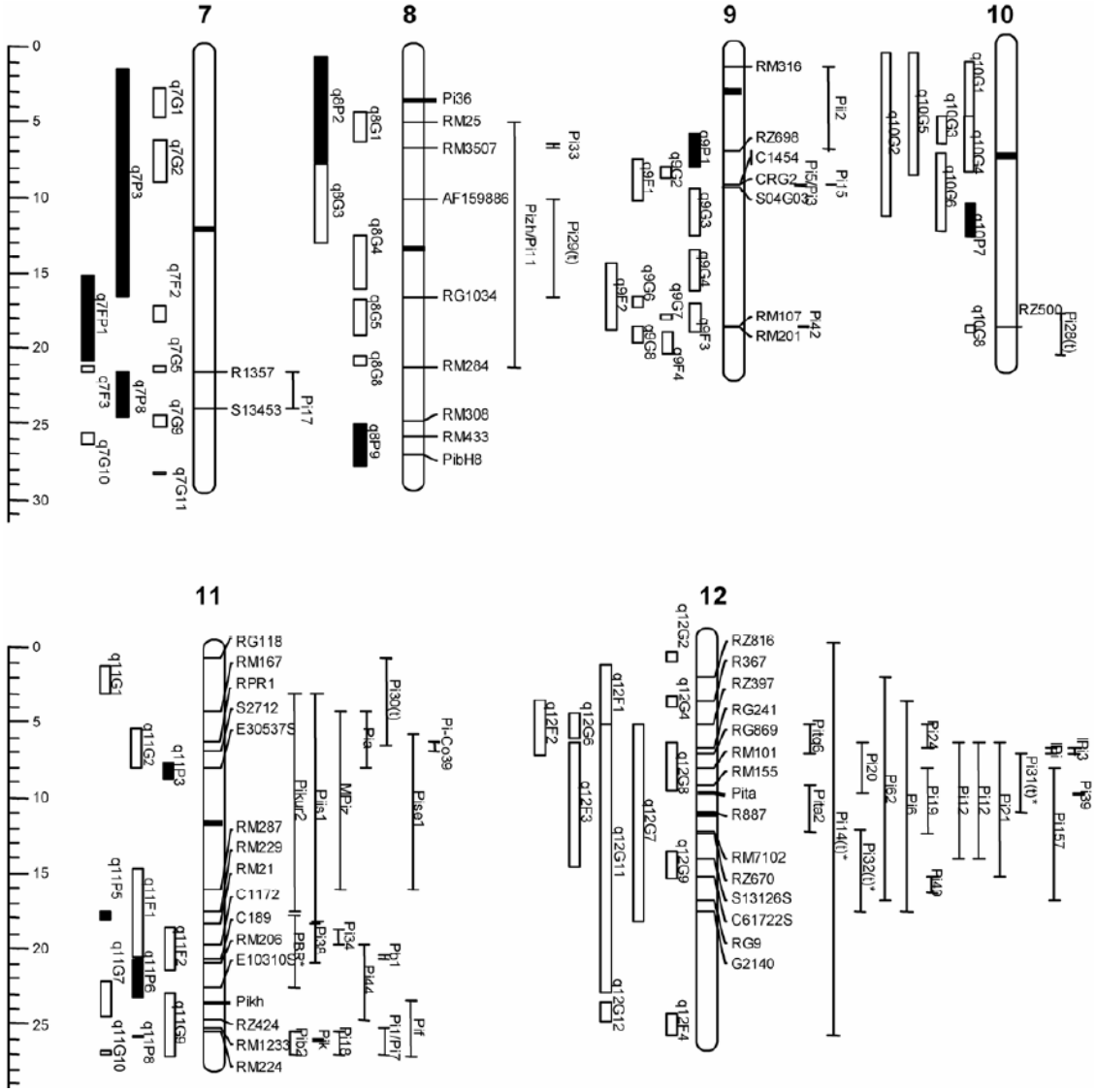
R3902
RG648
RM400
RM340
RM412



Chromosome size is shown by the scale on the left (in million base pairs) based on Gramene. The positions and names of markers are indicated by labelled bars on the chromosomes. The positions and names of resistance genes are indicated to the right of each chromosome. MetaQTL are symbolized by filled (partial-resistance metaQTL) or open (other resistance metaQTL) bars. Field QTL and greenhouse QTL are analyzed separately: the letters F, G, and P in QTL names represent field, greenhouse and partial respectively.

Figure 2.3 Physical maps of resistance genes and meta-quantitative trait loci (QTL) of resistance to rice blast

2.6 Brown spot of Rice

Brown spot, caused by the fungus *Bipolaries oryza* is one of the prevalent fungal diseases of rice and significantly reduces the yield and milling quality of grain (Datnoff *et al.*, 1992). The great Bengal Famine, which contributed to the famine of south Asia in 1942 (Padmanabhan, 1973), is testimony to this. Brown spot was reported for the first time in Iran at 1957 (Behdad, 1982). Brown spot has been reported in all rice-growing areas in the world. It is especially common in rainfed (Singh and Singh, 2000) and upland areas (Gupta and O'Toole, 1986). Yet, the fact that brown spot is the "poor farmer's" disease (Zadoks, 2002) anywhere the crop encounters drought, macro-nutrient deficiency (Ou, 1985) or both actually tells much more of the importance of the disease. Surveys show that brown spot causes a 5% yield loss across all lowland rice production situations in South and Southeast Asia (Savary *et al*., 2000b). In Japan, the disease is not considered destructive. In the United States also, the disease is not a serious one but in the rice- growing pockets, the annual loss since 1965 was no less than 0.5% of the total production.

2.6.1 Causal organism of Brown spot disease

At first the causal agent of Brown spot disease was named by Breda de Haan *Helminthosporium oryzae* (Gangopadhyay and Padmanabhan, 1987). The asexual stage is *Bipolaris oryzae* (Breda de Haan) Shoemaker (Dela Paz *et al*., 2006). The sexual stage is *Cochliobolus miyabeanus* (S. Ito & Kurib.) Drechsler ex Dastur. The disease can occur at all crop development stages. The pathogen infects the coleoptile, leaves, leaf sheath, panicle branches, glumes and spikelets. The disease causes seedling blight, with small, circular, yellow brown or brown lesions that may girdle the coleoptile and distort primary and secondary leaves (Webster and Gunnell, 1992).

Nowadays the graminicolous *Helminthosporium* species are divided into three genera

based on colony, conidiophores and conidial morphology, type of conidial germination and the type of hilum structure: *Bipolaris, Drechslera and Exserohilum*. Their telemorphs were from ascomycetes and consist of: *Cochliobolus, Pyrenophora and Setosphaeria*, respectively (Sivanesan, 1987).

2.6.2 Disease biology and epidemiology of Brown spot disease

The pathogen infects the coleoptile, leaves, leaf sheath, panicle branches, glumes, and spikelets. The disease causes seedling blight, with small, circular, yellow brown or brown lesions that may girdle the coleoptile and distort primary and secondary leaves (Webster and Gunnell, 1992). Typical classical brown spot symptoms are observed at tillering stage and beyond: small and circular foliar lesions that are initially dark brown to purple-brown. Lesions are often surrounded by a brown or yellow-brown halo, which is a toxin produced by the pathogen (Vidyasekaran *et al*., 1986). Lesions on susceptible varieties are 5 to 14 mm long (Webster and Gunnell, 1992), causing leaf wilting. Rice plants growing in nutrient-deficient and poorly drained soil are predisposed to brown spot infection. Brown spot is usually found in fields where farmers cannot afford to buy inputs. Plants that grow in sandy soil were also reported to be susceptible to brown spot (Ou, 1985). Brown spot is also favored by reduced water supply, particularly when the rice crop is direct-seeded (Savary *et al.,* 2005). This may be because rice plants in direct-seeded rice have a shallow root system (Castillo, 1962) and consequently may become more sensitive to water stress.

2.6.3 Management of Brown spot disease

Brown spot can be managed by improving soil fertility through regular monitoring of nutrients in the soil and the application of required fertilizers. The application of calcium silicate slag before crop establishment has been recommended

for soils that are low in silicon (Datnoff *et al.,* 1992). These fertilizers, unfortunately, are often costly and always take many cropping seasons before becoming effective. The use of resistant varieties would be the most economical means of controlling brown spot. Several studies have been made on genotypic variability in rice for brown spot resistance (Yoshii and Matsumoto, 1951; Oohata and Kubo, 1974; Deren *et al*., 1994). In those studies, some rice varieties e.g., Tadukan and Tetep offered sufficient quantitative resistance to brown spot and are agriculturally useful (Oohata and Kubo, 1974). No major genes with resistance to brown spot have yet been identified. However, varieties with partial resistance and three quantitative trait loci (QTL) for disease resistance have been identified (Sato *et al*., 2008).

2.6.4 Sources of Host Plant Resistance (HPR) for Brown spot disease

The search for sources of resistance to brown spot is a long-standing effort (Nagai and Hara, 1930; Chakrabarti, 2001) which still continues today. For instance, working on *Oryza sativa* species Satija *et al.,* 2005 was identified 15 entries out of 124 which were classified as resistant (less than 5% severity). Conversely, Hossain *et al.,* 2004 identified one resistant variety out of 29 entries. It however is felt that the sources of resistance amongst *Oryza sativa* entries are few and recent research (Goel *et al.,* 2006) has been exploring other pools, especially, *O. nivara*.

2.6.5 The genes identified for Brown spot disease

Early studies showed that resistance or susceptibility could be associated with a limited number of genes. For instance, Balal *et al*., 1979 found that two dominant genes were associated with resistance, while one gene was associated with susceptibility. Despite these findings, Adair (1941) suggested that resistance was recessive, involving several genes. At the Central Rice Research Institute, Cuttack (India), after screening of 1000 varieties for 9 years against *B. oryzae*, varieties CH13,

CH45, CH20, T141, T498-2A, T988, T2114, T2118, T960, Bam10, IET 13238, CR10-4025, CR84-30, JBS83, JBS21, JBS218, JBS238, JBS568, JBS781, JBS1510, JBS1199 etc. were found resistant. Padmanabhan (1973) confirmed that resistance in CH 13 is governed by three pairs of recessive genes indicating its horizontal nature. Inheritance of field resistance was studied at the seedling, adult plant and kernel stages in six crosses including two resistant (*Pi1* and YNA282) and 2 susceptible varieties (Giza 171 and Sakha 1) by (Balal *et al.,* 1979). Seedling and adult plant leaf reactions were similar and governed by three genes (Her_1, Her_2 and Hes_1). The first two were dominant for resistance and the third for susceptibility. Kernel resistance was dominant over susceptibility and controlled by two genes ($Hekr_1$ and $Hekr_2$) carried by *Pi1* and *YNA* 282 respectively.

2.6.6 The partial resistance of Brown spot tolerance

Goel *et al.,* 2006 analysing the inheritance of resistance of brown spot resistance from crosses involving *O. nivara* germplasm, hypothesized that additive, dominant, as well as epistatic gene interactions were involved. Three quantitative trait loci (QTLs) were detected in Tadukan (*qBS2, qBS9* and *qBS11*), located on chromosomes 2, 9 and 11, respectively (Sato *et al*., 2008). The *qBS11* being considered a major effect on brown spot resistance. However, Katara *et al*., 2010 identified 10 QTLs, some of which may be common to the results by Sato *et al.,* 2008.

2.7 The mapping and basic of QTLs

A Mendelian trait is determined by a single gene (or few genes), following classical Mendelian inheritance patterns, such as 3:1 for a phenotypic ratio from a trait controlled by a single dominant gene in an F_2 family. In contrast, multiple genes could determine a quantitative trait and its value is continuous, such as plant height and human weight. Quantitative traits are very common and are important both in applied

and theoretical studies. In simple terms, QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley, 1993; Young, 1996). A significant difference between phenotypic means of the groups, depending on the marker system and type of population, indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait. The QTL and marker will be usually be inherited together in the progeny and the mean of the group with the tightly-linked marker will be significantly different ($P < 0.05$) to the mean of the group without the marker.

The QTL studies are to locate QTL along chromosomes; this process is generally called QTL mapping. The detection and location of QTL have applications in many aspects of biological studies. By locating and characterizing the effects of individual QTL; the genetic architecture for a trait and its related biological function can be refined. The theory of QTL mapping was first described by Sax (1923), who noted that seed size in bean, a complex trait, was associated with seed coat color, a simple, monogenetically-controlled trait.

2.7.1 Genetic markers

In a broad sense, a genetic marker refers to any heritable character that can be used to distinguish one individual from another in a population. In current QTL mapping practice, variation at the DNA level is typically used because it is the most abundant and easily scored type of variation due the rapid development of genome technology. Among markers, RFLP, SSR and SNP are commonly used for mapping QTL. The term microsatellite refers to DNA sequences with repeating units of 1-6

nucleotides. For example $(GA)_n$ and $(CTG)_n$ are microsatellites, where n is the number of repeating units. They are often multiallelic, are usually locus specific and are evenly distributed along chromosomes and randomly distributed throughout the genome (Roder *et al*., 1998, McCouch *et al*., 2002). McCouch *et al*., 2002 reported that in a new set of 2240 rice SSR the largest proportion of SSR showed to poly(GA) motifs (36%), followed by poly(AT) (15%) and poly(CCG) (8%) motifs. AT-rich microsatellites had the longest average repeat tracts, while GC-rich motifs were the shortest. There is approximately one SSR every 157 kb in the rice genome. Microsatellites show high levels of polymorphism compared to other marker systems in rice.

2.7.2 Maps and map construction

A genetic map describes orders and positions of identifiable landmarks on DNA. These landmarks might be genes or genetic markers. Two types of map are commonly used in practice, genetic and physical maps. For QTL studies both are extensively used for fine mapping and physical characterization of QTL. A genetic map and a physical map provide similar information on marker or gene order along the chromosomes. Availability of the complete sequence makes it possible to determine directly the order and spacing of the genes, which is a type of physical map (Weeks and Lange, 1987). Software has also been developed to construct genetic maps; a popular one is MAPMAKER (Lander *et al*., 1987). Molecular marker technologies permit plant geneticists to construct high-density genetic maps for any species amenable to genetics and use them for detecting, mapping and estimating the effects of QTL. The analysis involves testing DNA markers throughout a genome for the likelihood they are linked with a QTL. Individuals in an appropriate mapping population (F_2, backcross, recombinant inbred) are analyzed for DNA marker

genotypes and the phenotype of interest (Young, 1996). For each DNA marker, the individuals are split into classes according to marker genotype. Mean and variance parameters are calculated and compared among the classes. A significant difference between means suggests that there is a relationship between the DNA marker and the trait of interest. In other words, the DNA marker is probably linked to a QTL. QTL mapping, like any genetic study, is only as good as its phenotypic scoring method. There are powerful computer software programs are now available to analyze QTL mapping results (Nelson, 1997; Manly *et al.,* 2001; Broman *et al.,* 2003; Wang *et al.,* 2005) and better DNA marker systems have been developed to simplify the technique and increase marker density.

2.7.3 The QTL mapping methods

There are various statistical methods have been developed for QTL mapping. The most commonly used methods for QTL mapping are based on the maximum-likelihood method.

2.7.3.1 Single- Marker Analysis (SMA)

Single-marker analysis (also single-point analyses) is the simplest method for detecting QTLs associated with single markers. Single marker analysis tests the association between marker genotypes and trait values. The statistical methods used for single-marker analysis include *t*-tests, analysis of variance (ANOVA) and linear regression. Linear regression is most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker. This method does not require a complete linkage map and can be performed with basic statistical software programs. SMA often fails to give reliable estimates of numbers and positions of QTLs and the magnitude of their effects (McMillan and Robertson, 1974, Lander and Botstein, 1989).

2.7.3.2 Simple Interval Mapping (IM)

The simple interval mapping (SIM) method makes use of linkage maps and analyses intervals between adjacent pairs of linked markers along chromosomes simultaneously, instead of analyzing single markers (Lander & Botstein, 1989). Interval mapping uses two observable flanking markers to construct an interval within which to search for QTL along the chromosomes. The use of linked markers for analysis compensates for recombination between the markers and the QTL and is considered statistically more powerful compared to single-point analysis (Lander & Botstein, 1989; Liu, 1998). When a peak has exceeded a threshold LOD value, there is evidence that a QTL has been found at that location (Zeng, 1994). Many researchers have used MapMaker/QTL (Lincoln *et al*., 1993) and QGene (Nelson, 1997) to conduct SIM.

2.7.3.3 Composite Interval Mapping (CIM)

More recently, composite interval mapping (CIM) has become popular for mapping QTLs. This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Jansen, 1993; Jansen & Stam, 1994; Zeng, 1994). The main advantage of CIM is that it is more precise and effective at mapping QTLs compared to single-point analysis and interval mapping, especially when linked QTLs are involved. Many researchers have used QTL Cartographer (Basten *et al*., 1994), MapManager QTX (Manly *et al*., 2001) and PLABQTL (Utz & Melchinger, 1996) to perform CIM.

2.7.3.4 Multiple Interval Mapping (MIM)

Multiple interval mapping uses multiple marker intervals simultaneously to fit various putative QTL directly into the model for mapping QTL. Kao and Zeng, 1999 developed MIM. MIM tends to be more powerful than SMA and CIM. Multiple interval mapping leads to more accurate QTL position and QTL effect estimates (Mayer, 2005). MIM is appropriate for the identification and estimation of genetic architecture parameters, including the number, genomic positions, effects and interactions of significant QTL and their contribution to the genetic variance.

Materials and Methods

CHAPTER - III

MATERIALS AND METHODS

The present study entitled **"QTL mapping for bacterial leaf blight, sheath blight, blast and brown spot tolerance using RIL population of Rice (*Oryza sativa* L.)."** was carried out in the Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The details of experiment are explained below.

3.1 Materials

3.1.1 Plant materials

The plant materials used in present study was recombinant inbred line (RIL) mapping population derived from the parent Danteshwari × Dagad deshi. The mapping population were provided by Dr S. B. Verulkar, Professor and Head, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur. The parent Danteshwari is a highly susceptible *indica* rice cultivar and Dagad deshi, an *indica* rice cultivar that has moderate resistance properties for diseases. The salient features of parents were summarized in (Table 3.1).

Table 3.1 Characteristic features of parents

S.N.	Parent	Pedigree	Reaction to diseases	Salient Features
1	Danteshwari	Shamridhi × IR 8608-298	Resistant to BLB and moderate resistant to Brown spot, Susceptible to ShB and leaf Blast	High yielding, resistance to gall midge, long slender grain.
2	Dagad deshi	Land race	Susceptible to BLB and Brown spot, Resistant to ShB and leaf Blast	Strong culm, broad leaves, bold seeded.

3.1.2 Isolates

The causal organism of Bacterial blight *Xanthomonas oryza* pv. *oryzae* and *Rhizoctonia solani* cause sheath blight used in present study isolated by Dr. Toshy

Agrawal and Dr. A.S. Kotasthane, Professor, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur from district Dhamtari and Raipur of Chhattisgarh.

3.2 Methods

122 RILs population derived from Danteshwari × Dagad deshi of F_{14} generation were evaluated for different diseases during *Kharif*-2013. The mapping populations along with their parents were evaluated for disease resistance, under field condition using Randomized Block Design (RBD) with three replications. The nursery of rice seedlings was prepared before 30 days of transplantation. The labeled field prepared as clean weeds, well plough, paddled and given basal dose of fertilizers for requirement of Nitrogen, Phosphorus and Potash etc. The plant-to-plant and row-to-row space has taken as 15 ×15cm and 20 × 20cm respectively. The normal package of practices was followed.

3.2.1. The phenotypic observations of mapping population and parents for bacterial leaf blight trait

3.2.1.1. Pathogen inoculation and disease scoring of Bacterial leaf blight

The local isolate of Bacterial blight *Xanthomonas oryzae* pv. *Oryzae* was inoculated at maximum tillering stage. The *Xoo* isolates were revived from the stock culture maintained at 4 ^{0}C. The culture was grown on Wakimoto's Medium for 3 days at 30^{0}C. The inoculum was prepared by suspending the bacterial mass with sterilized distilled water to a concentration of about 10^{9}cells/ml and was immediately used for inoculations with the help of scissor. Disease score was evaluated 21 days after inoculation. The mean lesion length and base on SES qualitative (0-9) scale, score data were recorded as in Table 3.2 (IRRI, 2002).

Table 3.2 Disease scoring and the reactions for Bacterial leaf blight

Score	Percentage of infected leaf area (%)	Reaction
0	0	Highly Resistant (HR)
1	1-5	Resistant (R)
3	6 – 12	Moderately Resistant (MR)
5	13 – 25	Moderately Susceptible (MS)
7	26 – 50	Susceptible (S)
9	75	Highly Susceptible (HS)

The entire inoculation experiment was replicated three times for population of the isolates. The *Xanthomonas* inoculation was carried out as described by clip inoculation technique (Kauffman *et al*., 1973). The inoculated plant grown in natural field condition. The component length of disease lesion was estimated and was considered for the traits. The diseased lesion of fifteen plants from each line was used for estimating of disease.

3.2.2. The phenotypic observations of mapping population and parents for Sheath blight

3.2.2.1. Pathogen inoculation and disease scoring of Sheath blight

The local isolate of *Rhizoctonia solani* isolated from ground soil of Raipur was used for screening. The fungus was maintained on oat meal agar medium for the production of sclerotia. The pure culture of *R. solani* was maintained in Petri dishes on Potato Dextrose Agar medium and transfer in rice bran for mass multification. The RIL lines screened by inoculation of fungus *Rhizoctonia solani* isolate at 30 days after transplanting during the month August. The observations on diseased lesion were recorded by measuring lesion size in centimeter (cm) after 10th day of inoculation from randomly selected six plants and affected tillers per plant of each RIL line from three replication. Grayish-green lesions may enlarge and coalesce with other lesions,

mostly on lower leaf sheaths. The disease lesion length and width were measured with the scale from one end to another end covering whole infected region of the sheath tissues. The length and width of the biggest lesion also taken for analysis. The length of sheath, plant height and total tiller per plant were also recorded to work out percentage length lesion to the sheath (Channamallikarjuna *et al*., 2010).

3.2.3. The phenotypic observations of mapping population and parents for leaf Blast disease

The experiment was carried out under field conditions plant disease screening section at Ambikapur between June and July, 2013. Evaluation of partial resistance to leaf blast in the 122 Danteshwari × Dagad deshi crossed RILs population along with parent was conducted at College of Agriculture, Ambikapur, Indira Gandhi Krishi Vishwavidyalaya in 2013. About 50 seeds of each line and the parental cultivars were sown in a 150 cm-length row with 10 cm spacing on July, 2013. A complete randomized block design was used with two replications. As Swarna is highly susceptible to Blast, to induce leaf blast development, the rice cultivar Swarna was used as a spreader by planting this cultivar after every ten lines and surrounded by one row around whole population. The population was allowed to grow for natural occurrence of the disease. The disease severity of leaf blast in each of the population and the parental cultivars was evaluated on 13.08.2013 based on 0-9 score as in Table 3.3 (IRRI, 2002). The scores ranged from 0 (no lesion) to 9 (highly affected).

Table 3.3 Disease scoring and the reactions for leaf Blast of rice

Score	Predominant lesion type
0	No lesions observed
1	Small brown specks of pin-point size or larger brown specks without sporulating center
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area
5	Typical blast lesions infecting 4-10% of the leaf area
6	Typical blast lesions infection 11-25% of the leaf area
7	Typical blast lesions infection 26-50% of the leaf area
8	Typical blast lesions infection 51-75% of the leaf area and many leaves are dead
9	More than 75% leaf area affected

3.2.4 The phenotypic observations of mapping population and parents for Brown spot

The 122 F_{14} RIL population and their parent Danteshwari and Dagad deshi grown in field as a randomized complete block design with three replicates for each treatment with spacing taken as 15 × 15cm plant to plant and 20 ×20cm row to row respectively. The plants were allowed to grow for 60 to 90 days in a field condition upto heading stage. Natural occurrence of the disease severity was assessed visually as percentage of leaf area with brown spot symptoms. Typical leaf spots are small, oval or circular and dark brown. The percentage of spots cover area were recorded and further categorized based on 0-9 score as in Table 3.4 (IRRI, 2002).

Table 3.4 Disease scoring and the reactions for Brown spot of rice

Score	Affected leaf area (%)
1	No incidence
2	Less than1
3	1-3
4	4-5
5	11-15
6	16-25
7	26-50
8	51-75
9	76-100

3.3 Statistical analysis of phenotypic data

The data recorded on all the traits related to disease resistance in both the seasons were statistically analyzed. Major statistical procedures followed were:

A. Mean: Mean is the average value of observation of population. It represents the standard average value over fluctuation in the environment**.**

Mean was calculated by the following formula:

$X = \sum Xi / n$

Where, $\sum Xi$ = Summation of all the observations

n = Total number of observations

B. Correlation coefficient

The correlation coefficients were worked out to determine the degree of association among different traits. Correlation coefficient (r) was calculated for disease resistance contributing characters by using

$$r_{(x,y)} = \frac{Cov.(x, y)}{\sqrt{Var(x) . Var(y)}}$$

Where,

$r_{(x,y)}$ = Correlation coefficient between character x and y

Var(x) = Variance of x character

Var(y) = Variance of y character

3.4 Genotyping of RIL population and construction of linkage map

The genotyping work was done in the following heading-

3.4.1 Isolation of genomic DNA

The Genomic DNA isolated from leaf of young succulent single plant of parents (Danteshwari and Dagad deshi) and 122 RILs population using modified CTAB protocol (Keb-Llanes *et al*., 2002). The tender leaves of single plant of parents and RILs were collected from field, extracted DNA and stored for feature uses.

Genomic DNA isolation Protocol leaf of young succulent single rice plant:

1. Weigh around 5-6g of plant tissue.
2. Leaves were cut into small pieces and crushed into Tissue lyzer (*Mo Bio Laboratories ltd*). Leaf powder (approximately 1.5ml) transferred immediately into 50 ml centrifuge tube.
3. Once the sample was prepared then adds 5 ml EBA, 15 ml EBB, and 2 ml of 20% SDS.
4. Vortex the sample and incubated at 65°C for 10 min.
5. Then added 15 ml of chloroform: isoamyl alcohol (24:1) and gently shake the mixture by inversion for 15 min.
6. Centrifuge at 3000 rpm for 20 min to separate the phase and transferred the upper phase to new tube.
7. Repeated the chloroform extraction (step 5) one more time.
8. Then added 2/3 volume of pre-chilled isopropanol and incubated at room temperature for 30 min or longer until DNA was precipitated.
9. Centrifuge for 10 min at 3000 rpm and collected DNA pellet.
10. Washed the DNA pellet with 70% EtOH and air dried for 10 min.
11. Resuspended DNA pellet in 5 ml of TE buffer and the pellet was dissolved.
12. Then added 15 µl of RNase (10 mg/ml) and incubated at 37^{0}C for 30 min.

13. Then added 1/10 volume of 3M sodium acetate and 2 volume of pre-chilled absolute ethanol mixed gently and incubated at room temperature for 30 min.
14. Centrifuge the DNA pellet for 8-10 min at 3000 rpm.
15. Then added 1ml of 70%EtOH and washed the DNA pellet.
16. Centrifuge for 3 min at 3000 rpm and discarded the 70% alcohol and placed the tube upside down on paper towel to get rid of excess of ethanol.
17. Then pellet was resuspended in 500 µl of TE buffer and incubated overnight. Centrifuge at 3000 rpm for 5 min. and supernatant was transferred to new 1.5 ml micro centrifuge tube. DNA stored at -20^{0}C until used.

3.4.2 Quantification and estimation of purity of DNA

The DNA was quantified using Nanodrop spectrophotomer (ND1000). Two micro liter of isolated DNA was placed over tip of Nanodrop. The absorbance ratio (A_{260}/A_{280}) was recorded for each sample to find out the purity of DNA. The Pure DNA and RNA has a ratio of approximately 1.8 and 2.0, respectively. If there is contamination with protein or phenol the ratio will be significantly less than this value (< 1.8). A ratio greater than 2.0 indicates a high proportion of RNA in the DNA sample.

3.4.3 Dilution of DNA samples

After quantification, the DNA was diluted with TE such that the final 3 of DNA was approximately 40ηg/µl. The diluted DNA was subsequently used for PCR amplification.

3.4.4 PCR amplification using SSR primers

3.4.4.1 PCR reaction

The optimized PCR protocol was used for identify the informative SSR markers on the basis of parental polymorphism. There were 254 SSR RM markers

surveyed for parent Danteshwari and Dagad deshi (Appendix II). The list of polymorphic SSR markers used for RIL population genotyping given in (Table 3.7).

Three µl of diluted DNA of parent was dispensed at the bottom of PCR tube. Cocktail was prepared separately in an eppendoff tube as described in Table 3.5 and 17 µl of cocktail was added to each tube. Amplification was carried out in Thermal Cycler of MJ Research Pvt. Ltd., USA for 35 cycles. The details of PCR thermal profile for amplification are summarized in table 3.6. 20 µl of PCR amplified and SSR product was mixed with 7µl of 6X loading dye and 5µl of this was loaded on 5% PAGE gel along with 100 bp ladder. Electrophoresis was done for 1 hour at 180 volts. Gels were stained using EtBr solution then visualized and photographed by using Gel Doc Unit, detailed below.

Table 3.5 PCR mix for one reaction (Volume 20µl)

S.No	Component	Stock concentration	Volume/reaction
1	DNA	40 µg/ml	3.0 µl
2	ADW		11.5 µl
3	10X Buffer	10X	2 µl
4	dNTP	1 mM	2 µl
5	Primer (Forward+ reverse)	5 µM	1 µl
6	*Taq* polymerase	1 U/µl	0.5 µl

Table 3.6 Temperature profile used for PCR amplification using SSR primers

Steps	Steps Temperature (°C)	Duration (min.)	Cycles	Activity
1	94ºC	4	1	Initial Denaturation
2	94ºC	1	30	Denaturation
3	55-68ºC	1	30	Annealing
4	72ºC	1	30	Extension
5	72ºC	5	1	Final Extension
6	4ºC	∞	1	Storage

Table 3.7 List of polymorphic SSR markers used for RIL population genotyping

S.N.	Marker	S.N.	Marker	S.N.	Marker	S.N.	Marker
1	RM-499	42	HvSSR3-56	83	HvSSR6-44	124	RM-108
2	HvSSR1-24	43	HvSSR3-71	84	HvSSR6-56	125	RM-242
3	HvSSR1-33	44	HvSSR3-85	85	RM-225	126	RM-288
4	HvSSR1-34	45	RM-517	86	HvSSR6-65	127	RM-553
5	HvSSR1-49	46	RM-7	87	RM-217	128	RM-278
6	RM-428	47	RM-232	88	RM-136	129	RM-201
7	HvSSR1-55	48	RM-411	89	RM-340	130	RM-245
8	RM-84	49	RM-135	90	RM-400	131	HvSSR10-1
9	RM-1	50	RM-55	91	RM-481	132	HvSSR10-5
10	HvSSR1-80	51	RM-85	92	HvSSR7-40	133	HvSSR10-17
11	HvSSR1-87	52	RM-307	93	HvSSR7-43	134	RM-222
12	HvSSR1-89	53	HvSSR4-26	94	HvSSR7-46	135	HvSSR10-34
13	RM-259	54	HvSSR4-35	95	RM-125	136	RM-171
14	RM-243	55	HvSSR4-38	96	HvSSR7-53	137	RM-228
15	RM-572	56	HvSSR4-39	97	RM-2	138	RM-484
16	RM-24	57	HvSSR4-42	98	RM-11	139	HvSSR11-1
17	RM-449	58	RM-564	99	RM-234	140	HvSSR11-2
18	RM-5	59	RM-273	100	RM-248	141	HvSSR11-3
19	RM-212	60	RM-348	101	RM-337	142	HvSSR11-13
20	RM-3825	61	RM-317	102	RM-152	143	RM-202
21	RM-302*	62	RM-559	103	HvSSR8-29	144	RM-229
22	RM-486	63	HvSSR5-13	104	RM-310	145	RM-21
23	RM-14	64	HvSSR5-23	105	RM-44	146	RM-26334
24	RM-109	65	HvSSR5-31	106	RM-483	147	RM-206
25	RM-485	66	HvSSR5-39	107	RM-72	148	RM-254
26	HvSSR2-1	67	HvSSR5-48	108	RM-515	149	RM-224
27	HvSSR2-12	68	HvSSR5-51	109	RM-256	150	RM-20
28	HvSSR2-23	69	HvSSR5-52	110	RM-230	151	HvSSR12-35
29	HvSSR2-27	70	HvSSR5-56	111	RM-433	152	HvSSR12-36
30	HvSSR2-78	71	HvSSR5-65	112	RM-281	153	HvSSR12-40
31	RM-174	72	HvSSR5-66	113	HvSSR9-5	154	HvSSR12-48
32	RM-492	73	RM-163	114	RM-444	155	HvSSR12-51
33	RM-475	74	RM-440	115	HvSSR9-7	156	RM-277
34	RM-341	75	RM-459	116	HvSSR9-19	157	RM-511
35	RM-221	76	RM-188	117	HvSSR9-25	158	RM-260
36	HvSSR3-6	77	RM-421	118	HvSSR9-27	159	RM-519
37	HvSSR3-9	78	RM-178	119	HvSSR9-37	160	RM-28305
38	HvSSR3-35	79	RM-26	120	HvSSR9-57	161	RM-270
39	HvSSR3-40	80	RM-274	121	RM-296	162	RM-17
40	HvSSR3-41	81	RM-87	122	RM-434		
41	RM-231	82	HvSSR6-35	123	RM-410		

Note: Hv= highly variable

3.5 Polyacrylamide Gel Electrophoresis

For better separation and visualization of PCR amplified microsatellite products, 5% polyacrylamide gels (vertical) were used, since polyacrylamide gels have better resolution. *CBS-SCIENTIFIC* electrophoresis unit used for casting gel. Glass plates were prepared before making the gel solution. Both glass plates (outer and inner notched glass plates) were cleaned thoroughly with warm water, detergent and then with deionised water.

3.5.1 Assembling and pouring of gel

- Gasket was fixed to the three sides of the outer plate (without notches). Spacers of 1.5mm thickness were placed along the sides by just attaching the gasket of outer plate.
- Later, notch plate was kept on the outer plate so that spacers were between the two plates. Clamps were put on the three sides of plates leaving notch side of unit. It was checked with water to found any leakages.
- For casting gel was prepared just prior to pouring. For preparation take 65 ml of 5% PAGE solution then added 700 µl of ammonium per sulphate 10 % (APS) and 70 µl of TEMED (N-N-N-N-Tetramethylethylenediamine) to initiate the polymerization process.
- The contents were mixed gently by swirling, but bubbles were avoided. Before pouring, assembly was kept on the bench top so that it made 45 degree angle with bench top.
- Then gel solution was poured from notch side with maximum care to avoid air bubbles. Comb of 1.5 mm thickness (60 wells) was inserted with tooth side in the gel.

- Later assembly was kept for polymerization for 20-30 min.

3.5.2 Electrophoresis

- After polymerization process, gasket was removed and assembly was kept in the electrophoresis unit with electrophoresis unit clamps so that notch side facing inner side of the unit and facing other plate without notch to outer side.
- TBE (1 X) was poured in upper tank in the unit and the rest was poured in bottom chamber.
- Comb was removed with care so that it cannot disturb the wells formed.
- At last, 7µl loading dye (6 X) was added to PCR products.
- Finally, 5µl of each sample were loaded into the wells for facilitating the sizing of the various alleles. Ladder (100bp) was loaded in the first well.
- Gel was run at 180 volts till the dye reached bottom of the gel.

3.5.3 Visualization of bands

After electrophoresis, clamps were removed and glass plates were separated without damaging the gel.

- Gel was taken out from plate into staining box with care by flipping the gel with help of spatula and by pouring little amount of water for easy removal.
- Ethidium bromide solution (prepared by adding 10µl to 200 ml double distilled water was poured into the staining box to stain the gel.
- It was agitated for about five minutes to stain the gel.
- Gel stained with Ethidium Bromide was washed two times with double distilled water to have clear images.
- The gels were scanned with the help of *BIO-RAD* gel doc XR^{+}.

3.6 Scoring of banding pattern

The banding pattern of population developed by each set of SSR scored as in Table 3.8.

Table 3.8 Scoring SSR banding pattern of population

S. No.	Code	Type of band
1.	A	Danteshwari like allele
2.	B	Dagad deshi like allele
3.	H	Both alleles
4.	O	Other type

3.7 QTL mapping

The position of QTLs on Chromosomes was identified with the help of Single marker analysis (SMA) and composite interval mapping (CIM) were performed by QTL cartographer software (version 2.5) (Wang *et al*. 2007). The phenotypic and genotypic data was analyzed using QTL cartographer-2.5 with a threshold value of 3.0 LOD and linkage map was subsequently constructed.

3.7.1 Single marker analysis

Single maker analysis considered one locus at a time and fits the following regression model:

$y = b0 + b1x + e,$

Where,

y = Phenotypic value of a line

$b0$ = Population mean

$b1$ = Additive effect of the locus on the trait

e = Residual error

x = Genotypic code at the locus being tested for the line considered

3.7.2 Composite interval mapping

The composite interval analysis was performed using Mixed linear model, scanning the genetic map and estimating the likelihood of a QTL. Background markers were selected by forward and backward stepwise regression. Default cutoff LOD score of 3 were used for QTL identification.

A method of composite interval mapping based on mixed linear model was applied to detect QTLs. The model being tested with CIM is:

$$y = b0 + b1x1 + b2x2 + \ldots + bnxn + bixi + e$$

where,

$b1, b2, \ldots, bn$ = Additive effects of the n cofactors

$x1, x2, \ldots, xn$ = Coefficients for the n cofactors (the -1 or +1 genotypic scores at those loci indicating whether a line has parent 1 or parent 2 genotype at the marker locus)

bi = Additive effect of the interval position being tested

xi = Coefficient for the interval position being tested, based on the two-locus genotypes of the flanking marker loci and the position inside the interval being tested

3.8 QTL cartographer 2.5

QTL Cartographer 2.5 (Wang *et al*. 2007) is a suite of programs for DOS, UNIX, MacOS or Windows. A freely available program to map quantitative traits using a map of molecular markers. QTL Cartographer is distinguished by its menu-driven interface, its more detailed documentation, and its resampling methods. Windows QTL Cartographer (WinQTLCart) maps QTLs in cross populations from inbred lines. WinQTLCart includes a graphic tool for presenting mapping results and can import and export data in a variety of formats. This program implements the

following statistical methods: single-marker analysis, interval mapping, composite interval mapping, Bayesian interval mapping, multiple interval mapping, multiple trait analysis and multiple trait MIM analysis.

3.9 QTL IciMapping 3.2

ICIM (QTL IciMapping) is software which constructs genetic linkage maps and maps QTL by simple interval mapping and inclusive composite interval mapping. It can also map segregation distorsion loci, analyse QTL by environment interaction in biparental populations and map QTL in Nested Association Mapping populations. New features and improvements in Version 3.2 include:

- Dominant/recessive markers are considered in recombination frequency estimation, map construction and QTL mapping.
- A new functionality called IMP is implemented, which used to build the integrated map from multiple genetic linkage maps sharing common markers.
- A new tool called 2 point REC is implemented, which used to estimate the pair-wise recombination frequency in bi-parental population.
- Figures of linkage maps are improved. The presence of a putative QTL was declared if the LOD threshold was larger than 2.5 using QTL IciMapping 3.2.

3.10 *In silico* analysis of the QTLs region

- The DNA sequence of the QTLs region from the genome database GRAMENE of *Oryza sativa subsp. japonica* cv. *Nipponbare* was used for *in silico* analysis (www.gramene.org).

3.11 Reagents and Buffers

1. Extraction Buffer A:

Chemicals	Stock concentration	Working concentration	Volume required per 1000 ml
CTAB	-	2%	20 g
Tris-HCl (pH 8.0)	1M	100 mM	100 ml
EDTA (pH 8.0)	0.5M	20 mM	10 ml
NaCl	5 M	1.4M	280 ml
PVP	-	4%	40 g
After adding required volumes of all the stock chemicals the final volume was made up to 1000 ml with autoclaved distilled water. Before using the solution added 700 µl β mercaptoethanol.			

2. Extraction Buffer B:

Chemicals	Stock concentration	Working concentration	Volume required per 1000 ml
Tris-HCl (pH 8.0)	1M	100 mM	100 ml
EDTA (pH 8.0)	0.5M	50 mM	25 ml
NaCl	5 M	100 mM	20 ml
After adding required volumes of all the stock chemicals volume was made up to 1000 ml with autoclaved distilled water. Before using the solution added 700 µl β mercaptoethanol.			

3. TE buffer:

Chemicals	Stock concentration	Working concentration	Volume required per 1000 ml
Tris-HCl (pH 8.0)	1M	10 mM	10 ml
EDTA (pH 8.0)	0.5M	1 mM	2 ml
After adding required volumes of all the stock chemicals adjusted the final volume was made upto 1000 ml with autoclaved distilled water.			

4. RNase solution (10 mg/ml):

Dissolved 10 mg of RNase powder in 1 ml of TE buffer by boiling. Allowed to cool at room temperature and stored in freezer.

5. 3M Sodium acetate (pH 5.2):

3M sodium acetate was prepared by dissolving 24.61 g of anhydrous sodium acetate in 30 ml autoclaved distilled water. After adjusted the pH to 5.2 with glacial acetic acid the final volume was made up to 100 ml with autoclaved distilled water.

6. 1M Tris-HCl solution (pH 8.0):

Dissolved 121.14 g of Tris base in 800 ml distilled water and adjusted the pH to 8.0 with Conc. HCl. and final volume was made up to 1000 ml with distilled water and sterilized by autoclaving.

7. 0.5 M EDTA solution (pH 8.0):

Dissolved 186.1 g of disodium EDTA in 800 ml distilled water along with 17g NaOH pellets and adjusted the pH to 8.0 with NaOH pellets itself. Final volume was make up to 1000 ml with distilled water. Sterilized by autoclaving.

8. 20 % SDS:

Dissolved 20 g SDS in 100 ml autoclaved distilled water.

9. 5 M NaCl:

Dissolved 73.05 g NaCl in 200 ml DW and make up the final volume to 250 ml with DW.

10. Chloroform: Isoamyl alcohol: prepared freshly as 24:1 v/v.

11. Ice-cold Isopropanol

12. Absolute Ethanol

13. Ethanol (70%) Preparation of solution for PAGE

14. 40% PAGE gel solution (100 ml)

Stock	**For100 ml**
Acrylamide	38 g
Bisacrylamide	2 g

Autoclave double distilled water was used to make up volume to 100 ml. For 5% PAGE gel were prepared by using 12.5 ml of 40% stock solution make up in 100 ml in 1X TBE buffer.

15. Ammonium persulphate (APS): 10% APS was prepared by mixing following components

Stock	For 10 ml
Ammonium persulphate	1 g
Distilled water	10 ml

16. Gel contains:

Contain	ml/ µL
5% PAGE solution	65ml
APS	700 µL
TEMED	70 µL

17. 10 X TBE buffer

Components	Requirement per 1000 ml
Trizma base	108 gm
Boric Acid	52 gm
0.5 M EDTA (pH 8.0)	40 ml

18. 1 X TBE: Diluted 100 ml of 10 X TBE to 900 ml with distilled water.

19. 0.5 M EDTA solution (pH 8.0):

Dissolved 186.1 g of disodium EDTA in 800 ml distilled water along with 17g NaOH pellets and adjusted the pH to 8.0 with NaOH pellets itself. Final volume was make up to 1000 ml with distilled water. Then, sterilized by autoclaving.

20. Formamide dye: Formamide dye was prepared by mixing following components

Stock	For 10 ml
Formamide	9.8 ml
0.5 M EDTA	200 µl
Xylene cyanol	0.0025 g
Bromophenol blue	0.0025 g

21. Ethidium Bromide stock solution (10 mg/ml): 1g Ethidium bromide was dissolved in 100 ml distilled water by stirring for several hours and stored it in a dark bottle at room temperature.

22. Composition of Wakimoto's Medium:

S.N.	component	Amount
1	Peeled potato	300 gm
2.	Sucrose	20 gm
3.	Peptone(Bacteriological)	5 gm
4.	$Ca(NO_3)2\ 4H_2O$	0.5 gm
5.	$Na_2HPO_4\ 12H_2O$	1.87gm
6.	Agar	15 gm
7.	Distilled water make upto	1000 ml
The pH was adjusted to 6.8-7.0 prior to sterilization		

23. Composition of PDA:

S.N.	component	Amount
1	Potato	250 gm
2	Dextrose	20 gm
3	Agar	20 gm
Boil and makeup with distilled water upto to 1000 ml		

Results

CHAPTER- IV

RESULTS

Rice is one of the most important staple foods for the increasing world population, especially in Asia. The diseases are among the most important limiting factors that affect rice production, causing annual yield loss conservatively. The resistant cultivars and application of pesticides have been used for disease control. Due to the breakdown of the resistance in the face of high pathogenic variability of the pathogen population need to develop strategies providing durable resistance, giving protection for a long time and over a broad geographic area. The polygenic quantitative resistance much more durable than qualitative resistance, because each gene involved has a small effect on host resistance. The accumulation of such small effects may process longer life span in crop production than the resistance conferred by a single *R* gene.

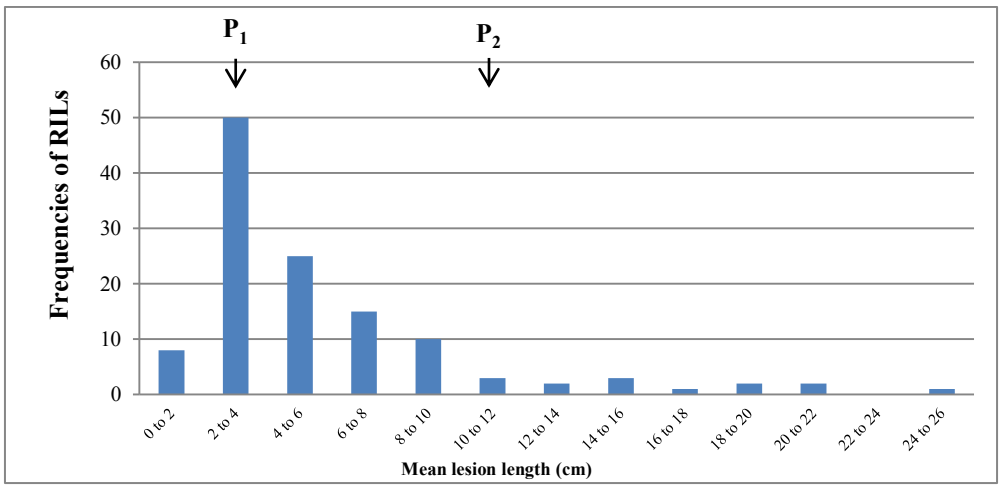
Two potential *indica* genotypes, Danteshwari: a high yielding popular rice cultivar but resistance to few diseases and Dagad deshi: a tall deep rooted, poor yielder and resistance to few diseases. The parents along with their derived F_{14} RILs were used for the phenotypic evaluation of four diseases such as Bacterial leaf blight, Sheath blight, leaf Blast and Brown spot traits. The genotyping of the RILs population further help us to detect QTLs for above diseases resistance.

4.1 The distribution of resistance for Bacterial leaf blight in the RILs population

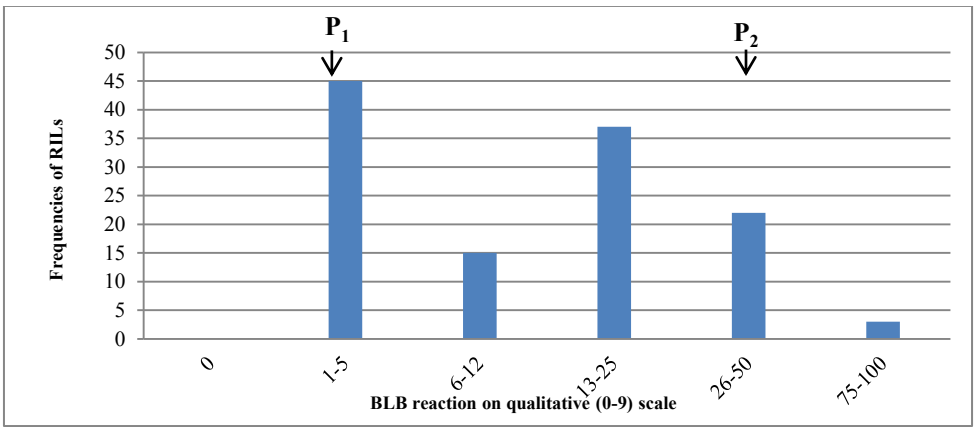
The frequency distribution of disease reaction of 122 RILs population derived from Danteshwari × Dagad deshi was examined to determine if its normality for local *Xoo* isolate used in the experiment. The analysis indicated that nearly normal distribution was followed for RILs population (Figure 4.1). A higher percentage of lines showed scores of 1, 3, 5 and 7 qualitatively (0-9 SES scale; IRRI, 2002) to *Xanthomonas oryzae* pv. *oryzae* (*Xoo)* local isolate used in the experiment. On the basis of mean lesion length, higher percentage of lines showed range (2 to 8cm) on

frequency distribution graph. The phenotypic screening result for Bacterial blight resistance of many RILs showed resistance toward local *Xoo* isolate used in the experiment. There were few lines such as line number 71, 83, 88, 94, 107, 109 and 115 which showed high resistance to the *Xoo* isolate used, indicating that a combination of several genes was required to achieve different level of resistance.

The parental lines Danteshwari and Dagad deshi showed significant differences in their resistance level in the experiment. The mean lesion length of 2.28cm for Danteshwari and 11.34cm for Dagad deshi were obtained. The parent Danteshwari showed high resistance reaction toward Bacterial blight *Xoo* isolate and the parent Dagad deshi showed highly susceptible (Fig. 4.2).

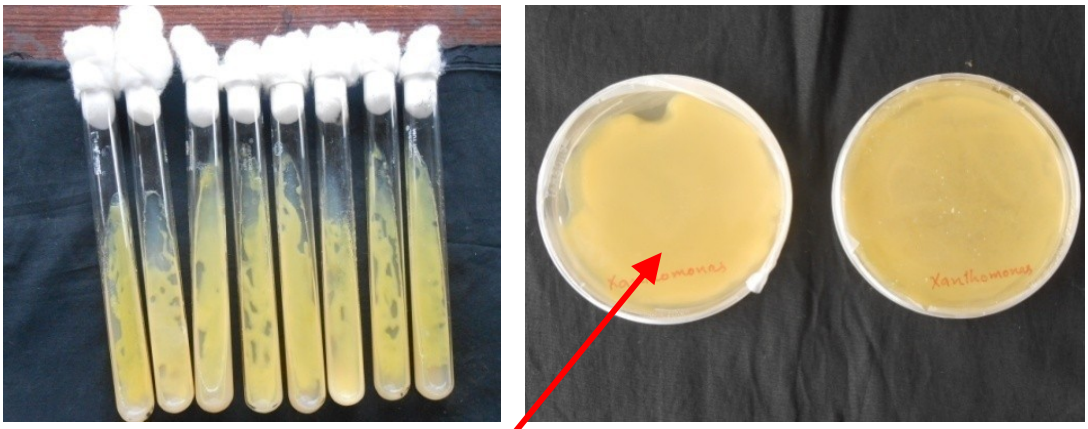


a) Mean lesion length



(b) BLB reaction on qualitative (0-9) scale

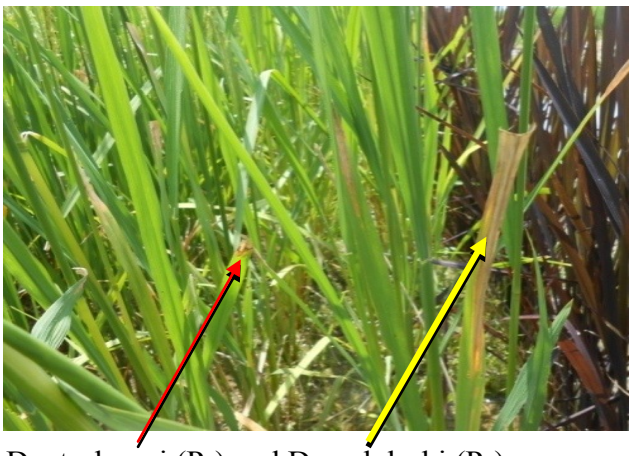
Figure 4.1 The frequency distributions of the traits such as mean lesion length and BLB reaction on qualitative (0-9) scale



a) Local *Xanthomonas oryzae* pv. *oryzae* (*Xoo)* isolate used for inaculation

b) Resistant line

Highly succeptable line



b) Parents Danteshwari (P_1) and Dagad deshi (P_2)

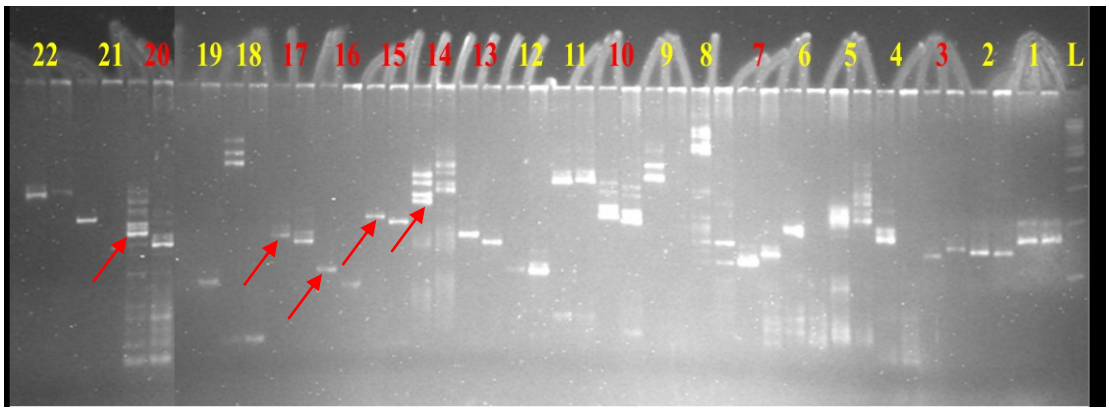
Figure 4.2 The occurrence of disease reaction on RIL population by artificial inoculation of *Xoo* isolate

4.2 Genotyping of RIL population and construction of linkage map

The polymorphism survey was conducted between the parents Danteshwari and Dagad deshi by using 254 SSR RM markers randomly distributed on all 12 rice chromosomes (www.gramene.org; Orjuela *et al*., 2009). Among 254 markers, 58 markers found parental polymorphic (Table 4.1 and Fig. 4.3).

Table 4.1 SSR markers used for parental polymorphism (Danteshwari and Dagad deshi)

Serial	SSR marker
CH# 1	
1	RM3252
2	RM3746
3	RM8071
4	RM129
5	RM8139
6	RM3341
7	RM7405
8	RM11307
9	RM1095
10	RM5794
CH# 2	
11	RM6842
12	RM12368
13	RM4355
14	RM6375
15	RM6509
16	RM13541
17	RM7485
CH# 3	
18	RM5474
19	RM3392
20	RM5626
21	RM16147
22	RM7389
CH# 4	
23	RM7585
24	RM16368
25	RM16559
26	RM5979
27	RM17303
28	RM17388
CH# 5	
29	RM3381
CH# 6	
30	RM6467
31	RM3765
CH# 8	
32	RM23251
CH# 9	
33	RM23736
34	RM257
35	RM24718
CH# 10	
37	RM25149
38	RM1126
39	RM1375
40	RM6673
41	RM4771
CH# 11	
42	RM26063
43	RM26105
44	RM332
45	RM26643
46	RM26652
47	RM26998
48	RM27318
49	RM27326
50	RM3577
CH# 12	
51	RM3323
52	RM5927
53	RM27542
54	RM27877
55	RM28607
56	RM1159
57	RM6411
58	RM1227



86 **Figure 4.3 The informative SSR markers identified on the basis of parental polymorphism**

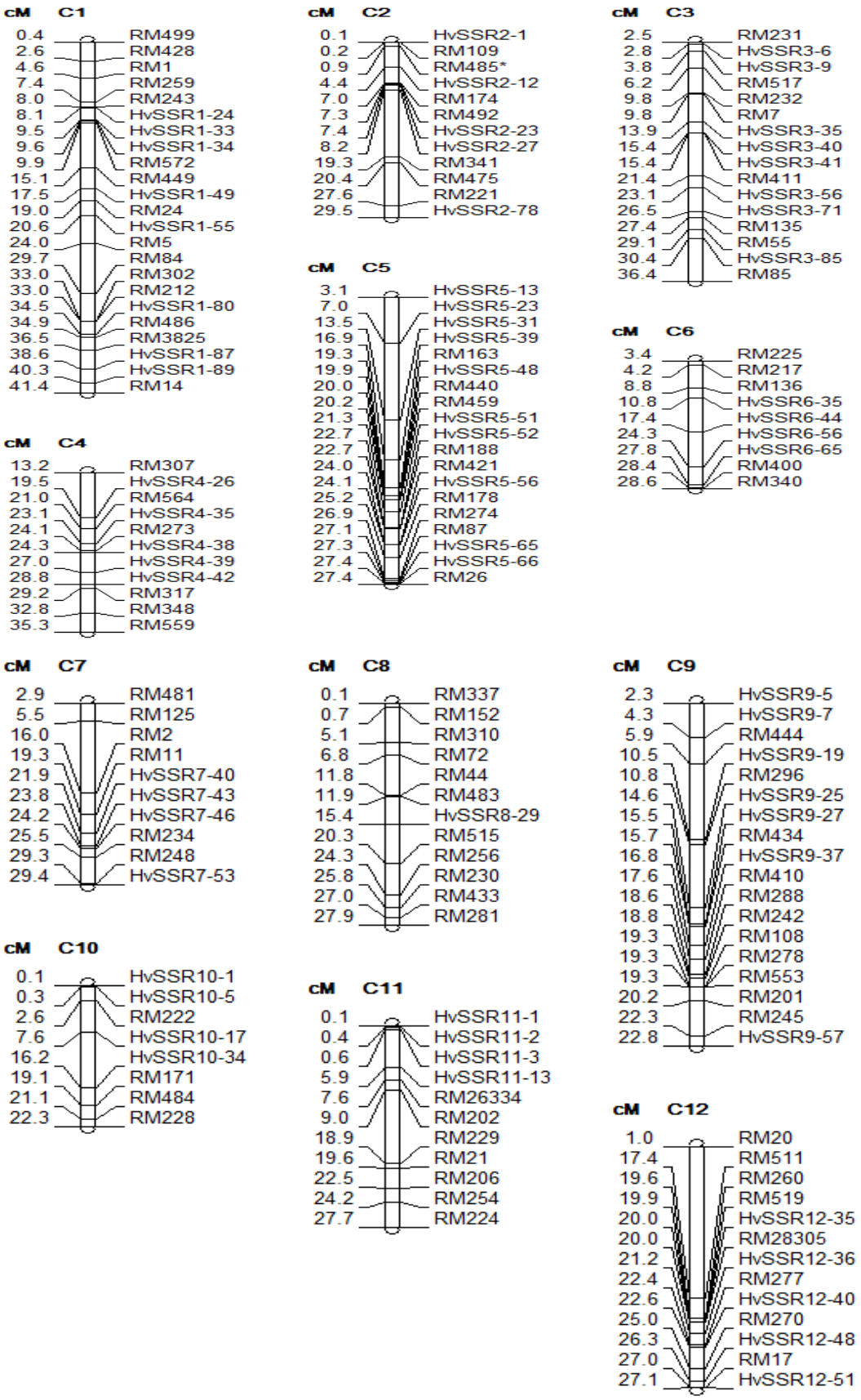


Figure 4.4 The linkage map constructed from 162 polymorphic markers

A linkage map of 162 SSR polymorphic markers (RM and HvSSR) was constructed by using QTL cartographer 2.5. A total of 162 well distributed polymorphic SSR (RM and HvSSR) (McCouch *et al*., 2002) markers between Danteshwari and Dagad deshi were used to construct a linkage map (Verma, 2013; Thesis). All of 162 clearly polymorphic markers were used in segregation analysis of 122 RILs population. The genotypes at markers were scored for each line based on the banding patterns. Based on these data, the genetic linkage map was constructed as shown (Figure 4.4). The map covered 335.8 cM for all 12 chromosomes with an average interval of 29.65 cM between the adjacent markers. The order of the markers in each chromosome was consistent with the order of the Nipponbare map (www.gramene.org). The genotypic data of 162 polymorphic SSR (RM and HvSSR) markers were further used for QTL mapping in the experiment.

4.3 Single Marker Analysis for BLB resistance

The Single Marker Analysis tests the association between marker genotypes and trait values using F-test in QTL cartographer 2.5. This analysis fits the data to the simple linear regression model y = b0 + b1 x + e. The results given estimate of b0, b1 and the F statistic for each marker. It was interested in whether the marker is linked to a QTL. This was determining if b1 is significantly different from zero. The F statistic compares the hypothesis H0: b1 = 0 to an alternative H1: b1 not 0. The pr(F) a measure of how much support there is for H0. A smaller pr(F) indicates less support for H0 and thus more support for H1. Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and **** respectively. The following markers were highly associated with mean lesion length and reaction qualitative SES (0-9) scale for Bacterial leaf blight resistance (Table 4.2).

Table 4.2 The markers that were highly associated BLB resistance

Chr.	Markers significance at 0.01% level (****)	Traits
1	RM499, RM449, HvSSR1-80, RM486, RM3825, HvSSR1-87, HvSSR1-89	The mean lesion length
9	RM288, RM242, RM108	
11	RM224	
12	RM260, RM519, HvSSR12-35, RM28305	
1	RM499, RM3825	The reaction qualitative (0-9) scale
3	HvSSR3-6, RM232, HvSSR3-40, HvSSR3-41	
4	HvSSR4-26, HvSSR4-38, HvSSR4-39, RM348	
8	RM230, RM433, RM281	
11	HvSSR11-1, RM224	
12	RM260, RM519, HvSSR12-35, RM28305, HvSSR12-40	

4.4 QTLs mapping through Composite Interval Mapping for BLB

The genotypic data and phenotypic data of field condition of Bacterial blight were analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2 softwares. The presence of a putative QTL declared if the LOD threshold was larger than 3 for the traits using QTL cartographer 2.5 while 2.5 using QTL IciMapping 3.2 software.

A total of 10 and 3 QTLs were identified for Bacterial blight resistance under different conditions such as mean lesion length and on the basis of BLB reaction qualitative (0-9) scale (IRRI, 2002), respectively using QTL cartographer 2.5 while 3 and 1 QTLs were identified using QTL IciMapping 3.2, out of which 1 QTL was found common within both the software. These loci were associated with LOD scores above the threshold value determine by permutation test for these traits of the experiment. The resistance loci mapped to 8 chromosomes out of 12 rice chromosome. The LOD score for QTLs found range from 3.03 to 8.83 for mapping with QTL cartographer 2.5 and 2.53 to 10.66 using QTL IciMapping 3.2 for Bacterial blight resistance. These QTLs were found to be present on

chromosome #1, 3, 4, 5, 6, 9, 10 and 12. The QTLs along with their LOD score and R^2 value worked out through composite interval mapping (Table 4.3, 4.4, 4.5 and 4.6) respectively.

Table 4.3 QTLs underlying BLB resistance mapped by QTL cartographer 2.5

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Additive	R^2%
Mean lesion length	*qBbr1a*	1	1.00	RM499	RM1	7.86	-1.37	13.53
	qBbr1b	1	39.20	HvSSR1-87	HvSSR1-89	5.33	-3.06	27.61
	qBbr3a	3	16.9	HvSSR3-40	RM411	4.39	-0.06	0.36
	qBbr3b	3	26.00	HvSSR3-56	HvSSR3-71	4.19	0.001	1.02
	qBbr4a	4	7.80	RM564	HvSSR4-35	4.56	-0.61	3.45
	qBbr5a	5	6.90	HvSSR5-23	HvSSR5-31	3.14	-0.11	0.11
	qBbr5b	5	15.8	HvSSR5-39	HvSSR5-48	3.03	0.01	0.02
	qBbr9a	9	5.60	HvSSR9-7	RM444	3.37	-0.75	1.20
	qBbr10a	10	16.20	HvSSR10-34	RM171	3.58	-0.46	0.37
	qBbr12a	12	19.00	RM511	RM519	4.22	-1.75	10.9

Table 4.4 QTLs underlying BLB resistance mapped by QTL IciMapping 3.2

Trait Name	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Add	PVE (%)
Mean lesion length	*qBbr1c*	1	40.38	HvSSR1-89	RM14	4.43	-1.81	12.46
	qBbr9b	9	18.28	RM410	RM288	2.53	-1.14	6.03
	qBbr12b	12	19.96	RM28305	HvSSR12-36	4.17	-1.50	11.26

Table 4.5 QTLs underlying Bacterial blight resistance on qualitative (0-9) scale by QTL cartographer 2.5

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Additive	R^2%
BLB reaction Qualitative (0-9) scale	*qBbr4b*	4	7.40	HvSSR4-26	RM564	5.00	-1.12	15.31
	qBbr6a	6	22.90	HvSSR6-44	HvSSR6-56	3.95	0.79	9.22
	qBbr12c	12	8.00	RM20	RM511	6.47	-1.91	52.81
	qBbr12a	12	18.40	RM511	RM260	8.83	-1.33	22.73

Table 4.6 QTLs underlying Bacterial blight resistance on Qualitative (0-9) scale by QTL IciMapping 3.2

Trait Name	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Add	PVE (%)
BLB reaction Qualitative (0-9) scale	*qBbr4c*	4	25.15	HvSSR4-38	HvSSR4-39	3.68	-0.88	9.43
	qBbr12a	12	18.97	RM511	RM260	10.6	-1.46	34.02

4.5 The loci associated with quantitative resistance for Bacterial leaf blight

There were two QTLs, *qBbr1a* and *qBbr1b* identified on chromosome 1 affected resistance to local isolate shown in (Figure 4.5). The highly significance QTL was identified between markers RM499 and RM1 with LOD value 7.86; explained 13.53% of phenotypic variation. This was the large effect estimated QTL for the trait mean lesion length. The second QTL, *qBbr1b* mapped between markers HvSSR1-87 and HvSSR1-89 with LOD score 5.33 and explained 27.61% of phenotypic variation. This was also a major effect QTL estimated. The negative value additive effect of both the QTLs showed that alleles from susceptible parent Dagad deshi. The third QTL, *qBbr1c* mapped between markers HvSSR1-89 and RM14 by QTL IciMapping 3.2 for Bacterial blight resistance with LOD value of 4.43 and also explain 12.46% of PVE. The alleles come from susceptible parent Dagad deshi.

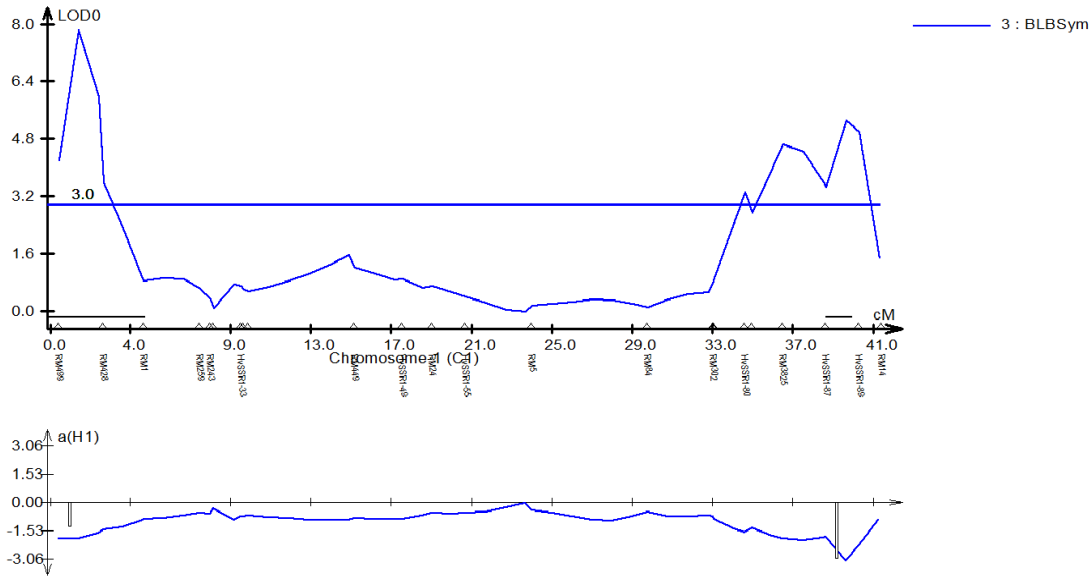


Figure 4.5 The QTLs mapped for mean lesion length trait identified on chromosome 1 for the Bacterial blight resistance using CIM. The bars indicate the most likely positions of the QTLs. The small graphic (bottom) showed the additive effect (-) value = both alleles from the susceptible parent Dagad deshi.

The one common significant QTL, *qBbr12a* was mapped on chromosome 12 for mean lesion length between markers RM511 and RM519 with LOD score

4.22, which explained 10.9% of phenotypic variation by QTL cartographer 2.5(Figure 4.6). The same QTL on the basis of BLB reaction qualitative (0-9) scale explained 14.17% phenotypic variation with high LOD score 8.83 by QTL cartographer 2.5 and with very high LOD score 10.66, explained 26.07% of PVE by QTL IciMapping 3.2. The QTL with major effect resistance to local *Xoo* isolate used in the experiment. The both qualitative scale and quantatively analysis result showed that this QTL was consistence in all type of analysis. The negative value of additive effect showed that alleles from susceptible parent Dagad deshi. Similarly, another QTL, *qBbr12b* specially identified by QTL IciMapping 3.2 on chromosome 12 between markers RM28305 and HvSSR12-36 with LOD value of 4.17. The phenotypic variance explained by the QTL was 11.26%. This allele also from susceptible parent Dagad deshi.

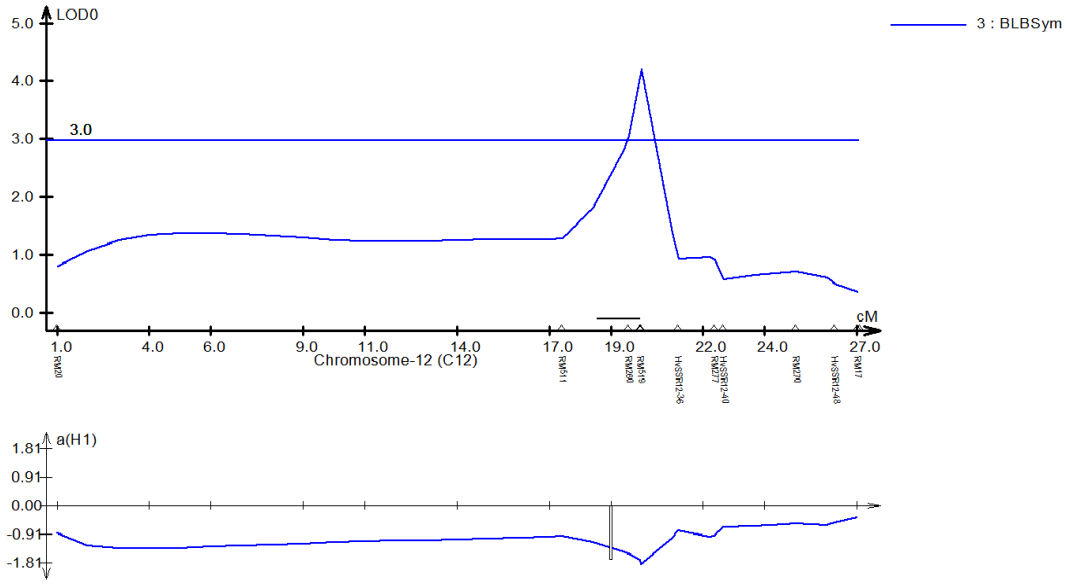


Figure 4.6 The common QTL mapped for mean lesion length trait identified on chromosome 12 for the Bacterial blight resistance using CIM. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance parent and (-) values = alleles from the susceptible parent.

There were two minor effect QTLs, *qBbr3a* and *qBbr3b* mapped between

92 markers HvSSR3-40 and RM411 with LOD value 4.39 and between markers HvSSR3-56 and HvSSR3-71 of LOD score 4.19 respectively. The positive value of

additive effect of *qBbr3a* showed that allele from resistant parent Danteshwari. Similarly, other minor significant QTLs, *qBbr5a* and *qBbr5b* mapped on chromosome 5. The allele for *qBbr5b* also from resistant parent Dentashwari. The allele from the resistance parent Danteshwari acted to decrease the measured trait bacterial blight resistance for mean lesion length. The two small effect QTLs, *qBbr9a* and *qBbr9b* mapped on chromosome 9 by QTL cartographer 2.5 and QTL IciMapping 3.2 respectively. The QTLs mapped between markers HvSSR9-7 to RM444 and RM410 to RM288 with very low R^2 values. The both QTLs showed negative additive effect mean both carry from susceptible parent Dagad deshi. The *qBbr10a* also a minor QTL showed on chromosome 10.

4.6 The loci associated with quantitative resistance for Bacterial blight resistance on (0-9) scale

One QTL, *qBbr4b* was mapped on chromosome 4 affect resistance to local *Xoo* isolate shown in (Figure 4.7). The *qBbr4b* locus was identified between markers HvSSR4-26 and RM564 specially reported by QTL cartographer 2.5 with LOD value 5.00. The QTL accounted 15.31% of phenotypic variation for Bacterial blight resistance on the basis of qualitative SES, IRRI (0-9) scale. It was relatively large effect QTL for local *Xoo* isolate used in this experiment. The negative value of additive effect showed that allele from susceptible parent Dagad deshi. Another minor QTL, *qBbr4c* positioned on chromosome 4 on the basis of BLB reaction qualitative (0-9) scale between markers HvSSR4-38 to HvSSR4-39 with LOD score 3.68 and explained 9.43% of the phenotypic variation by QTL IciMapping 3.2. The negative value of additive effect showed that the allele also from susceptible parent Dagad deshi. The *qBbr4c* also a minor effect for mean lesion length identified on chromosome 4.

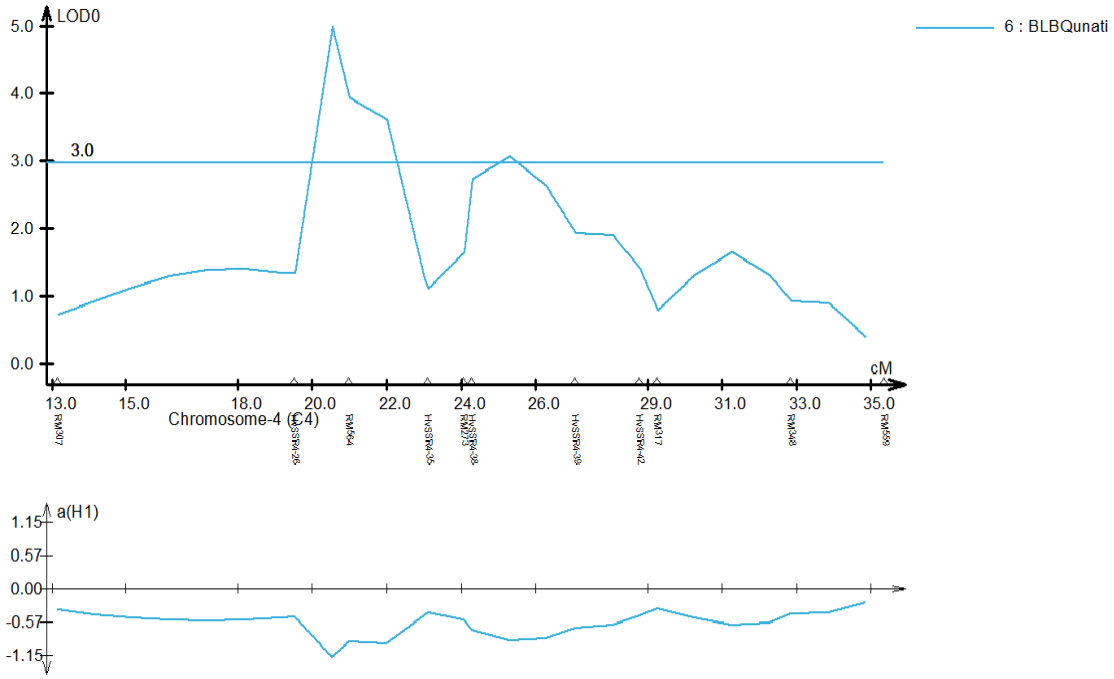


Figure 4.7 The QTL mapped on chromosome 4 for the Bacterial blight resistance trait using CIM. The bars indicate the most likely positions of the QTL.

A major significant QTL, *qBbr12c* affect resistance to local isolate was identified on chromosome 12 shown in (Figure 4.8). The QTL was mapped in the region between markers RM20 and RM511 with high LOD score 6.47 by QTL cartographer 2.5 under heavy infection condition. The phenotypic variance explained by the QTL was very high that was 52.8%. The very large effect QTL affect resistance to local *Xoo* isolate used in this experiment. The negative value of additive effect showed that allele from susceptible parent Dagad deshi. Similarly *qBbr6a* mapped between markers HvSSR6-44 and HvSSR6-56 with a LOD score of 3.95 and 9.22% phenotypic variance. The QTL showed positive additive effect mean carry from resistance parent Danteshwari.

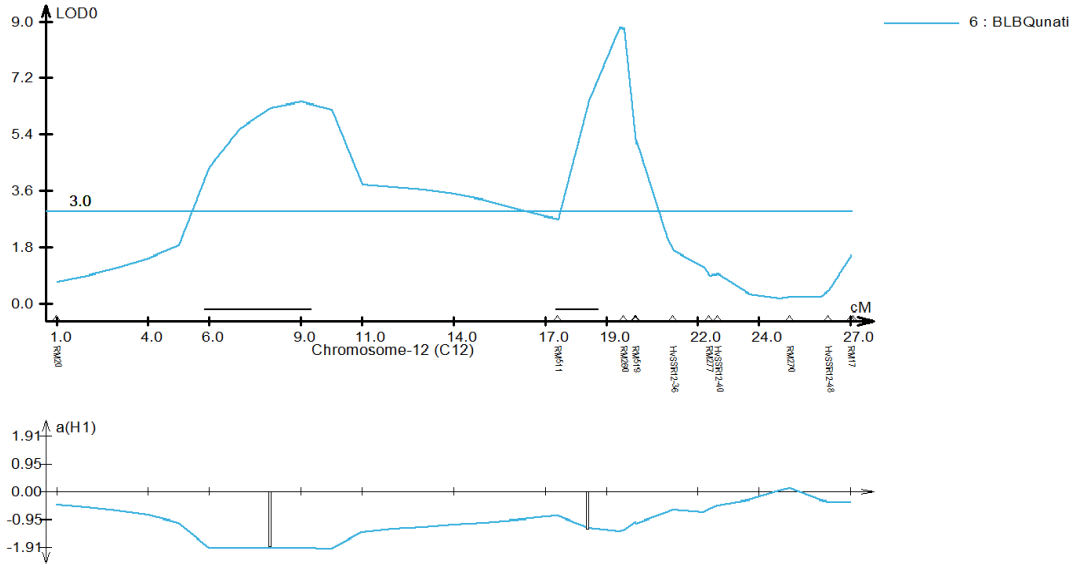


Figure 4.8 The QTLs mapped for Bacterial blight resistance trait identified on rice chromosome 12 using CIM. The bars indicate the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance parent and (-) values = alleles from the susceptible parent

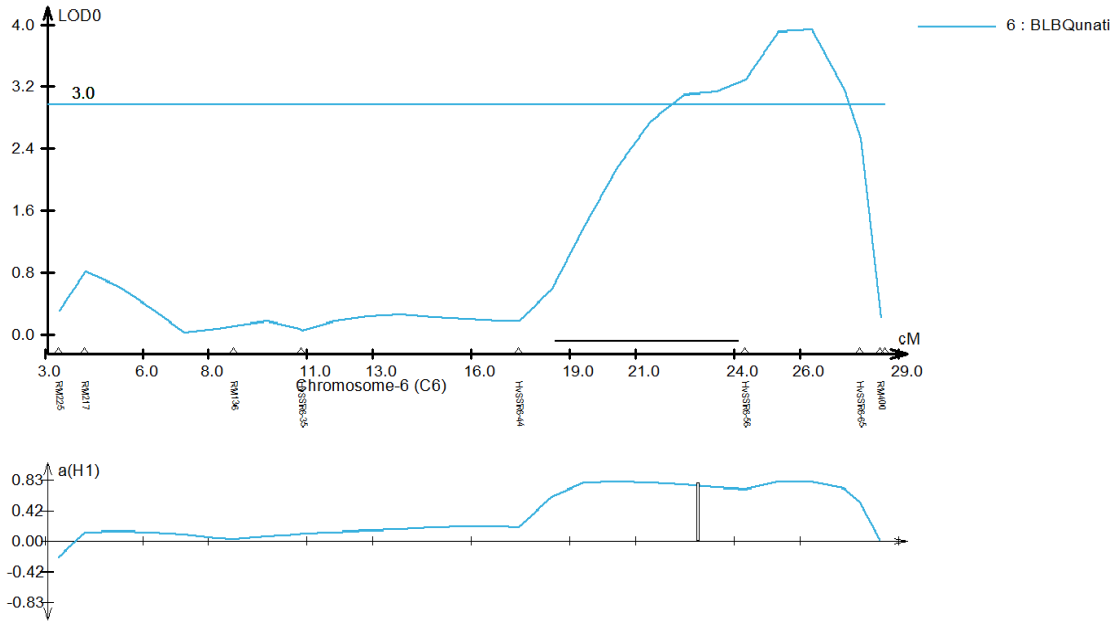
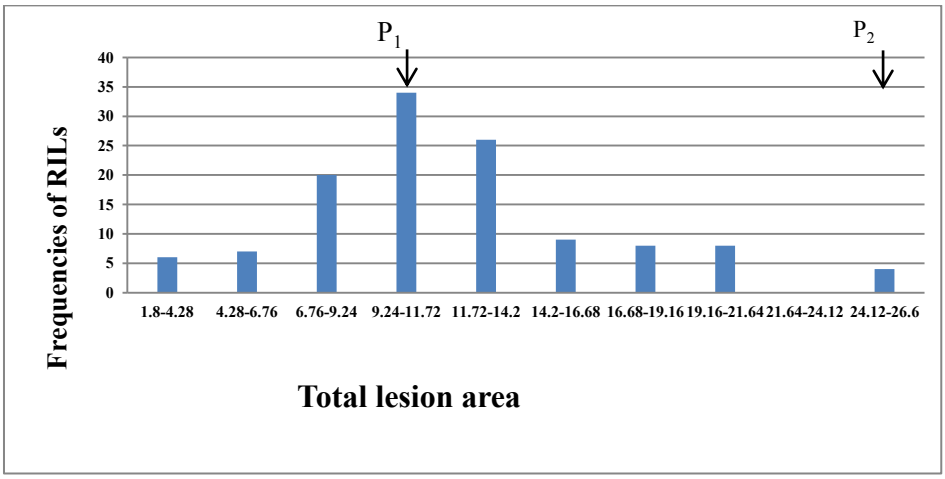


Figure 4.9 The QTL mapped for Bacterial blight resistance trait identified on rice chromosome 6 using CIM. The bar indicates the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance parent and (-) values = alleles from the susceptible parent

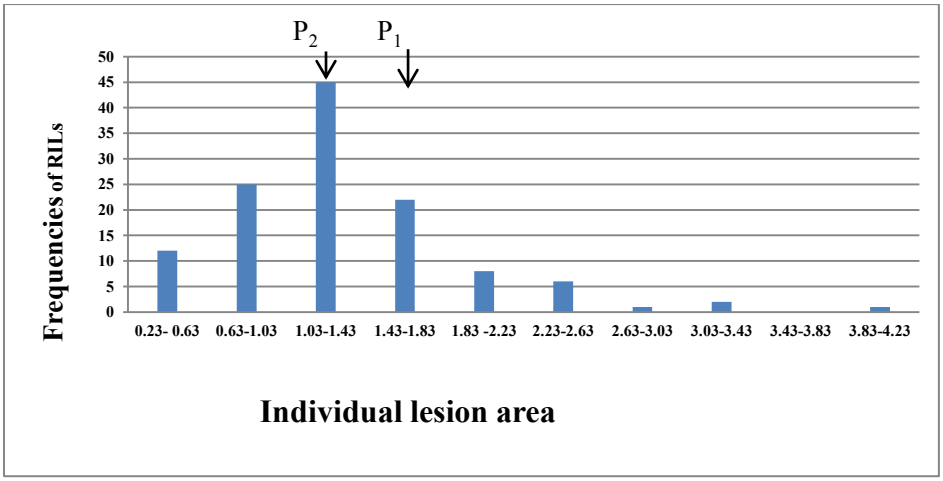
4.7 The distribution of resistance for Sheath blight in the RIL population

The Danteshwari × Dagad deshi derived mapping population 122 F_{14} RIL lines and parents were screened for Sheath blight resistance. The F_{14} RILs population exhibited significant phenotypic variance for traits to support QTL mapping. The frequency distribution of score obtained in the experiment was examined to determine if its normality. The Sheath blight disease index of RILs was continuously distributed, as expected for a quantitative trait shown in (Fig. 4.10). The resistance segregation in this experiment varied continuously. The parents Danteshwari and Dagad deshi showed significant differences in their resistance level in the experiment. The average individual disease lesion size of 1.62 cm^2 for Danteshwari and 1.05 cm^2 for Dagad deshi was obtained. Similarly, the percentage of lesion length of sheath length was 13.72 for Danteshwari and 7.82 for Dagad deshi. The parent Dagad deshi showed very much resistance for sheath blight and Danteshwari was highly susceptible in field condition shown in (Fig. 4.11).

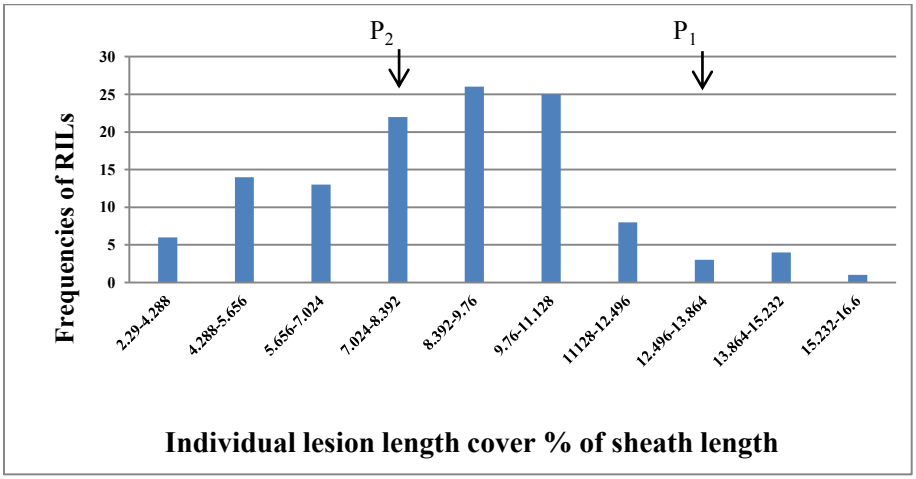
A higher percentage of lines were Sheath blight resistance scored middle range of the distribution toward local isolate of *Rhizoctonia solani* used in this experiment. On the basis of average disease lesion size and lesion length many lines showed resistance toward *Rhizoctonia solani* local isolate used in the experiment.



A) Total lesion area



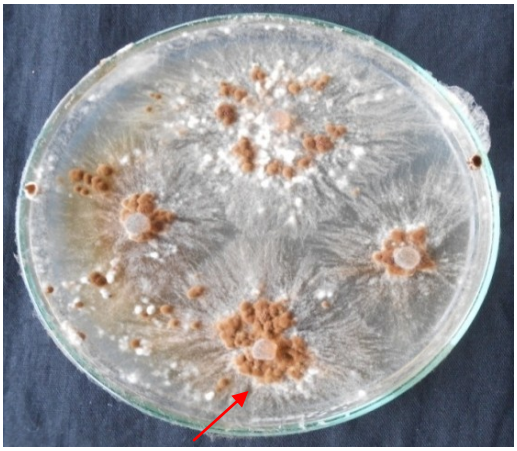
B) Individual lesion area



C) Individual lesion length cover % of Sheath length

Figure 4.10 The frequency distribution of Sheath blight response of 122 F_{14} RIL lines for total and individual lesion

A) Experiment on field



B) The *Rhizoctonia solani* local isolate

C) Highly resistant lines for *Rhizoctonia solani*

D) Very susceptible lines toward *Rhizoctonia solani*

Figure 4.11 The occurrence of Sheath blight disease reaction on RIL population by artificial inoculation

The Danteshwari × Dagad deshi derived mapping population provides a good basis to study and to analyze genetically complex and polygenic forms of disease resistance known as “Quantitative trait loci” (QTL) for Sheath blight of rice. Thus, the present investigation was carried out with the observations on the response of RILs population to Sheath blight resistance and the identification of putative QTLs (associated with Sheath blight resistance) with the molecular marker was carried out by QTL cartographer 2.5 and QTL IciMapping 3.2 softwares.

4.8 The Single Marker Analysis for Sheath blight resistance

The single marker analysis tests the association between marker genotypes and trait values using F-tests in QTL cartographer 2.5. This analysis fits the data to the simple linear regression model y = b0 + b1 x + e. The results gave the estimates for b0, b1 and the F statistic for each marker. This was interested in whether the marker is linked to a QTL. The pr(F) values showed significance at the 5%, 1%, 0.1% and 0.01% levels respectively. There were 20 markers associated with Sheath blight for total lesion area. Out of 20 markers, HvSSR4-35 and HvSSR4-38 on chromosome 4 and RM-459 on chromosome 5 were showed significant at 1% level. The only marker RM273 on chromosome 4 was found significant at 0.1% level for the trait total lesion area of Sheath blight resistance.

The markers for individual lesion area were not highly associated. The only marker HvSSR7-43 showed significance at 1% level out of 12 associated markers. There were 17 putative markers were indicated to be associated with the sheath blight resistance for trait individual lesion length cover % of sheath length. Out of 17, 2 markers such as HvSSR1-18 on chromosome 1 and RM222 on chromosome

10 showed significant at 1% level. Similarly, marker RM3825 and HvSSR1-87 were found significance at 0.1% and 0.01% respectively.

4.9 The QTL mapping through Composite Interval Mapping for Sheath blight

The genotypic data and phenotypic data of field condition for Sheath blight disease resistance was analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2 softwares. The QTL analysis results are presented in (Table 4.7 and 4.8). The presence of a putative QTL was declared if the LOD threshold was larger than 3 for the trait using QTL cartographer 2.5 while 2.5 using QTL IciMapping 3.2.

A total of 7 QTLs were identified for Sheath blight disease resistance using QTL cartographer 2.5 using composite interval mapping while 2 QTLs were identified using QTL IciMapping 3.2, out of which 2 QTLs were common with both the softwares. These loci were associated with LOD score above the threshold values determine by permutation test for the trait of the experiment. The QTLs were found to be present on chromosomes 1, 2, 3, 4, 9 and 12. The LOD score for QTLs ranged from 0.02 to 7.3 for mapping with QTL cartographer 2.5 and 3.03 to 4.6 using QTL IciMapping 3.2. The QTLs along with their LOD score and R^2 value worked out through composite interval mapping (Table 4.7 and 4.8).

4.10 The loci associated with quantitative resistance for Sheath blight resistance of rice

One major significant common QTL, *qSBR4-1* was identified on chromosome 4 (Figure 4.12) for total lesion area for Sheath blight resistance between markers HvSSR4-35 and HvSSR4-38 with LOD score of 3.49 with a QTL peaking at RM273, explained 12.1% of the phenotypic variation in QTL cartographer 2.5 and the same QTL mapped between markers RM 273 and HvSSR4-38 with LOD score of 3.04, explained 12.94% of the phenotypic variation

in QTL IciMapping 3.2 under heavy infection condition. The QTL showed negative additive effect in both the software (Table 4.7 and 4.8). This means that the allele transferred from the susceptible parent Danteshwari.

Table 4.7 QTLs underlying Sheath blight resistance mapped by QTL Cartographer 2.5

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Additive	R^2%
Total lesion area	*qSBR4-1*	4	10.9	HvSSR4-35	HvSSR4-38	3.49	-1.9589	12.1
Individual lesion area	*qSBR2-1*	2	7.2	RM174	RM492	0.02	0.0392	0.07
	qSBR3-1	3	5.7	RM517	RM232	3.97	-0.0708	2.21
	qSBR9-1	9	3.6	HvSSR9-7	RM444	3.06	-0.1280	5.24
	qSBR12-1	12	17.4	RM511	RM260	5.30	-0.0476	0.11
Individual lesion length	*qSBR1-2*	1	2.2	RM499	RM1	3.19	0.3641	3.62
cover % of Sheath length	*qSBR1-1*	1	38.1	RM3825	HvSSR1-87	7.30	1.3597	26.64

Table 4.8 QTLs underlying Sheath blight resistance mapped by QTL IciMapping 3.2

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Add	PVE (%)
Total Lesion area	*qSBR4-1*	4	24.14	RM273	HvSSR4-38	3.04	-1.94	12.95
Individual lesion length cover % of Sheath Length	*qSBR1-1*	1	38.38	RM3825	HvSSR1-87	4.6	1.19	19.18

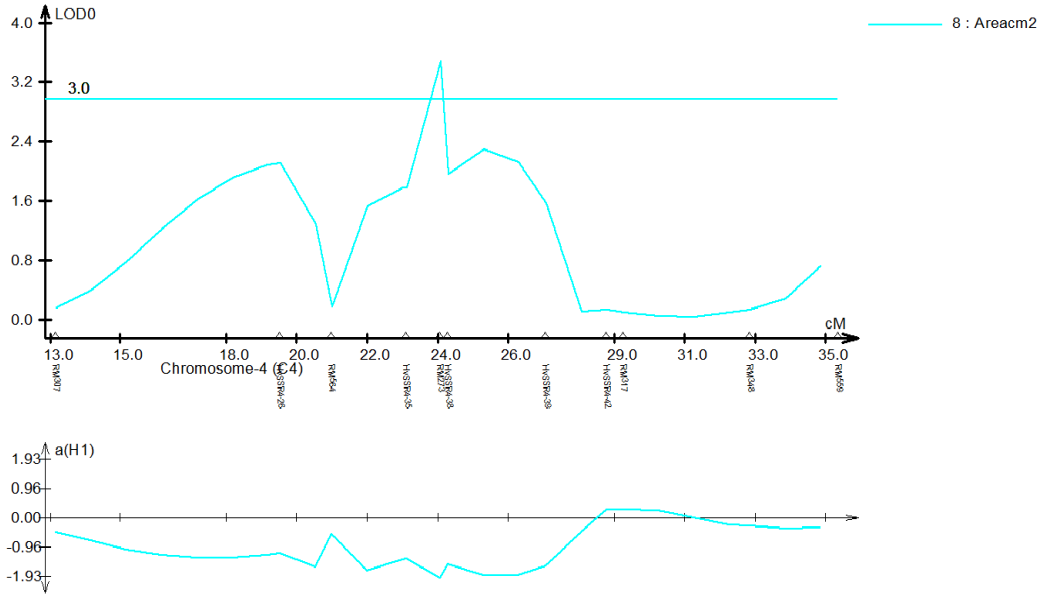


Figure 4.12 The QTL *qSBR4-1* mapped for the Sheath blight trait identified on rice chromosome 4 using CIM in experiment. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance parent and (-) values = alleles from the susceptible parent

The major significant common QTL, *qSBR1-1* was identified for individual lesion length cover percentage of sheath length on chromosome 1 between markers RM3825 and HvSSR1-87 with LOD score of 7.3 with a QTL peaking at HvSSR1-87, explained 26.64% of the phenotypic variation in QTL cartographer 2.5 (Figure 4.13). The same QTL with LOD score 4.64, explained 19.17% of the PVE by QTL IciMapping 3.2. The QTL was a large effect for Sheath blight disease resistance. The QTL showed positive additive effect. The allele from the resistance parent Dagad deshi acted to decrease the measured trait to increase Sheath blight resistance.

Another significant QTL, *qSBR1-2* for trait individual lesion length cover percentage of sheath length associated with Sheath blight resistance was detected on chromosome 1(Figure 4.13). The QTL was mapped in the region between markers RM499 and RM1 with LOD score 3.19 and a QTL peak at marker RM428 by QTL cartographer 2.5 under heavy infection condition. The percentage of

phenotypic variance explained by the QTL was 3.62%. The QTL also showed positive additive effect means that the allele from the resistance parent Dagad deshi acted to decrease Sheath blight susceptibility.

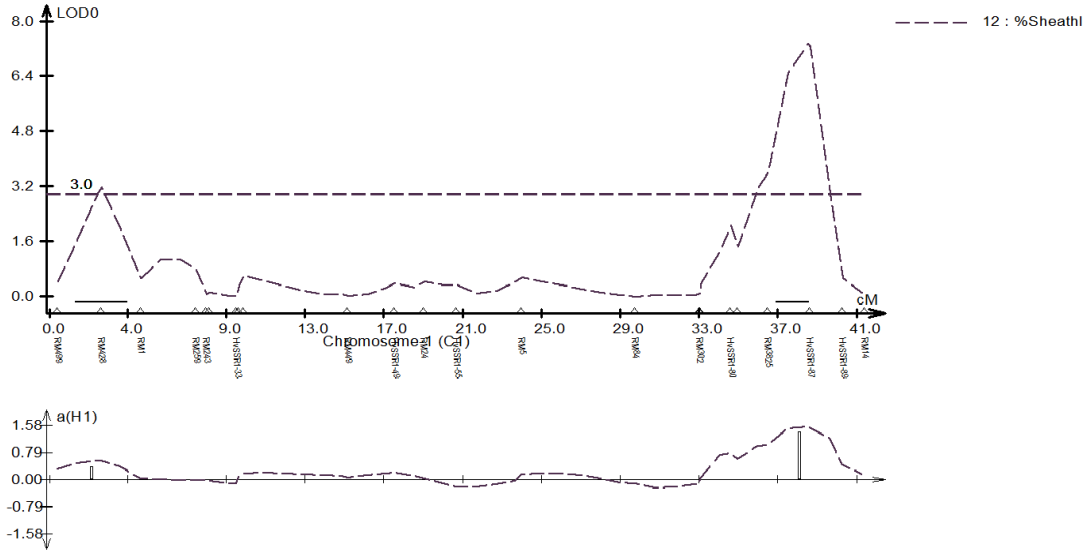


Figure 4.13 The QTLs, *qSBR1-1* and *qSBR1-2* mapped for the Sheath blight trait identified on rice chromosome 1 using CIM in experiment. The bars indicate the most likely positions of the QTLs. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance parent and (-) values = alleles from the susceptible parent.

There were four small effect QTLs such as *qSBR2-1, qSBR3-1, qSBR9-1* and *qSBR12-1* mapped for individual lesion area on chromosome 2, 3, 9 and 12 respectively (Fig. 4.14). The very small effect *qSBR2-1* mapped between markers RM174 and RM492 showed positive additive effect mean carried from resistant parent Dagad deshi. The *qSBR9-1* mapped between HvSSR9-7 and RM444 with moderate phenotypic variance. The QTL, *qSBR12-1* for the trait detected on chromosome 12 between markers RM511 and RM260 with high LOD score 5.3. The small percentage of phenotypic variance explained by each QTLs were 0.07, 2.21, 5.24 and 0.11 percentage respectively. These were significant small effect

QTLs showed negative additive effect mean alleles from susceptible parent Danteshwari.

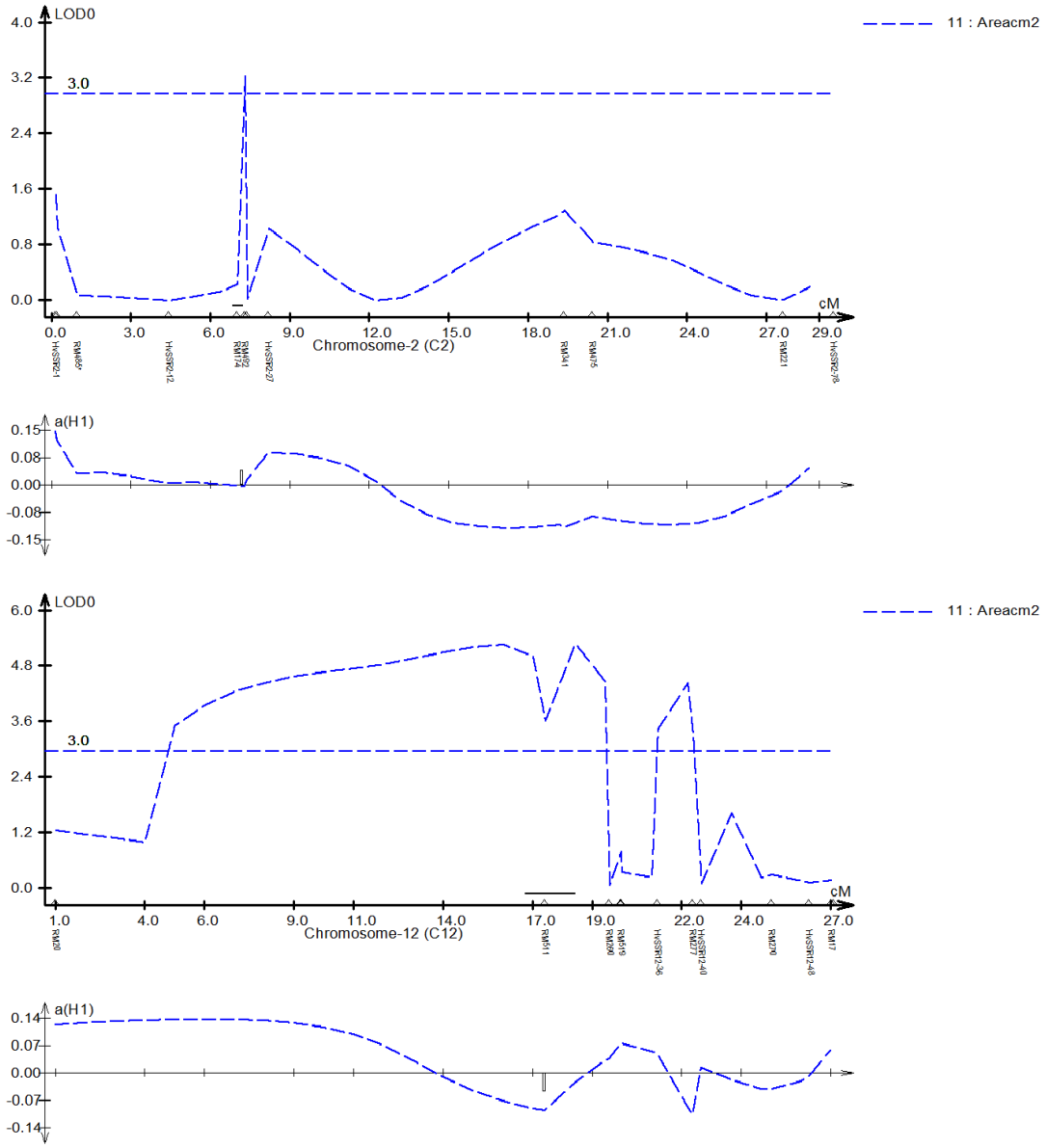


Figure 4.14 The QTL mapped for the Sheath blight trait identified on rice chromosome 2 and 12 using CIM in experiment. The bars indicate the most likely positions of the QTL.

4.11 The QTLs analysis for other traits tillers per plant and plant height

A total of two QTLs were identified for tillers per plant and one QTL for plant height under same conditions using QTL cartographer 2.5 using composite interval mapping (Table 4.9 and 4.10). These loci were associated with LOD score

above the threshold values i.e. 3 determine by permutation test for the trait of the experiment. The resistance loci mapped to 2 chromosomes out of 12 rice chromosome. The QTLs were found to be present on chromosomes 1 and 3. The LOD score for QTLs ranged from 4.00 to 5.30 for tillers per plant and for plant height was 12.19. The QTLs along with their LOD score and R^2 value worked out through composite interval mapping shown in (Table 4.9 and 4.10).

Table 4.9 QTLs underlying traits tillers per plant and plant height by QTL cartographer 2.5

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Additive	R^2%
Tillers/ Plant	*qTN1.1*	1	36.10	RM486	RM3825	4.00	0.61	11.91
	qTN3.1	3	0.00	RM231	HvSSR3-6	5.30	0.83	16.53
Plant height	*qPH1.1*	1	37.10	RM3825	HvSSR1-87	12.19	-11.86	47.0

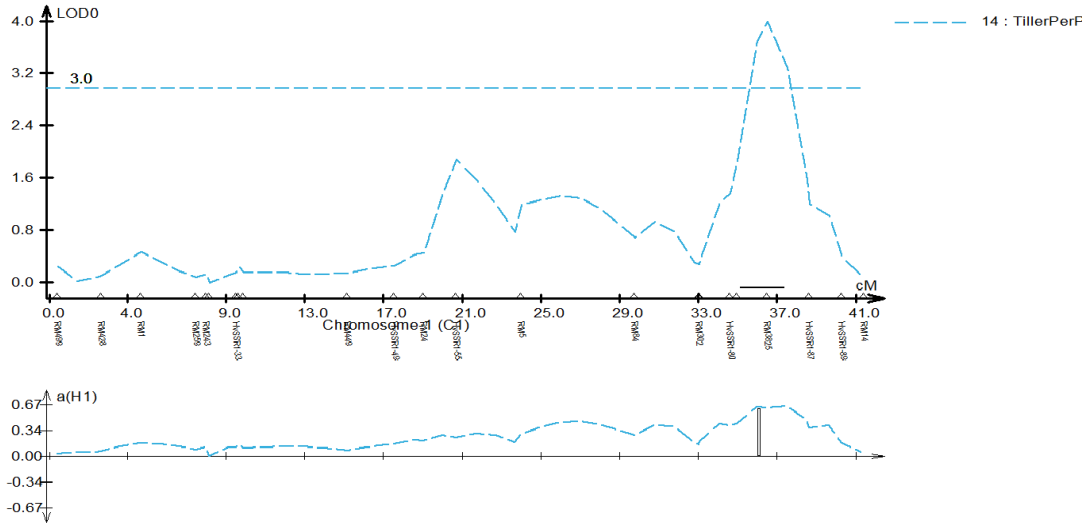
Table 4.10 QTLs underlying traits tillers per plant and plant height by QTL IciMapping 3.2

Trait Name	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Add	PVE (%)
Tillers/ Plant	*qTN1.1*	1	36.38	RM486	RM3825	2.78	0.52	8.99
	qTN3.1	3	2.45	RM231	HvSSR3-6	4.21	0.77	15.28
Plant height	*qPH1.1*	1	37.38	RM3825	HvSSR1-87	8.32	-11.66	35.55

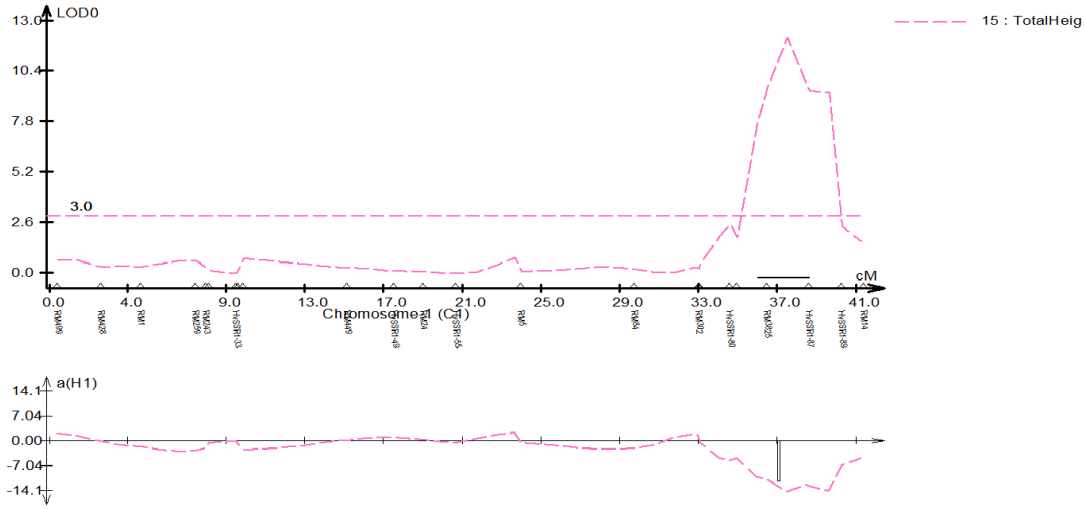
The significant major QTL, *qPH1.1* for plant height mapped on chromosomes 1 with LOD value 12.19; with a QTL peak marker RM3825 and explained very high phenotypic variance of 47%. The same QTL explain 35.55% phenotypic variance by QTL IciMapping 3.2. The height of parent Dagad deshi was very high and Danteshwari showed very low.

A QTL, *qTN1.1* for tiller number was mapped on chromosomes 1 with LOD values 4.00 and explained 11.19% of phenotypic variance. Another QTL such as *qTN3.1* for tiller number mapped on chromosome 3. The *qTN3.1* mapped between markers RM231 and HvSSR3-6 with LOD value 5.3, explained 16.53% of phenotypic variation on chromosome 3. There were many number of tiller

showed by parent Danteshwari and small number by Dagad deshi. The QTLs, *qTN1.1* and *qTN3.1* showed positive additive effect. This means that the alleles from the parent Danteshwari acted to increase the measured trait (i.e. tiller number) (Figure 4.15 A and B). The QTL for tillers and plant height found side by side on chromosome 1 and QTL, *qSBR1-1* for individual lesion length cover % of sheath length coincided with plant height QTL.



(A) Tiller number



B) Plant height

Figure 4.15 The QTL mapped for the agronomic traits plant height and tiller per plant identified on rice chromosome 1 using CIM. The bars indicate the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance.

4.12 The distribution of resistance in the RILs population for the trait leaf blast

The frequency distribution of score obtained in the experiment with natural disease occurrence was examined to determine if its normality. The phenotypically screened the structural population RIL derived from Danteshwari × Dagad deshi for blast resistance. Analysis indicated that approximately normal distribution was followed for the trait Blast resistance of RILs (Fig. 4.16).

Maximum number of RILs shown blast resistance scores of 2, 4, 6 or 8 on SES (0-9) scale (IRRI, 2002) reaction caused by the filamentous fungus *Magnaporthe oryzae* naturally. The resistance segregation in the experiment varied dramatically. There were many lines showed resistance toward natural leaf Blast disease. The parent Dagad deshi showed very resistance toward Blast disease reaction and Danteshwari found highly susceptible. The check Swarna used in this experiment was highly susceptible to Blast disease scored such as 9 on SES (0-9) scale shown in (Fig. 4.17). Interestingly, among lines few lines showed highly resistance to the disease occurrence, indicating that a combination of many genes was required to achieve different level of resistance.

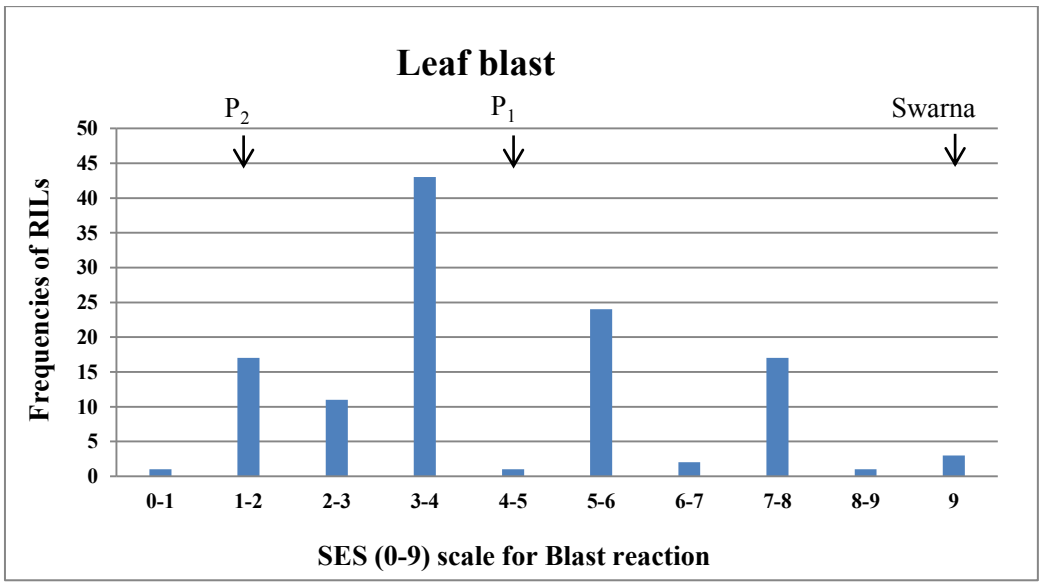


Figure 4.16 The frequency distribution of RIL for disease Blast reaction

A) 122 RIL line+P1+P2

B) Disease reaction with parents

C) Highly resistant lines

D) Highly susceptible check rice variety Swarna

Figure 4.17 The natural occurrence of disease on RIL population used for screening (yellow arrow indicating susceptible lines)

4.13 Single Marker Analysis for leaf blast

The single marker analysis tests the association between marker genotypes and trait values using F-tests in QTL cartographer 2.5. There were 13 putative markers indicated to be associated with the leaf Blast resistance. Among, them markers such as HvSSR2-12 on chromosome 2; RM410, RM242 and RM553 on

chromosome 9; RM277 and HvSSR12-51 on chromosome 12 were significance at the 1% level.

4.14 The QTL mapping through Composite Interval Mapping for leaf blast

The genotypic data and phenotypic data of field condition of blast disease were analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2 softwares. The QTL analysis results are presented in (Table 4.11). The presence of a putative QTL was declared if the LOD threshold was larger than 3 for the trait using QTL cartographer 2.5 while 2.5 using QTL IciMapping 3.2.

A single QTL was identified for Blast resistance using RILs population by QTL cartographer 2.5 while no QTLs were identified using software QTL IciMapping 3.2. The locus was associated with LOD score above the threshold value determines by permutation test for the trait of the experiment. The resistance loci mapped to only 1 chromosome out of 12 rice chromosomes. The QTL was found to be present on chromosome 10. The LOD score for QTL found 3.65, mapping with QTL cartographer 2.5. The QTL along with their LOD score and R^2 value worked out through composite interval mapping (Table 4.11).

Table 4.11 QTLs underlying Blast resistance mapped by QTL cartographer 2.5

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Additive	R^2%
Blast reaction	*qLB10.1*	10	9.5	HvSSR 10-17	HvSSR 10-34	3.65	-0.9116	16.57

4.15 The explanation of loci associated with quantitative resistance for leaf Blast

The only major effect significant QTL, *qLB-10.1* on chromosome 10 was identified in the interval markers HvSSR10-17 and HvSSR10-34 (Figure 4.18). The LOD score of the QTL was 3.65 and explained 16.57% of the phenotypic

variation in QTL cartographer 2.5 under heavy infection condition. The QTL showed additive effect of value -0.91. The negative value of the additive effect showed that allele was inherited from susceptible parent Danteshwari.

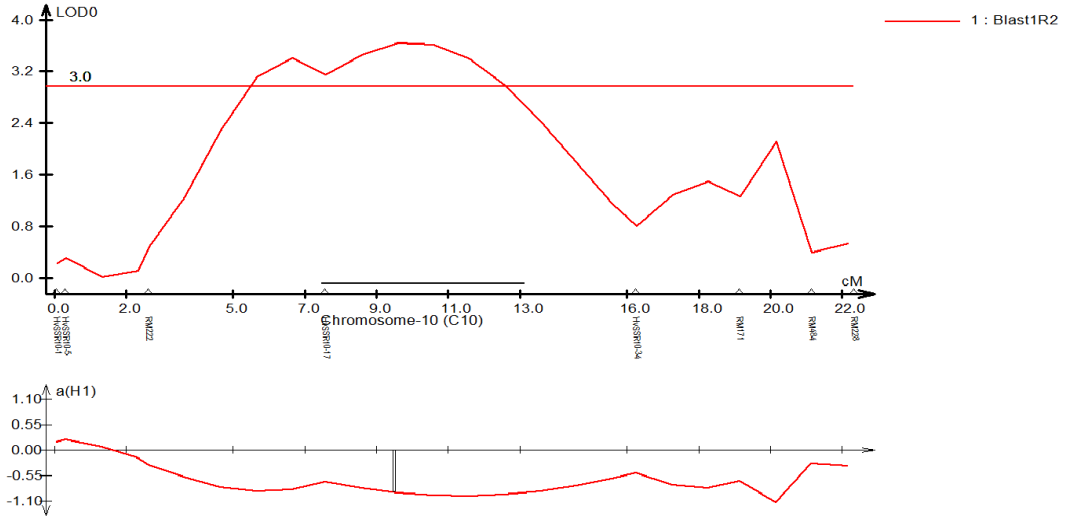


Figure 4.18 The QTL mapped for the Blast resistance trait identified on rice chromosome 10 using CIM in experiment. The bar indicates the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance.

4.16 The distribution of tolerance for the trait Brown spot in RIL population

The parents along with RILs exhibited marked variation for the reaction to Brown spot. The parent Danteshwari showed slightly resistance on the basis of score for Brown spot comparatively Dagad deshi. The frequency distribution indicated that nearly normal distribution was followed for the trait Brown spot tolerance (Fig. 4.19). The resistance segregation in the experiment varied. The RILs exhibited abnormal segregation, which indicated that neither parent contained all the alleles for resistance or susceptibility. Maximum number of RIL lines in the experiment showed Brown spot tolerance scores of (0-9 scale of SES; IRRI, 2002) from 2 to 8. The Danteshwari × Dagad deshi derived 122 F_{14} generations RIL population was screened for Brown spot tolerance. The natural occurrence of disease was accounted for score shown in (Fig.4.20). Among 122 lines, many lines

were shown tolerance toward Brown spot. No line showed very high resistance for Brown spot tolerance.

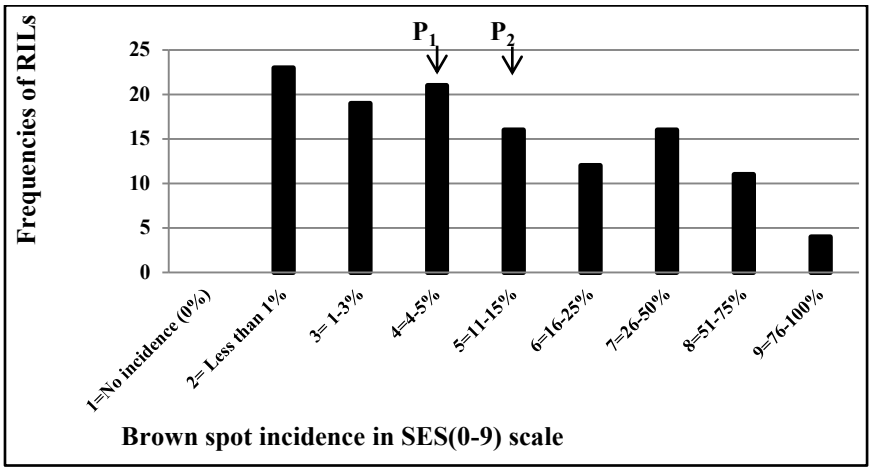
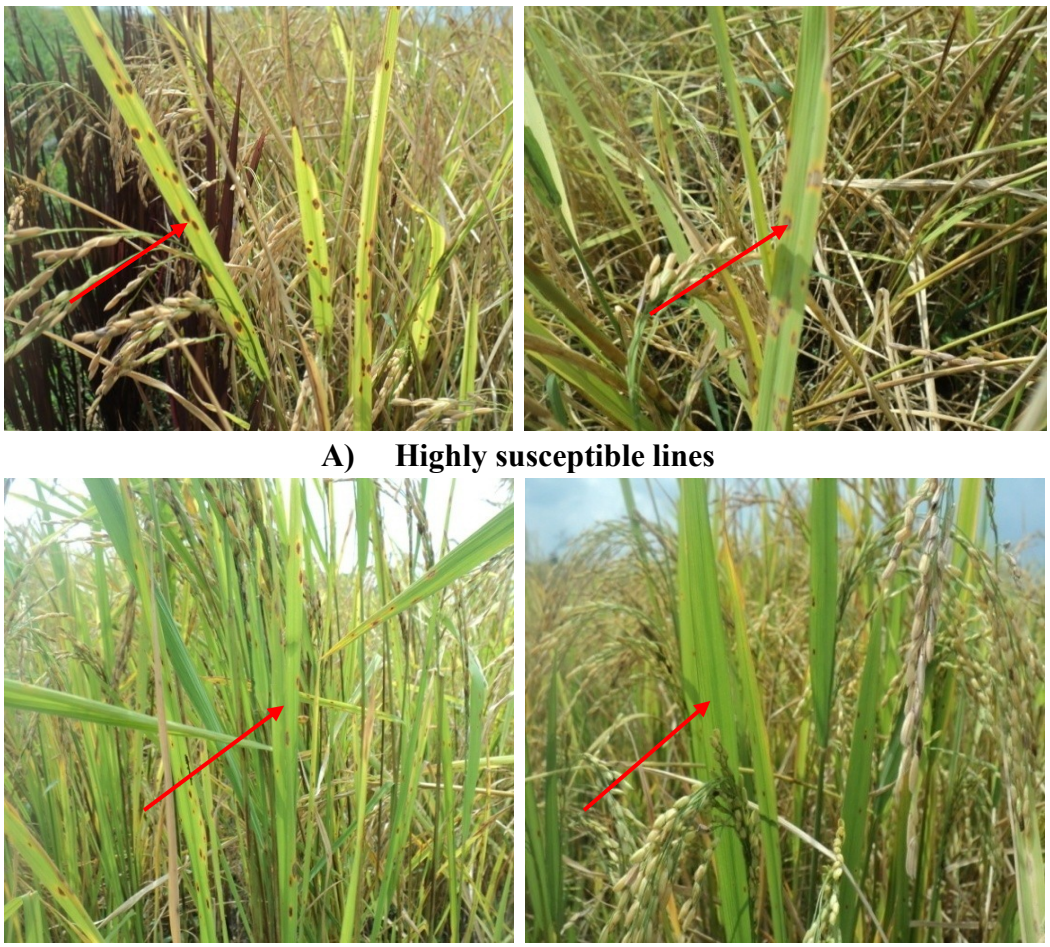


Figure 4.19 The frequency distribution of disease Brown spot resistance across 122 RIL lines in the experiment



A) Highly susceptible lines

B) Succeptable line C) Resistant line

Figure 4.20 The natural occurrence of disease Brown spot on RIL population

4.17 The Single Marker Analysis for Brown spot tolerance

The single marker analysis tests the association between marker genotypes and trait values using F-tests in QTL cartographer 2.5. The markers significance at the 5%, 1%, 0.1% and 0.01% levels respectively. There were 35 putative markers were indicated to be associated with the brown spot tolerance. The highly associated markers for Brown spot tolerance was HvSSR1-55 on chromosome 1 significance at 0.1% level. The other markers significant at 1% level (Table 4.12).

Table 4.12 Markers that were highly associated with traits for Brown spot tolerance

Chr.	Markers significance at 1% level (**)
1	HvSSR1-87
2	HvSSR2-78
3	RM232, HvSSR3-40, HvSSR3-41
7	RM125
11	RM254
12	RM260, RM519, HvSSR12-35, RM28305, RM17

4.18 The QTL mapping through Composite Interval Mapping for Brown spot tolerance

The genotypic data and phenotypic data of field condition of Brown spot disease were analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2 softwares. The presence of a putative QTL was declared if the LOD threshold was larger than 3 for the trait Brown spot using QTL cartographer 2.5 while 2.5 using QTL IciMapping 3.2.

One QTL was identified for Brown spot tolerance using QTL cartographer 2.5 while two QTLs were identified using QTL IciMapping 3.2 under heavy infection condition. These loci were associated with LOD scores above the threshold value determine by permutation test for the Brown spot tolerance trait of the experiment. QTLs for brown spot were found to be present on chromosome 1, 11 and 12. The LOD score 3.17 of QTL for mapping with QTL cartographer 2.5

and found range 2.71-3.14 using QTL IciMapping 3.2. Two resistant alleles identified from parent Danteshwari. The QTLs along with their LOD score and R^2 value worked out through composite interval mapping are shown in (Table 4.13 and 4.14).

Table 4.13 QTLs underlying Brown spot tolerance mapped by QTL Cartographer 2.5

Trait	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Additive	R^2 %
Brown spot tolerance	*qBS12*	12	16.40	RM20	RM511	3.17	0.5024	3.54

Table 4.14 QTLs underlying Brown spot tolerance mapped by QTL IciMapping 3.2

Trait Name	Putative QTL	Chr.	Position	Left marker	Right marker	LOD	Add	PVE (%)
Brown spot tolerance	*qBS1*	1	21.38	HvSSR1-55	RM-5	3.149	-0.8198	14.3593
	qBS11	11	25.05	RM254	RM224	2.718	-0.7860	13.9974

4.19 The description of loci associated with quantitative resistance for Brown spot disease tolerance

A minor effect significant QTL, *qBS12* locus was identified on chromosome 12 between markers RM20 and RM511, a QTL peak on marker RM511 with LOD value 3.17 and explained very low 3.54% of phenotypic variance (Figure 4.21). It had relatively small effect for disease resistance in experiment. The positive value (0.5024) of the additive effect showed that allele transferred from resistance parent Danteshwari. This means that the allele from the resistance parent Danteshwari acted to decrease the measured trait (i.e. disease reaction) or to decrease brown spot susceptibility.

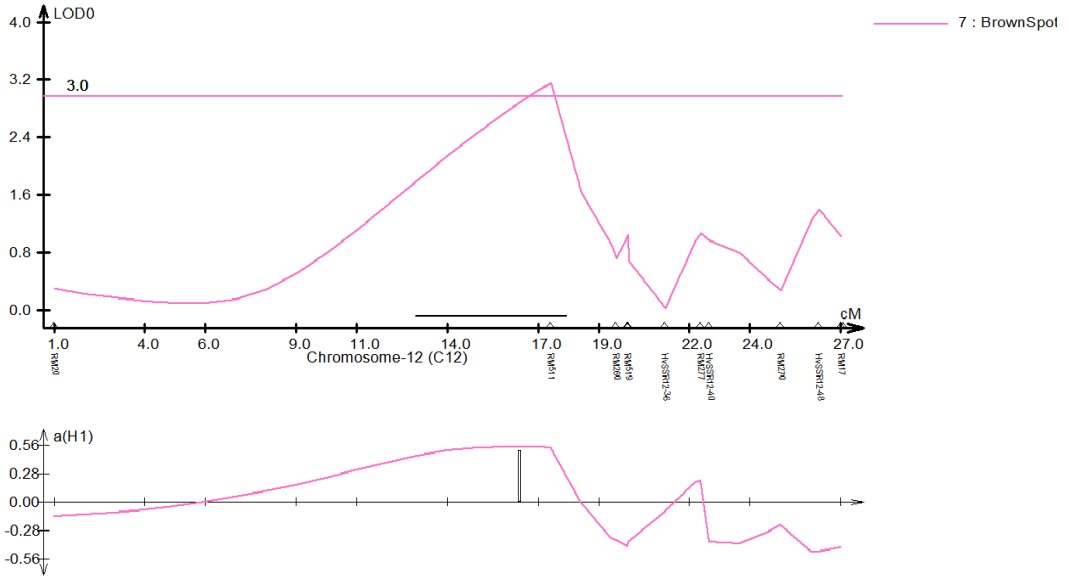


Figure 4.21 The QTL mapped for the Brown spot resistance trait identified on rice chromosome 12 using CIM in experiment. The small graphic (bottom) show the additive effect where (+) values = allele from resistance parent Dantehwari and (-) values = allele from susceptible parent Dagad deshi.

One significant QTL, *qBS1* was identified on chromosome 1 between markers HvSSR1-55 and RM5 with LOD score of 3.14 and explained 14.35% of PVE in QTL IciMapping 3.2 under heavy infection condition. This can be considered as a major effect QTL related to brown spot which significantly affect Brown spot resistance. The negative value of the additive effect showed that allele transferred from susceptible parent Dagad deshi. Similarly, the QTL, *qBS11* was mapped on chromosome 11 between markers RM254 and RM224, with LOD value 2.71 and explained 13.99% of phenotypic variance. The negative value of the additive effect showed that allele from susceptible parent Dagad deshi.

4.20 The diseases resistance in integrated form for all four diseases

Among all 122 F_{14} RIL lines used for screening of four diseases, the line number “71” and “77” found resistance for all four diseases e.g. Bacterial leaf

blight, Sheath blight, leaf Blast and Brown spot tolerance. The categorization of resistance toward various diseases given below (Table 4.15).

Table 4.15 Categories of lines for different diseases resistance

1	Lines with resistance against one disease
	Blast:- line #7, 9, 29, 39, 42, 45, 48, 60 BLB:- line #2, 16, 31, 53, 62, 83, 99, 107, 109, 115, 117, 120 Brown spot: - line # 12, 15, 61, 81, 85, 86, 114 Sheath:- line #8, 10, 20, 23, 24, 26, 27, 30, 51, 55, 59, 72, 76, 79, 84, 87, 91, 92, 95, 96, 97, 98, 100, 101, 104, 105, 112, 113
2	Lines with resistance against two diseases
	BLB & Brown Spot:- line #34, 35, 37, 40, 82 Brown Spot & Sheath blight: - line #93 BLB & Brown Spot:- line #P_1 (Parent Danteshwari) Blast & Sheath blight: - line #11, 14, 22, 25, 47, 74, 75, 118, P_2 (Dagad deshi) Blast & Brown Spot: - line #21, 65, 68
3	Lines with resistance against three diseases
	Blast, Brown Spot & Sheath blight: - line #46 Blast, BLB & Brown Spot: - line #70, 88 Blast, BLB & Sheath blight: - line #18, 73, 78 BLB, Brown Spot & Sheath blight: - line # 28, 94
4	Lines with resistance four diseases
	Blast, BLB, Brown Spot & Sheath blight: - line #71, 77

4.21 The region of chromosomes with multiple diseases resistance

There were region of chromosomes 1, 9 and 12 which showed multiple disease resistance in this experiment. The region of chromosome 1 between markers RM499 to RM1 found common for both diseases BLB and Sheath blight resistance (Fig. 4.22.1). Similarly, the region of chromosome 12 between markers RM20 and RM511 found common for both the diseases *i.e.* Brown spot tolerance and Bacterial leaf blight resistance. Another region of this chromosome between

markers RM511 and RM260 coincided for BLB and Sheath blight. The QTLs of both diseases overlapped in this region shown in (Fig. 4.22.14). In this study also found a region on chromosome 9 between markers HvSSR9-7 and RM444 common for Sheath blight and BLB resistance (Fig. 4.22.10).

4.22 Development of High Resolution Molecular Marker Map *in silico* of QTLs identified associated with tolerance to different rice diseases (BLB, leaf blast, Sheath blight, Brown spot)

The ability to quickly and reliably select desirable material and to eliminate individuals that contain deleterious alleles is critical to the success of a plant breeding program. The use of genetic markers to facilitate the identification of favorable (or deleterious) alleles in a collection of diverse genotypes is referred to as marker assisted selection (MAS) (Dubcovsky, 2004). When markers are closely linked to a trait of interest, they can be used to indirectly select for the trait, saving time, money and labour. Additional advantages to MAS include the ability to select for multiple alleles underlying a polygenic trait, the ability to select for traits that are difficult or very costly to score phenotypically, are expressed late in the life of the plant or that require progeny testing due to their recessive nature or a lack of heritability (Koebner and Summers, 2003). Indirect selection can be inefficient if recombination occurs between the gene/trait of interest and the marker, if the marker must first be mapped in a new population or if additional unwanted alleles show linkage to the marker in particular germplasm accessions (Varshney *et al*., 2005). In general, if a genetic marker is separated from the target gene by more than 1–2 cM, recombination will occur at unacceptable frequencies. Genes can be phenotypically selected in backcross breeding programs, combining several genes at once requires screening with the appropriate bacterial strains to differentiate the overlapping phenotypic effects of these genes (Mew *et al*., 1993). Some of the
116 BLB resistance genes, including *xa-5*, *xa-8*, *xa-9* and *xa-13*, are recessive and progeny testing is required to detect them in the heterozygous state. QTLs

associated with tolerance to different rice diseases **(**BLB, leaf blast, Sheath blight, Brown spot**)** were mapped to different genomic location between molecular marker intervals (**CH #1**:- RM499 to RM1, HvSSR 1-55 to RM5, RM3825 to HvSSR1-87, HvSSR1-89 to RM14; **CH #2**:- RM174 to RM492; **CH #3**:- RM517 to RM232, HvSSR 3-40 to RM411, HvSSR 3-56 to HvSSR 3-71; **CH #4**:- HvSSR4-26 to RM564, HvSSR4-38 to HvSSR4-39, HvSSR4-35 to HvSSR4-38; **CH #5:-** HvSSR 5-23 to HvSSR5-31, HvSSR5-39 to HvSSR5-48; **CH #6:-** HvSSR6-44 to HvSSR6-56; **CH #9:-** HvSSR9-7 to RM444, RM410 to RM288; **CH #10**:- HvSSR10-17 to HvSSR10-34, HvSSR10-34 to RM171; **CH # 11**:- RM254 to RM224; **CH #12**:- RM20 to RM511, RM511 to RM260, RM 28305 to HvSSR 12-36).

By way of these co-mapped markers, the map in this study is tied to the physical and sequence map developed by the International Rice Genome Sequencing Project (http://rgp. dna.affrc. go.jp/; http:// www. usricegenome. org/; http://genome.arizona.edu/fpc/rice/; http://www.gramene.org/) and the principal mapping populations used by the rice scientific community. Identification of map position was accomplished by identifying physical positions on the rice chromosome that simultaneously contained a hit from the two molecular markers flanking identified QTLs and therefore formed the basis for development of high resolution molecular marker map *in silico* of genomic location encompassing QTLs associated with tolerance to different diseases of rice (Fig.4.22.1-14). Following blast analysis and simultaneously that contained a hit with the identified molecular markers intervals (flanking molecular marker of the QTL) physical positions on the rice chromosome for the molecular marker was assigned. Physical region between the anchored molecular markers were searched for recently released IRGSP 1.0 SSR markers that generated a high resolution physical map of the region.

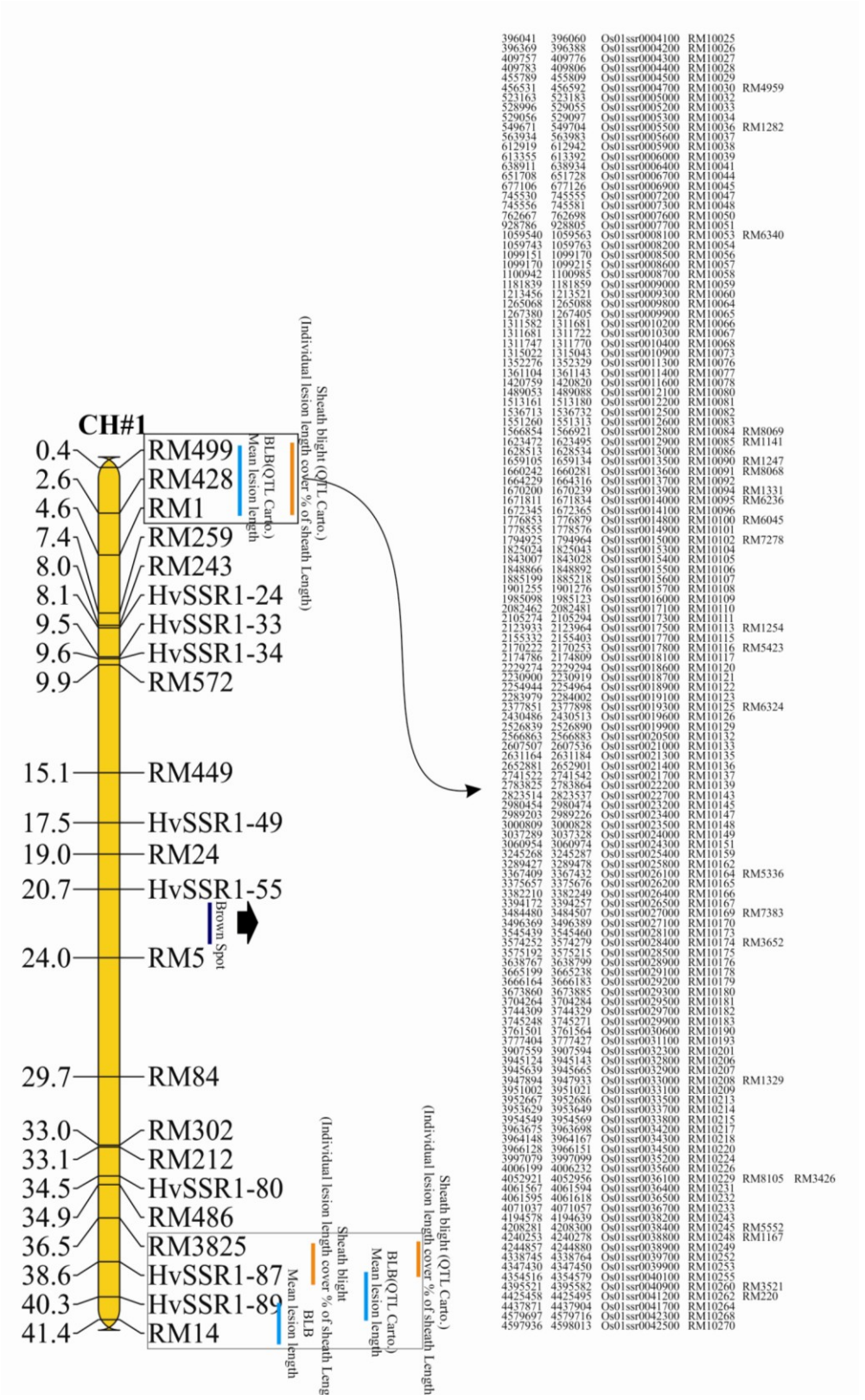


Fig. 4.22.1 Genetic locations and *in silico* analysis of QTLs for BLB trait mean lesion length and Sheath blight trait individual lesion length cover % of Sheath length on chromosome 1

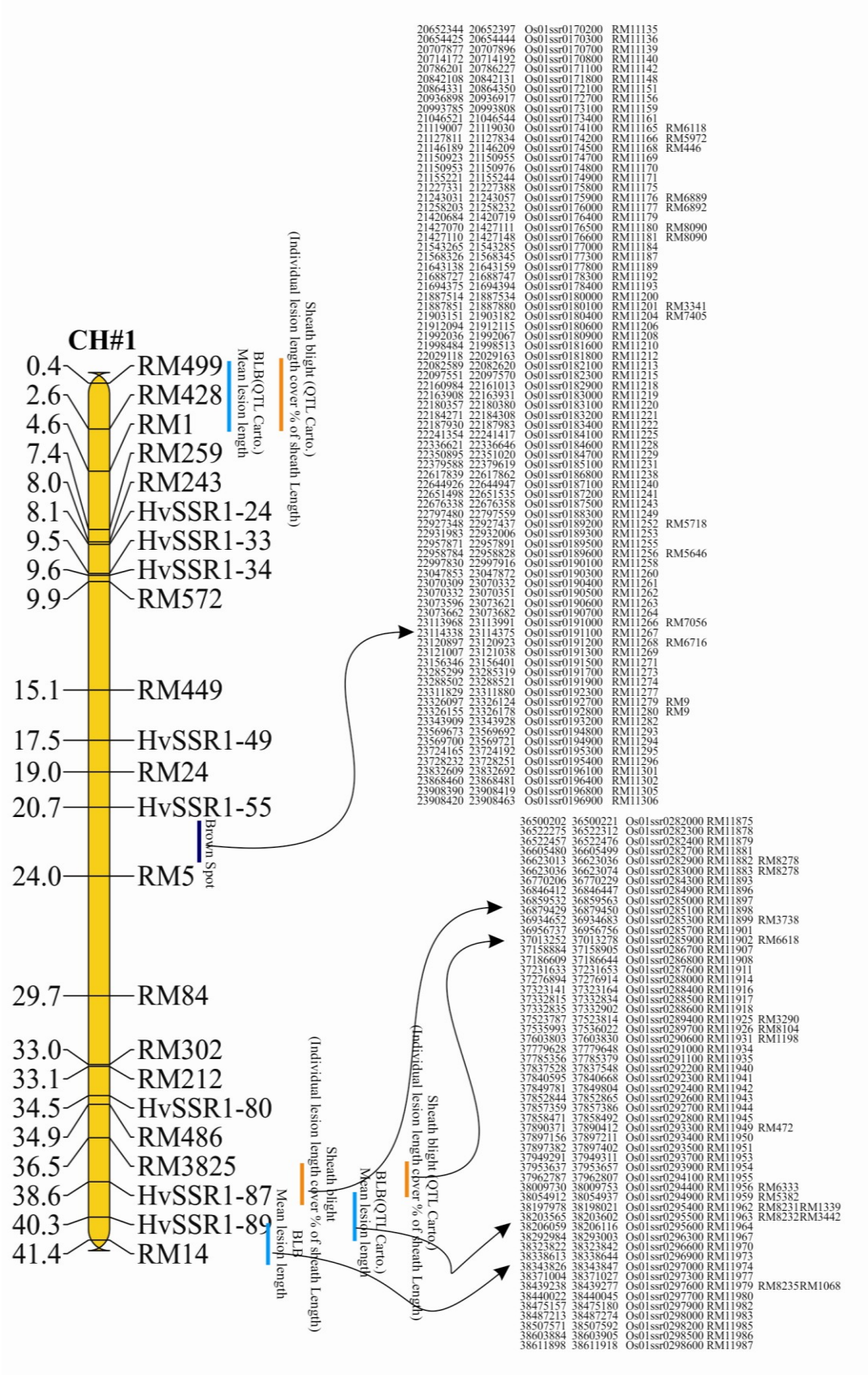


Fig. 4.22.2 Genetic locations of QTLs for Brown spot tolerance, BLB trait mean lesion length and Sheath blight trait individual lesion length cover % of Sheath length on chromosome 1 and *in silico* analysis

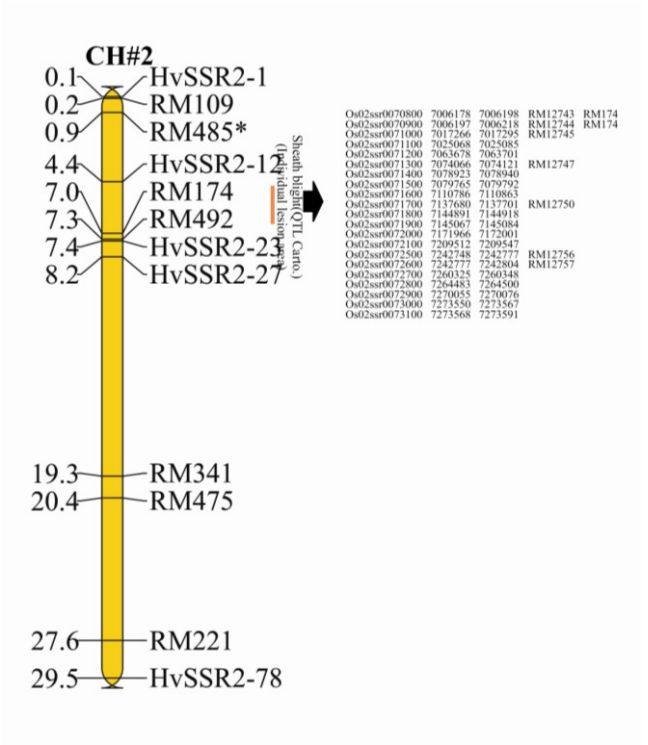


Figure 4.22.3 Genetic location and *in silico* analysis of Sheath blight QTL for trait individual lesion area on chromosome 2

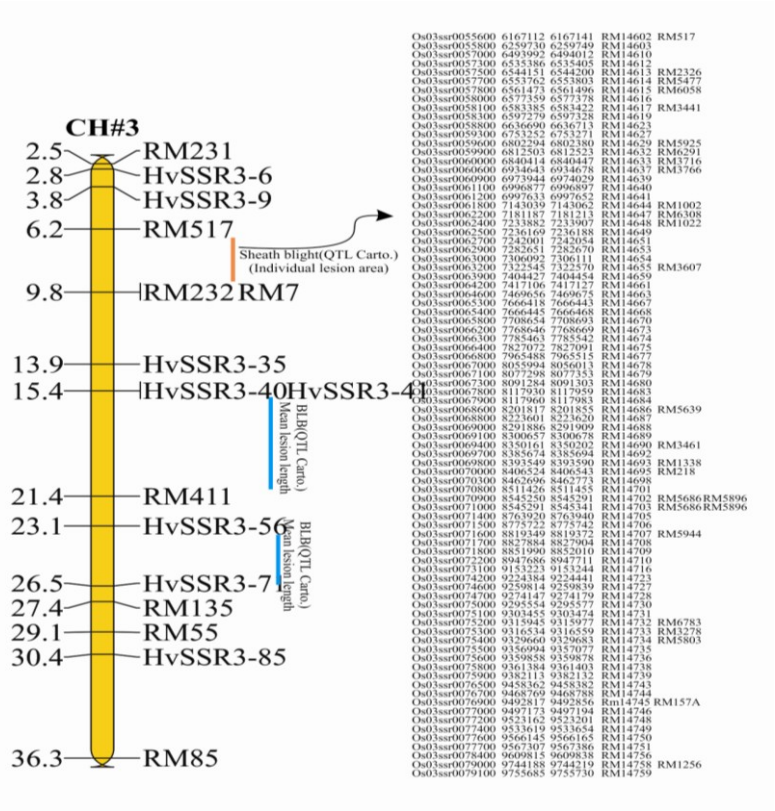


Figure 4.22.4 Genetic locations and *in silico* analysis of Sheath blight QTL for individual lesion area on chromosome 3

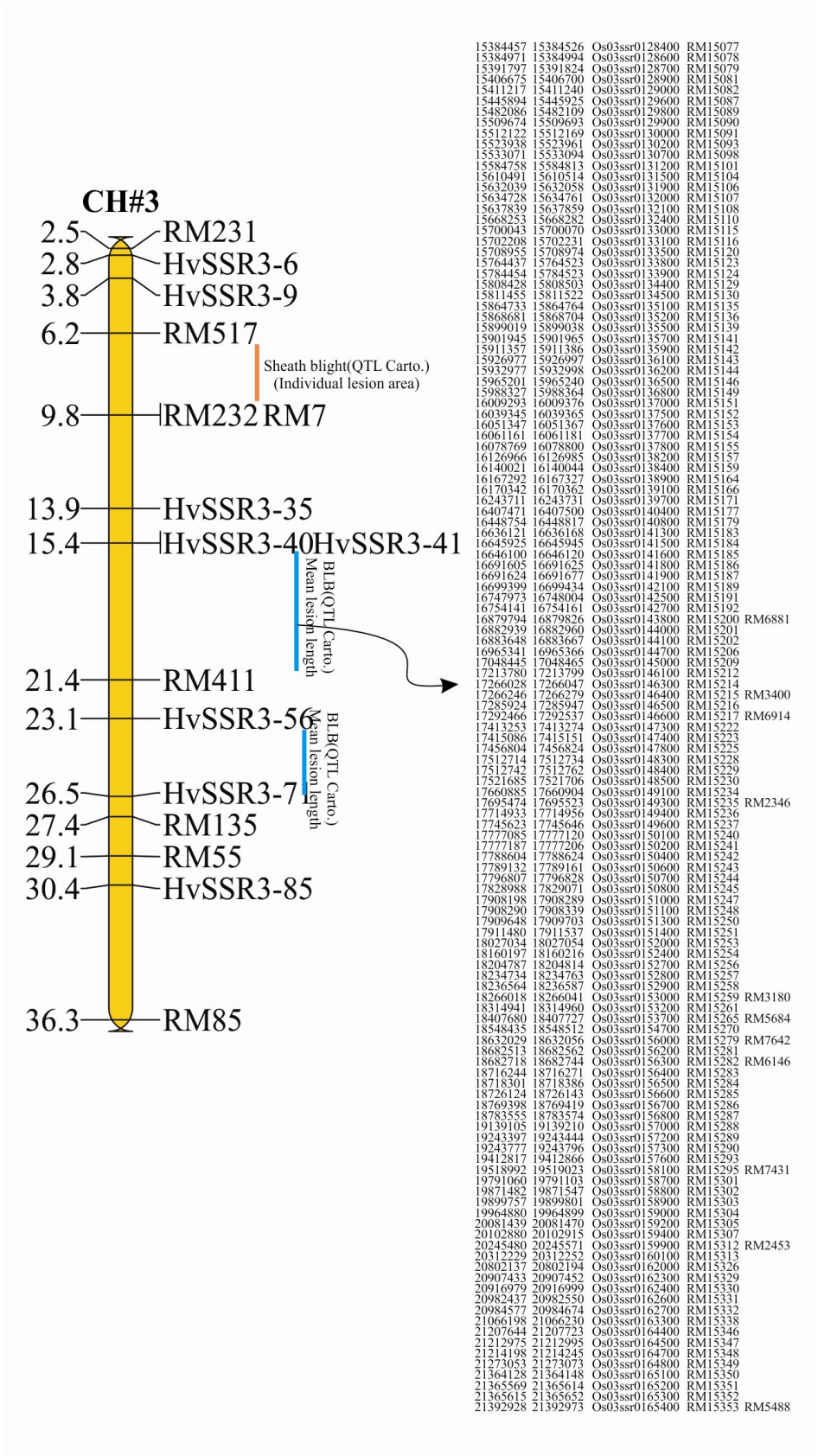


Figure 4.22.5 Genetic location and *in silico* analysis of QTLs for BLB trait mean lesion length on chromosome 3

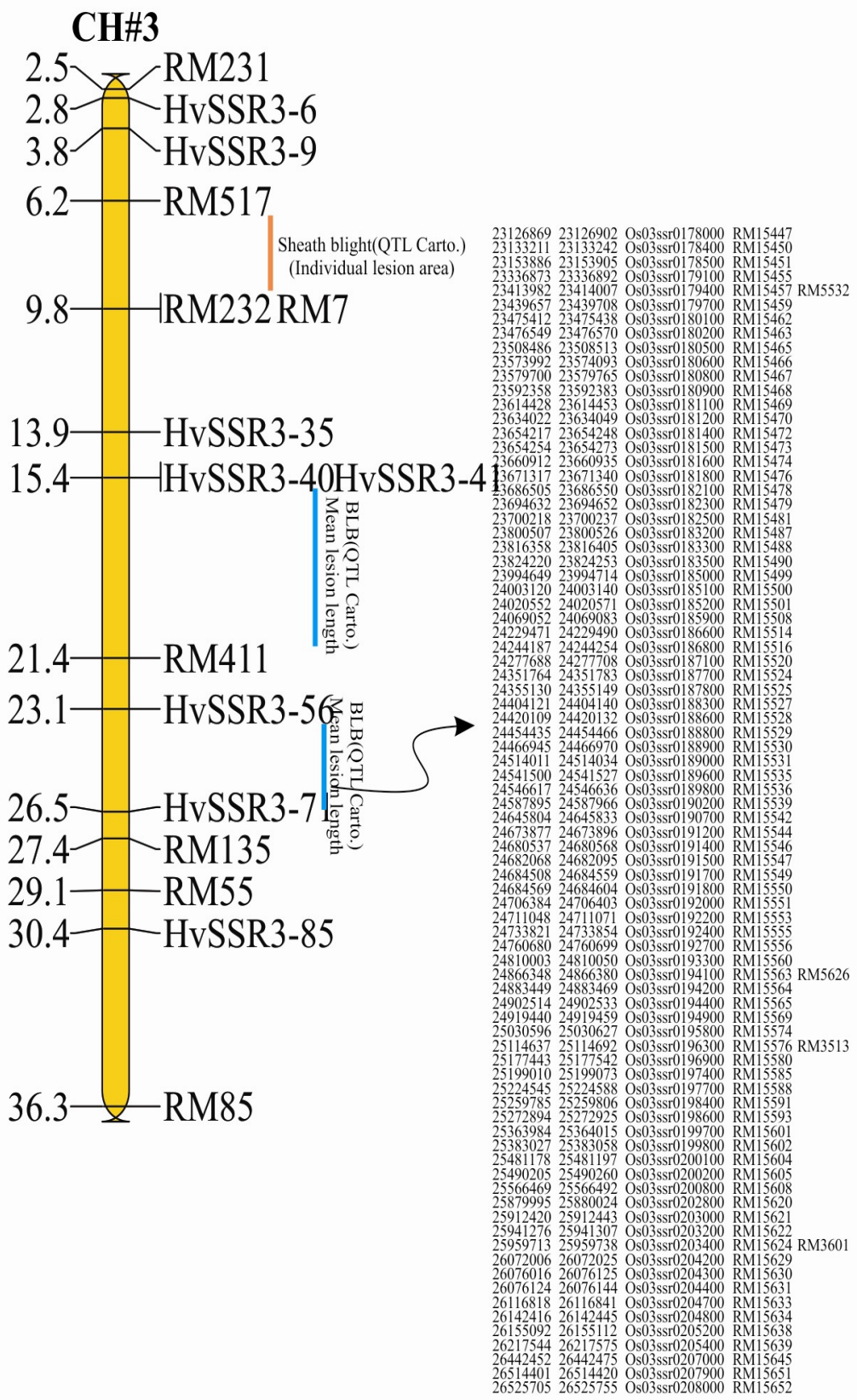
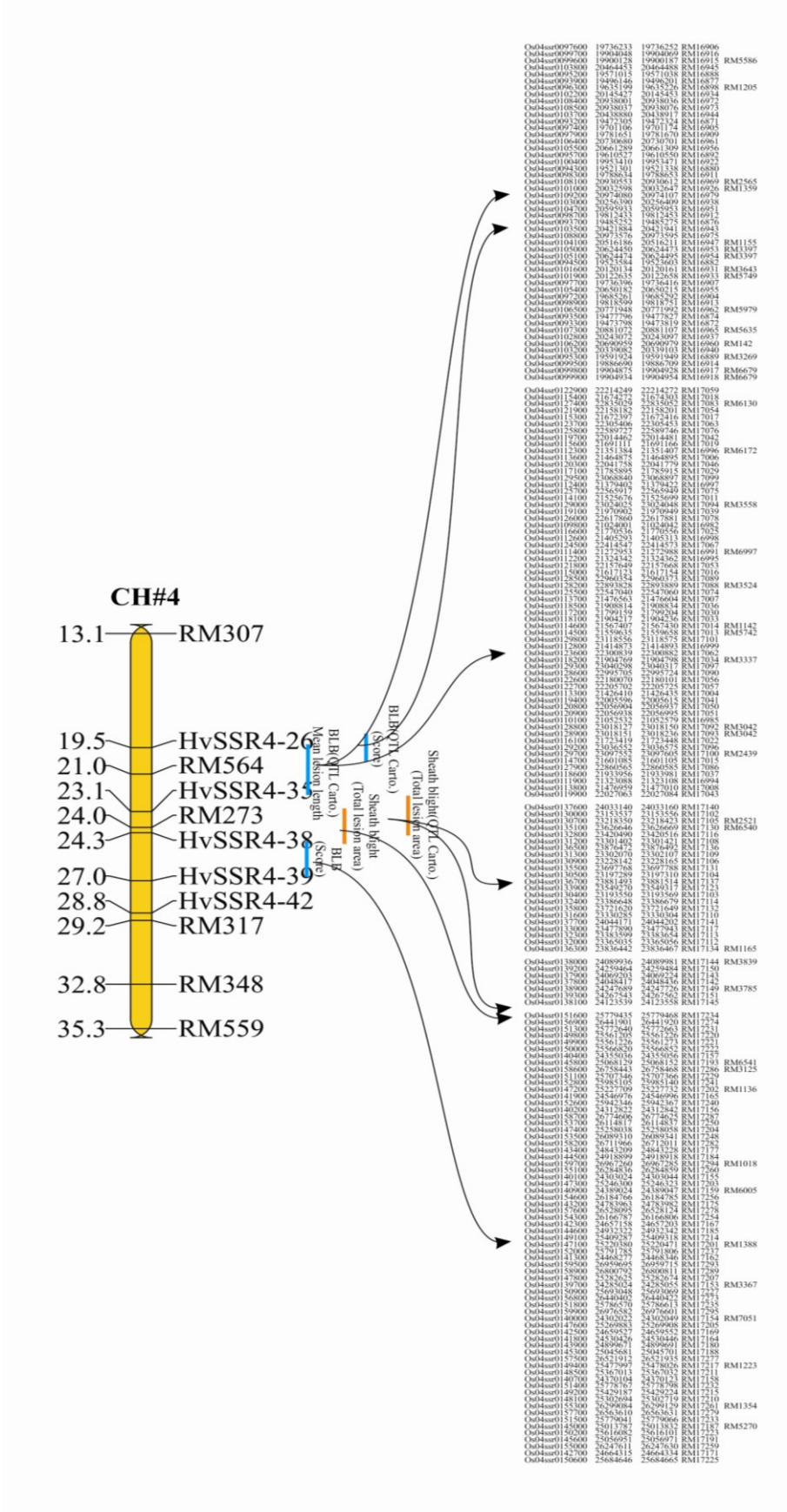


Figure 4.22.6 Genetic location and *in silico* analysis of QTL for BLB trait mean lesion length on chromosome 3



123 **Figure 4.22.7 Genetic locations of QTLs for sheath blight trait total lesion area and BLB trait mean lesion length, reaction qualitative (0-9) scale on chromosome 4 and *in silico* analysis**

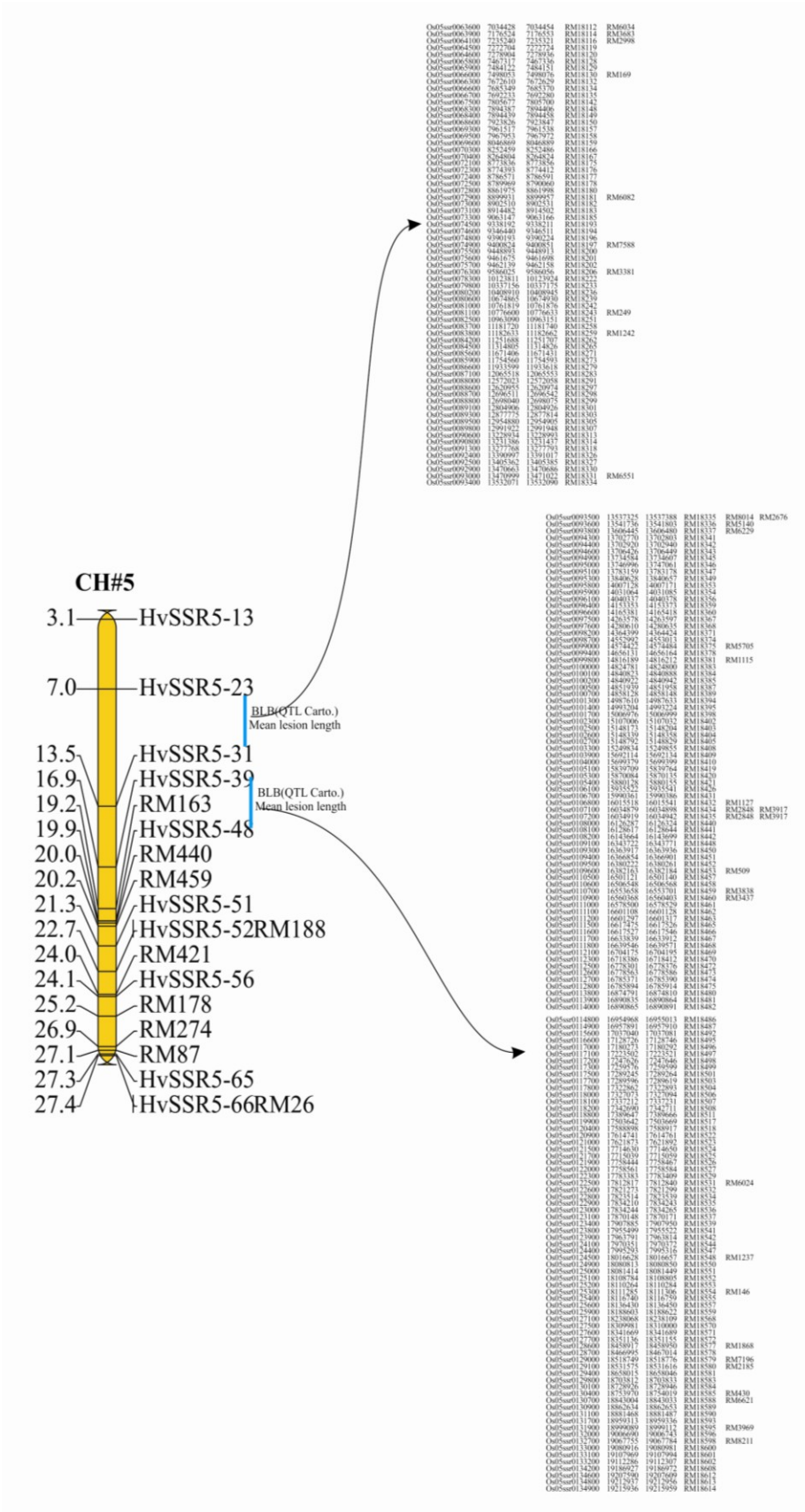


Figure 4.22.8 Genetic locations and *in silico* analysis of QTLs for BLB trait mean lesion length on chromosome 5

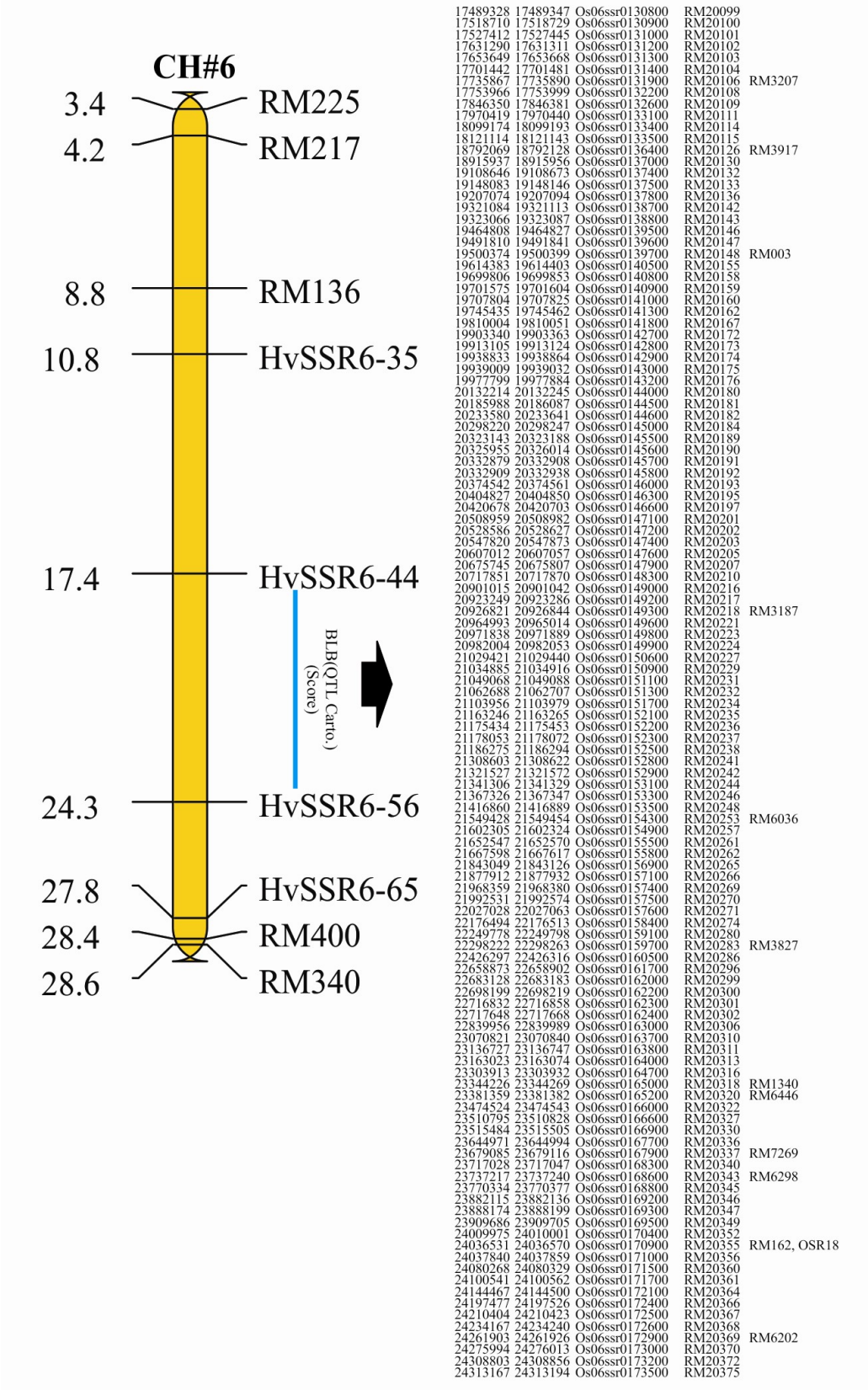


Fig. 4.22.9 Genetic location and *in silico* analysis of QTL for BLB reaction based on qualitative (0-9) score on chromosome 6

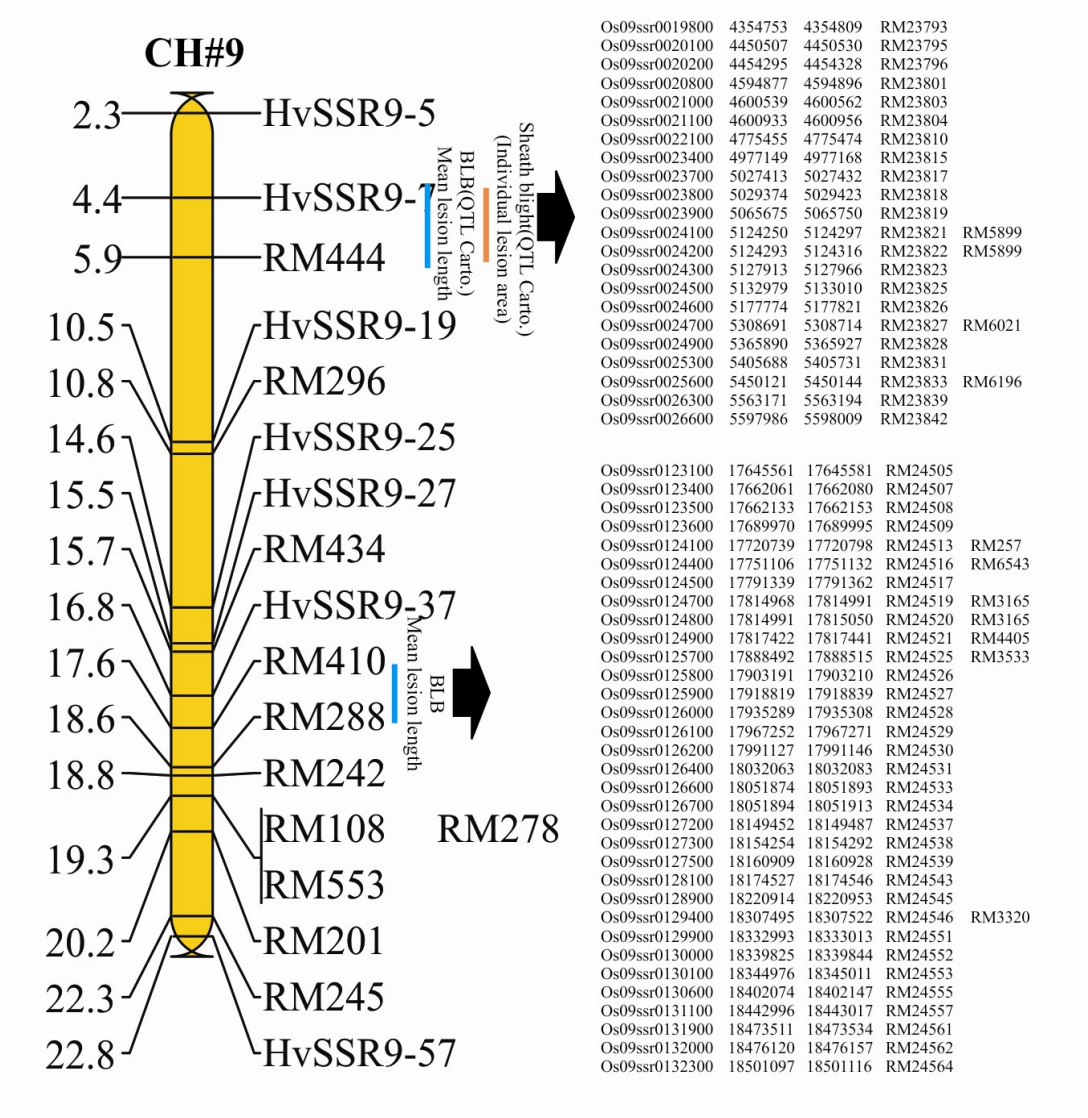


Fig. 4.22.10 Genetic location and *in silico* analysis of QTLs for BLB trait mean lesion length and sheath blight trait individual lesion area on rice chromosome 9

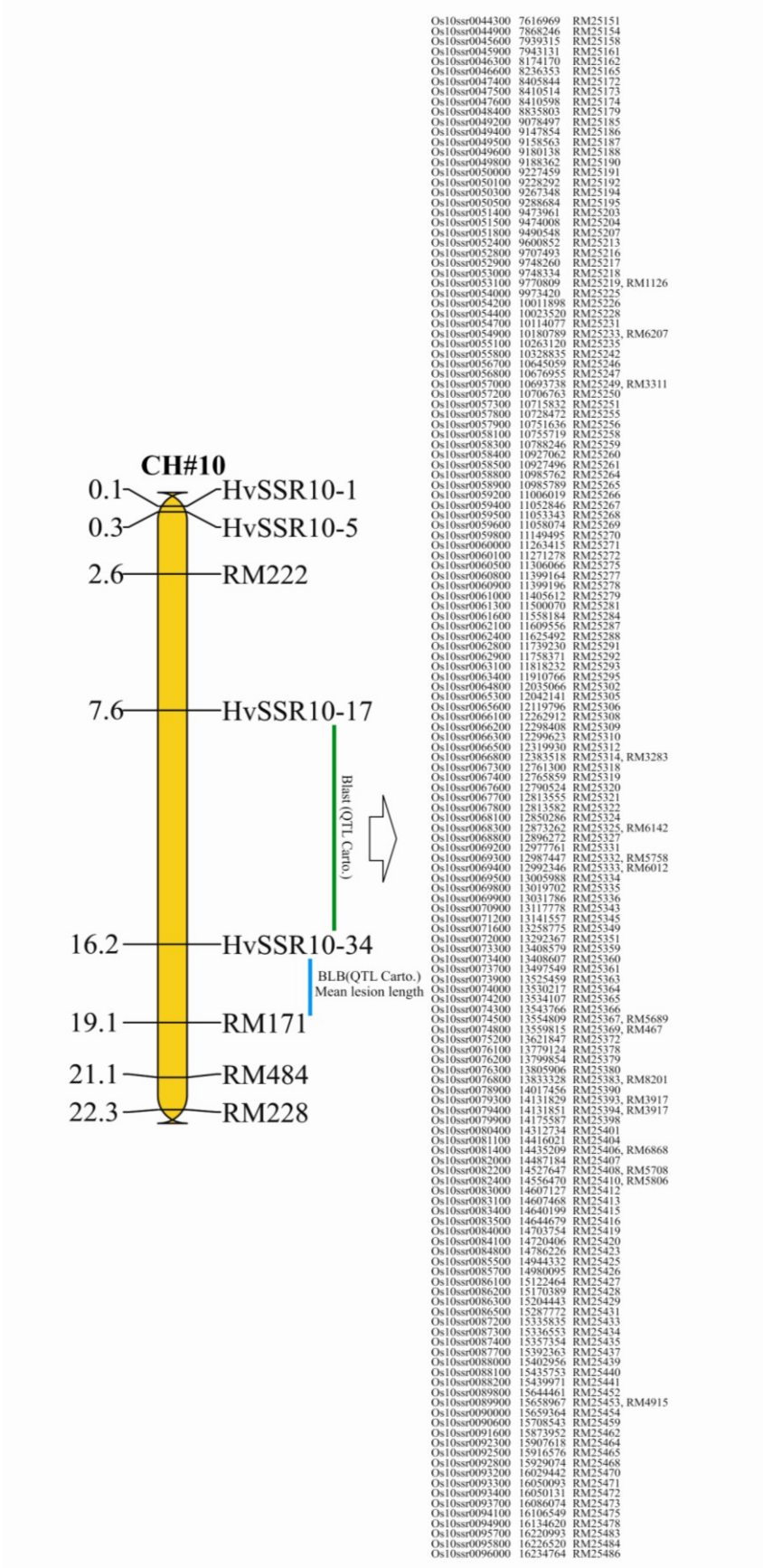
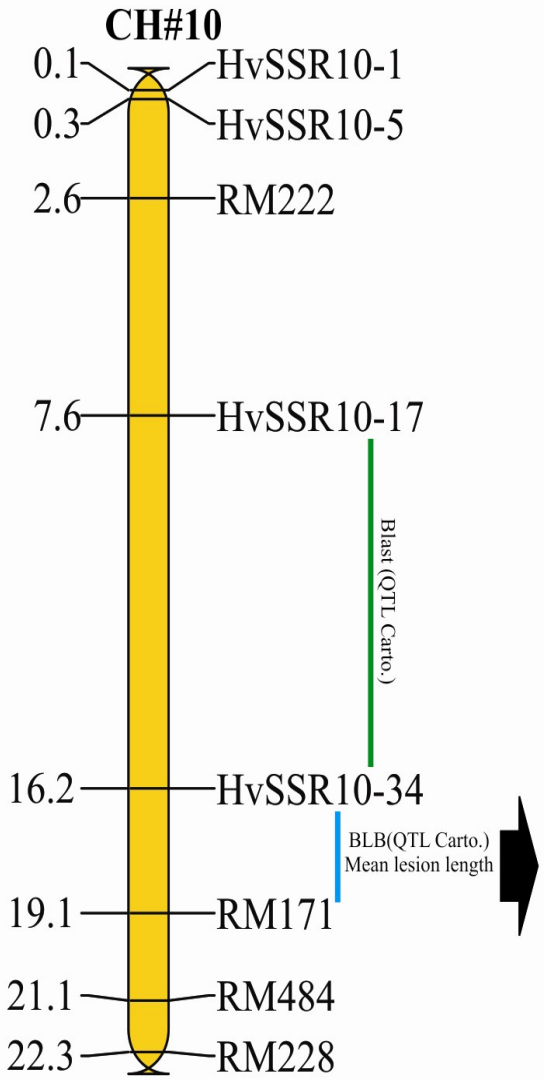


Fig.4.22.11 Genetic location of QTL for Blast reaction on rice chromosome 10 and *in silico* analysis



Os10ssr0096000	16234764	16234817	RM25486	
Os10ssr0096200	16281708	16281789	RM25487	
Os10ssr0096300	16281788	16281807	RM25488	
Os10ssr0096800	16352749	16352768	RM25491	
Os10ssr0097900	16501142	16501162	RM25499	
Os10ssr0098300	16535468	16535487	RM25501	
Os10ssr0099100	16641878	16641903	RM25507	
Os10ssr0099900	16716252	16716313	RM25512	RM1375
Os10ssr0100000	16738094	16738117	RM25513	
Os10ssr0100500	16805288	16805314	RM25516	RM6477
Os10ssr0100700	16863287	16863307	RM25517	
Os10ssr0100900	16870914	16870997	RM25519	
Os10ssr0101400	16938519	16938542	RM25522	
Os10ssr0101900	17026987	17027010	RM25527	
Os10ssr0102700	17081628	17081647	RM25533	
Os10ssr0102800	17081648	17081671	RM25534	
Os10ssr0103600	17185791	17185811	RM25538	
Os10ssr0103800	17217004	17217073	RM25539	
Os10ssr0104500	17270456	17270476	RM25540	
Os10ssr0104700	17357651	17357692	RM25541	RM3470
Os10ssr0104900	17374975	17375002	RM25542	RM5392
Os10ssr0105000	17380882	17380923	RM25543	
Os10ssr0105200	17474893	17474922	RM25545	RM5620
Os10ssr0105900	17565857	17565878	RM25550	
Os10ssr0106700	17630636	17630677	RM25557	
Os10ssr0106800	17631878	17631925	RM25558	
Os10ssr0107600	17752042	17752062	RM25564	
Os10ssr0107700	17773552	17773571	RM25565	
Os10ssr0108100	17794333	17794362	RM25567	
Os10ssr0108900	17838568	17838589	RM25574	
Os10ssr0109100	17842785	17842808	RM25576	RM1125
Os10ssr0110000	17918834	17918867	RM25581	RM5274
Os10ssr0110200	17922062	17922095	RM25582	
Os10ssr0110300	17922538	17922557	RM25583	
Os10ssr0110500	17925867	17925887	RM25585	
Os10ssr0110600	17926106	17926138	RM25586	
Os10ssr0110700	17941157	17941232	RM25587	
Os10ssr0110800	17941419	17941438	RM25588	
Os10ssr0111400	17980165	17980185	RM25591	
Os10ssr0111600	17998446	17998483	RM25592	RM1937
Os10ssr0111700	18001222	18001245	RM25593	RM5756
Os10ssr0111900	18005569	18005631	RM25594	RM6704
Os10ssr0112400	18032713	18032733	RM25598	
Os10ssr0112500	18032749	18032768	RM25599	
Os10ssr0112700	18085558	18085579	RM25600	RM258
Os10ssr0112800	18085634	18085654	RM25601	
Os10ssr0113100	18107438	18107457	RM25602	
Os10ssr0113200	18108735	18108755	RM25603	
Os10ssr0113300	18113675	18113694	RM25604	
Os10ssr0113400	18129331	18129354	RM25605	
Os10ssr0113700	18139044	18139083	RM25607	
Os10ssr0114100	18228026	18228046	RM25609	
Os10ssr0114400	18262976	18263003	RM25611	
Os10ssr0114500	18270172	18270195	RM25612	
Os10ssr0114600	18271237	18271286	RM25613	
Os10ssr0115100	18376038	18376064	RM25614	RM6150
Os10ssr0115200	18377781	18377801	RM25615	
Os10ssr0115400	18393370	18393390	RM25616	
Os10ssr0115600	18404283	18404303	RM25617	
Os10ssr0117400	18572351	18572419	RM25626	
Os10ssr0117900	18635301	18635370	RM25628	
Os10ssr0118200	18667582	18667603	RM25631	
Os10ssr0118700	18698788	18698814	RM25635	RM6474
Os10ssr0119100	18727248	18727271	RM25639	
Os10ssr0119400	18745638	18745670	RM25640	RM5629
Os10ssr0119500	18783568	18783630	RM25641	RM6737
Os10ssr0120000	18798625	18798650	RM25645	RM5373
Os10ssr0120200	18809916	18809937	RM25646	
Os10ssr0120400	18848497	18848517	RM25648	
Os10ssr0120600	18857250	18857273	RM25649	RM6132
Os10ssr0121000	18874730	18874750	RM25650	
Os10ssr0121100	18877064	18877085	RM25651	
Os10ssr0121700	18907154	18907177	RM25656	RM6469
Os10ssr0121800	18917202	18917234	RM25657	
Os10ssr0121900	18959447	18959468	RM25658	
Os10ssr0122200	18981519	18981539	RM25659	

Figure 4.22.12 Genetic location and *in silico* analysis of QTL for BLB trait mean lesion length on chromosome 10

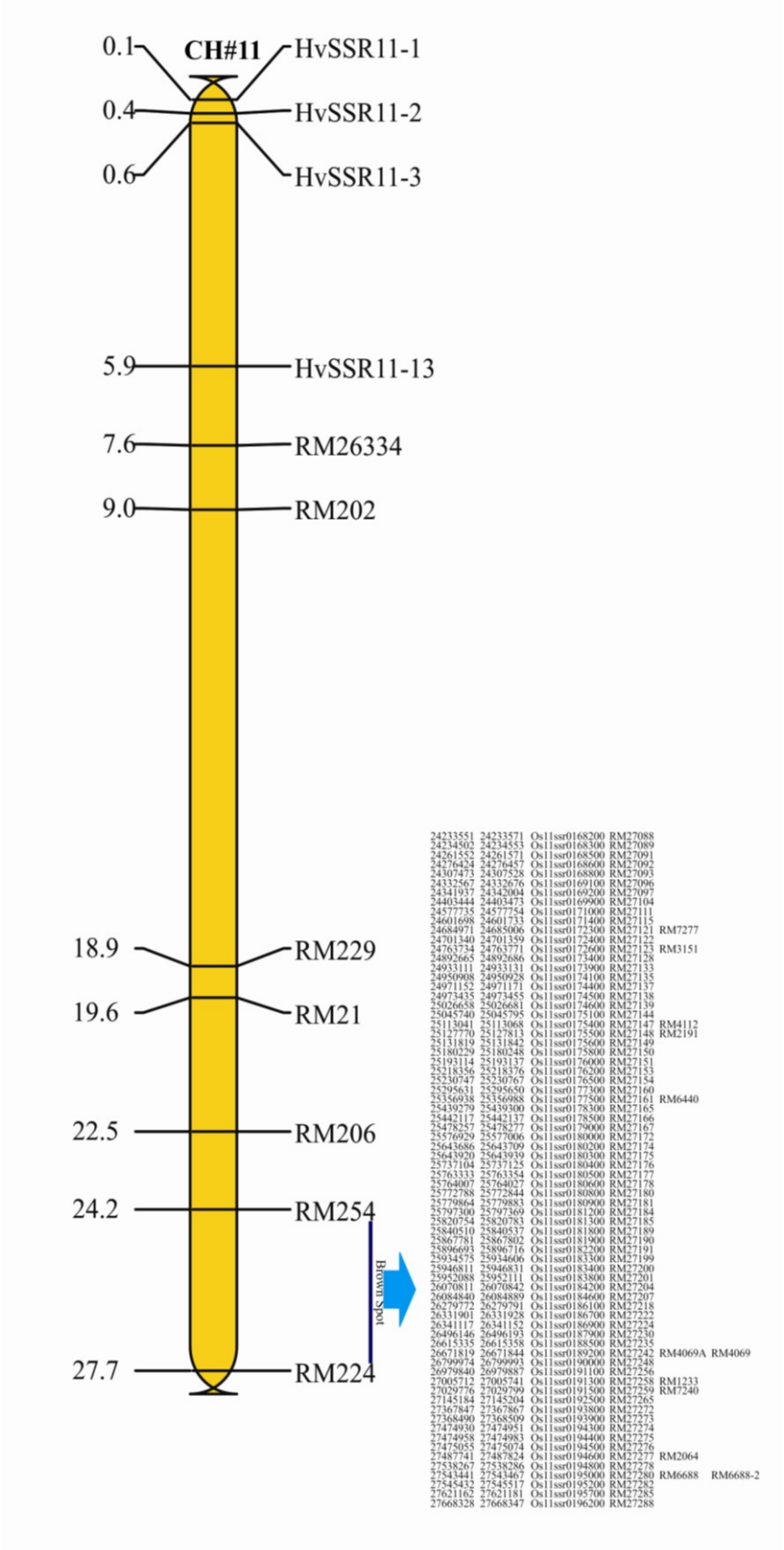


Figure 4.22.13 Genetic location of QTL for trait Brown spot tolerance on chromosome 11 and *in silico* analysis

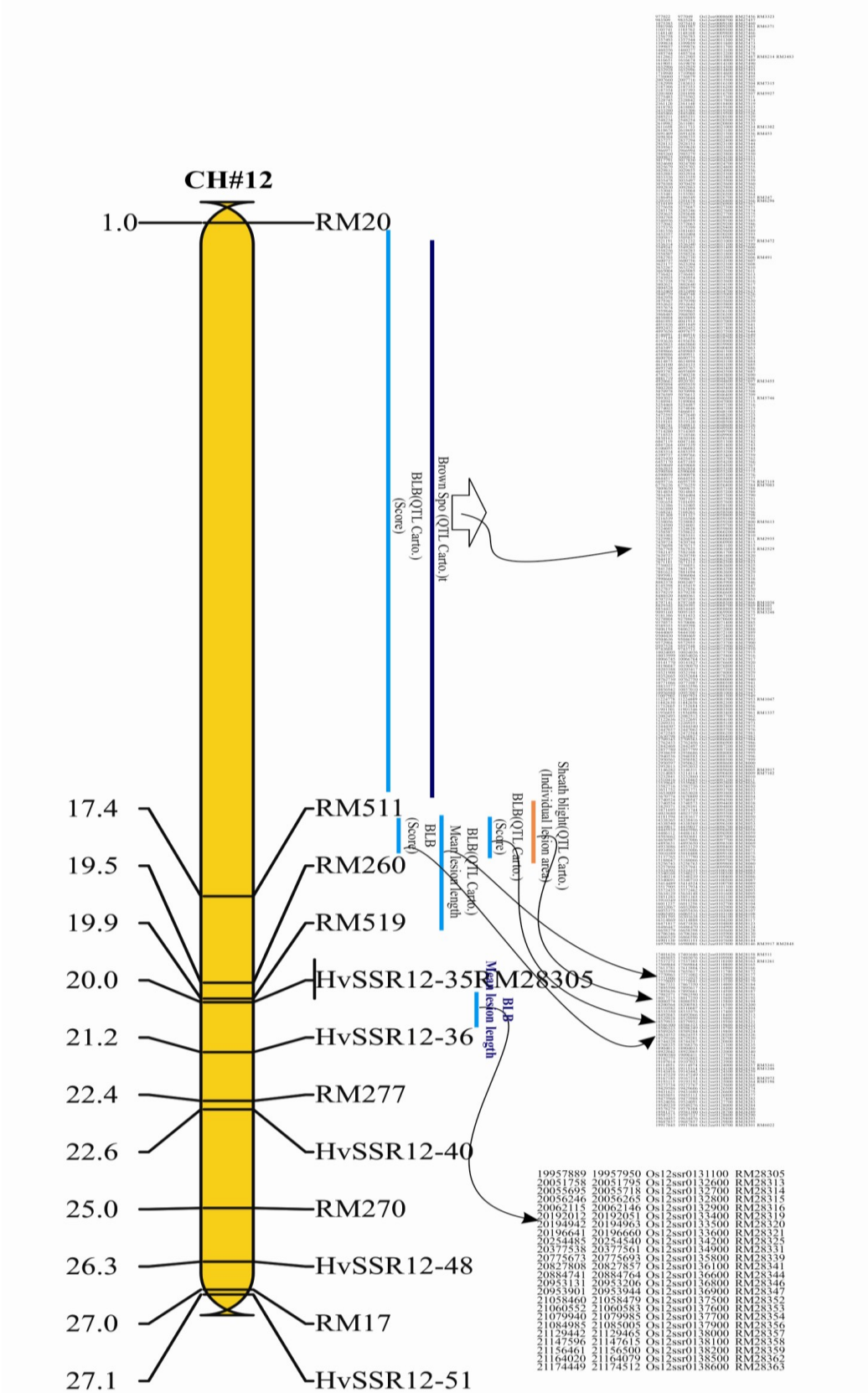


Figure 4.22.14 Genetic locations and *in silico* analysis of QTLs for Brown spot tolerance and BLB trait mean lesion length, reaction qualitative (0-9) score and Sheath blight trait individual lesion area on chromosome 12

Discussion

CHAPTER- V

DISCUSSION

Oryza sativa is a model tractable species among the members of the grass family. Much research has been devoted to understanding the biology of plant-pathogen interactions. The extensive genetic analysis of disease resistance in rice, coupled with the sequenced genome and genomic resources, provides the opportunity to seek convergent evidence implicating specific chromosomal segments and genes in the control of resistance. The molecular marker technique has proved valuable in identifying the loci involved in quantitative disease resistance and has provided insight into its complex relationship with associated factors. The efficiency of marker-aided selection in breeding programs depends on the strength of linkage between molecular markers and the target trait. Traditionally, anonymous molecular markers are used to establish linkage with a phenotype. However, even for tightly linked markers, the effectiveness of marker-aided selection is greatly diminished by the occasional uncoupling of the marker from the trait during many cycles of meiosis in a breeding program. Identification of QTLs and a close correspondence in both genomic locations for resistance against different diseases in other populations has been reported (Li *et al*., 1995; Causse, *et al*. 1994). In rice, doubled haploid (DH) population (IR64 /Azucena), provides a useful reference population for mapping candidate genes (Guiderdoni *et al*., 1992) and has been used for mapping disease (Albar *et al*., 1998; Prashanth *et al*., 1998; Wang *et al*., 2001) and insect resistance (Alam and Cohen, 1998) and tolerance to drought (Courtois *et al*., 2000). The positions of QTL for rice blast and sheath blight on this DH population were previously reported (Prashanth *et al*., 1998; Wang *et al*., 1994; Zou *et al*., 2000). Map

positions of quantitative trait loci (QTL) have been reported by different workers for

blast (Prashanth *et al*., 1998; Wang *et al*., 1994) and sheath blight (Zou *et al*., 2000), brown plant-hopper (BPH) (Alam and Cohen, 1998). A close correspondence was observed between the genomic locations conferring resistance to different diseases and pests of rice to that of the QTLs identified for brown spot (Hulbert *et al*., 2001), which is unlikely to be due to chance and reflect functional differences in member genes within the cluster of resistance gene families (Wang *et al*., 2001; Zou *et al*., 2000).

The development of molecular markers diagnostic for the selection of resistance genes is a goal of many rice breeding programs. Several of the major resistance genes to the bacterial leaf blight (BLB) pathogen, *Xanthomonas oryzae* pv. *oryzae* , have been tagged with restriction fragment length polymorphism (RFLP) or random amplified polymorphic DNA (RAPD) markers (McCouch *et al*., 1992; Ronald *et al.*, 1992; Yoshimura *et al.,* 1992; Yoshimura *et al*., 1995). However, both of these types of markers have limited application in a breeding program. Although RFLP markers are widely available for rice (Causse *et al*., 1994; Kurata *et al*., 1994), they have proven too technically cumbersome to be used for selection with large numbers of plants. Since RAPD markers are normally dominant, detect multiple loci and can have technical problems, they are less useful for selection. Therefore, new types of co-dominant, single-copy and polymerase chain reaction (PCR)-based markers are desirable. Recently, many RFLP markers have been converted into PCR-amplifiable sequence-tagged sites (STS) for specific locations in the rice genome (Inoue *et al*., 1994; Williams *et al*., 1991) and a group of microsatellite markers spread randomly throughout the rice genome has been developed (Wu and Tanksley, 1993; Panaud *et al*., 1996). PCR-based markers closely linked to BLB resistance genes would be very useful for efficient marker-assisted selection.

High-resolution mapping has been crucial to the success of map-based cloning projects in rice (Yoshimura *et al.,* 1998; Ashikari *et al.,* 1999; Yano *et al.,* 2000; Monna *et al.,* 2002). High-resolution maps are useful for the precise placement of a gene of interest and the analysis of regional and sub-regional rates of recombination (Rybka *et al.,* 1997). They can also be used to select appropriate combinations of markers for marker assisted selection in plant-breeding programs.

The genetic and molecular evidence have suggested that quantitative resistance can be pathogen race-nonspecific and even pathogen species-nonspecific, that is, broad-spectrum resistance (Kou and Wang, 2010). Mapping quantitative trait loci (QTLs) is a powerful tool for genetic dissection of QDR. The segregation data for all of the markers could be very useful for analysis of the phenotypic variations observed in many traits owned by the RI lines. In this study, using the segregation data for all mapped markers and rice diseases incidence, such as Bacterial leaf blight, Sheath blight, leaf Blast and Brown spot, it was able to identify a many QTLs conferring above diseases resistance.

5.1 Bacterial leaf blight resistance

The resistance of rice to specific *Xoo* race is governed by both major *R* genes with a qualitative effect that condition complete resistance (CR) and polygenes with a quantitative effect (quantitative trait loci, QTL) that condition partial resistance (PR) (Koch and Parlevliet, 1991b; Li *et al*., 2006). The F_{14} RIL population derived from the cross Danteshwari with Dagad showed nearly normal distribution as continuous variation for BLB (Fig. 4.1) and variation in the RIL for lesion length as in quantitative trait. In similar study, the lesion length on the RIL caused by CR4 and CXO8 showed a bimodal distribution, suggested involvement of major resistance gene(s). On infection with CR6, lesion length of the RILs exhibited continuous

variation and transgressive segregation was present in both directions, showed typical polygenic inheritance (Li *et al*., 1999).

The RILs showed two types of segregation in lesion length (LL), when against avirulent *Xoo* races 1 and 5; the RILs exhibited a bimodal distribution, suggesting involvement of a major R gene(s). When against virulent races 2, 3, 4 and 6, the RILs exhibited an approximately normal distribution with transgressive segregation toward both directions. For virulent races 2, 3, 4 and 6, the genotype × race interaction was more pronounced (*R*2=28.4%), indicating that the PR of rice to *Xoo* is race-specific. Similarly, the DHLs exhibited a bimodal distribution against avirulent *Xoo* races 1, 5, 7, 8, 9 and 10 and an approximately normal distribution against virulent races 2, 3, 4 and 6 again; the race-specificity of PR to virulent races (Li *et al*., 2006). In other study, the distribution of lesion length after inoculation with *Xoo* strain JL691 in a sample containing 500 randomly individuals from a F_2 population developed from a cross between Zhenshan 97 and Minghui 63. The distribution of the lesion length in the 500 plants was found bimodal (Yang *et al.,* 2003). A segregation ratio of 1 : 4 : 6 : 4 : 1 with respect to resistance, moderate resistance, moderate susceptibility, susceptibility and high susceptibility was noticed when the F_2 plants derived from the crosses Ajaya/TN1 and Ajaya/BPT 5204 were screened for BB resistance (Sujatha *et al*., 2011).

In this study, large numbers of QTLs detected by using single local *Xoo* isolate. There were 13 QTLs mapped for mean lesion length on chromosomes such as 1, 3, 4, 5, 9, 10 and 12 using RILs derived from Danteshwari and Dagad deshi. Li *et al.,* 2006 was mapped 22 QRL to 12 rice chromosomes in the RILs derived from Teqing × Lemont. In the DHLs, 26 QRL were mapped to 12 rice chromosomes. The IR64 alleles at 10 QRL were associated with resistance, whereas the Azucena alleles

at 6 QRL resulted in resistance. Resistance at the remaining 10 QRL was associated with the IR64 or Azucena alleles, depending on the *Xoo* races. This confirmed the presence of QTLs.

The two QTLs, *qBbr1a* and *qBbr1b* were identified on chromosome 1 between markers RM499 and RM1 with LOD value 7.86 explained 13.53% of phenotypic variation and between markers HvSSR1-87 and HvSSR1-89 with LOD score 5.33 and explained 27.61% of phenotypic variation affected resistance to local isolate. In the previous study, Li *et al*., 2006 also reported spread of quantitative resistance for bacterial blight on this chromosome. The *qBbr1a* mapped us found similar position of previously identified *QBbr1d* on chromosome 1(Li *et al.*, 2006). The QTL, *qBbr1c* mapped in our work found a different position that is in the telomeric region of same chromosome.

The *qBbr12a* was mapped for mean lesion length between markers RM511 and RM519 with LOD score 4.22, which explained 10.9% of phenotypic variation. The *qBbr12b* identified between markers RM28305 and HvSSR12-36 with LOD value of 4.17 and PVE 11.26%. The *qBbr12c* also identified between markers RM20 and RM511 with high LOD score 6.47 mapped in our work in different region on chromosome 12 as analysis in both RIL and DH population (Li *et al*., 2006). The *Xa25(t)* may be nearby region of *qBbr12c* of this study.

The *qBbr4a* mapped between markers RM564 and HvSSR4-35*, qBbr4b* between markers HvSSR4-26 to RM564 and *qBbr4c* positioned between markers HvSSR4-38 to HvSSR4-39 on chromosome 4 in our work but only the position of *qBbr4c* found nearly similar position. Similarly, *qBbr9a* and *qBbr9b* identified on chromosome 9. The position of *qBbr9b* nearly similar as worked out previously (Li *et al*., 2006). The *qBbr5a* and *qBbr5b* were found similar position on chromosome 5.

Ten putative QTLs were identified using both the whole data set and two data subsets. These QTLs fell on eight of the 12 rice chromosomes such as 2, 3, 4, 8, 9, 10, 11 and 12 and collectively explained more than 65% of the residual variation in mean fitness (LL) unexplained by *Xa4*. The resistance alleles at seven of the QTLs were from Teqing and three were from Lemont (Li *et al*., 1999). Similarly, QTLs on chromosomes 2, 3, 4, 9, 10 and 12 for bacterial blight resistance were also worked out in this study.

5.2 Sheath blight resistance

No gene conferring true resistance to sheath blight has yet been identified, as rice researchers working (Li *et al*., 1995). However, few resistant varities and lines such as Tetep, Jasmin 85, Teqing and Minghui 63 offer sufficient partial resistant to pathogen in field condition to be agriculturally useful (Pan *et al.,*1999; Li *et al*.,1995, Zou *et al*., 2000; Kunihiro *et al*., 2002). The genetic nature of sheath blight has been found to be complex and controversial issue in the earlier studies (Loan *et al*., 2005). On the contrary, genetics studies on the quantitative resistance to *R. solani* in rice have shown both polygenes and major gene inheritance (Sha and Zhu, 1989; Li *et al*., 1995; Zou *et al*., 2000).

In the present study, the disease index of the RILs population derived from Danteshwari × Dagad deshi for sheath blight response found continuously distributed as expected for a quantitative trait (Fig. 4.10). Thus, QTLs might be involved in resistance to Sheath blight. Similar, the Sheath blight response of 127 RIL population derived by single seed descent method from a cross between HP2216 (susceptible to *R. solani*) and Tetep (having a high degree of resistance to *R. solani*) also reported continuously distributed (Channamallikarjuna *et al*., 2010). The mean ShB severity on

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a subset of 256 F_5 RILs from Lemont/Jasmine 85 (LJRILs) in the microchamber and

mist-chamber assays were distributed normally, with the resistant and susceptible parents at the extreme ends (Liu *et al*., 2009). The frequency distributions of Sheath blight response ratings of 300 recombinant inbred lines (RILs) from the cross 'Lemont' × 'Teqing' in a 2-year replicated field experiment exhibited continuous variation for SBR with skewing toward resistance both years (Pinson *et al*., 2005).

In the other study, the $F_{2:3}$ progeny population exhibited significant phenotypic variance for Sheath blight disease scores were continuously distributed, as expected for a quantitative trait (Sharma *et al*., 2009). Phenotypic distribution of bacterial panicle blight (BPB) ratings averaged after removal of apparent "escapes" in the mapping population comprised of 300 RILs derived from a cross between Lemont (PI 475833) and Teqing (PI 536047) found normal in distribution with no clear cases of transgressive segregation (Pinson *et al*., 2010). The frequency distributions of lesion height (LH), actual lesion length (ALL) and disease ratings by inoculation with *Rhizoctonia solani* were continuous, typical of quantitative traits from 266 Teqing Near Isogenic Introgression Lines (NIILs) were developed by using Teqing as recurrent parent and Lemont as introgression parent (Loan *et al*., 2004). The disease ratings in the F_2 clonal population were continuously distributed from total of 128 F_2 clonal families and their parents were used for genetic analysis of disease resistance (Zou *et al*., 2000). The identification of genes that affect complexly inherited traits often difficult and the best approached through developing a genetic linkage map to identify quantitative trait loci (QTLs) (Tanksley and McCouch, 1997).

A major effect highly significant QTL, *qSBR1-1* was identified on chromosome 1 in the region between markers RM3825 and HvSSR1-87 with LOD score 7.3 and with a QTL peaking at HvSSR1-87, explained 26.64% of the phenotypic variation for Sheath blight resistance, the presence of QTLs on this

chromosome previously also reported (Pinson *et al*., 2005; Sharma *et al*., 2009; Liu *et al*., 2009 and Channamallikarjuna *et al*., 2010). Previously, Pinson *et al*., 2005 identified *qSB-1* on chromosome 1 with LOD value of 3.80 and 8.0% of PVE and found associated with morphological character heading date. Similarly, a QTL, *qSB-1* with a peak marker RM104, with LOD value 3-3.2 and 3.4-3.6 % PVE mapped on chromosome 1(Liu *et al*., 2009). Channamallikarjuna *et al*., 2010 was identified *qSBR1-1* on chromosome 1 a peak marker HvSSR1-68 with LOD value 2.9-3 and 8.1-15.0 % of PVE. All these study, confirmed its presence on the chromosome 1.

The significant QTL, *qSBR1-2* for trait individual lesion length cover percentage of sheath length associated with Sheath blight resistance was detected on chromosome 1. The QTL was mapped in the region between markers RM499 and RM1 with LOD score 3.19 and a QTL peak at marker RM428 by QTL cartographer 2.5 under heavy infection condition. The percentage of phenotypic variance explained by the QTL was 3.62% respectively. This was a minor effect QTL first time reported in this work.

One major significant common QTL, *qSBR4-1* was identified on chromosome 4 for total lesion area for Sheath blight resistance between markers HvSSR4-35 and HvSSR4-38 with LOD score of 3.49 and explained 12.1% of the phenotypic variation. Li *et al*., 1995 reported a QTL, *qSB-4* on chromosome 4 with a peak marker locus RG14-RG214 with LOD value 2.8 and 5% PVE. Similarly, two QTLs, *qSB-4-1* and *qSB-4-2* reported on chromosome 4 with LOD value 3 of 5% PVE and 4.6 of 7% PVE respectively.

There are two QTLs namely *qSBR2-1* and *qSBR3-1* were identified on chromosomes 2 and 3 respectively using composite interval mapping were also

previously reported about the presence of QTLs for Sheath blight resistance on these

chromosomes. However, the genomic location of these QTLs varies in different populations (Li *et al.,* 1995; Pan *et al*., 1999; Zou *et al*., 2000; Kunihiro *et al*., 2002; Pinson *et al*., 2005; Sharma *et al*., 2009 and Liu *et al*., 2009). These differences of genomic location could be due to the use of different sources of resistance to Sheath blight in different studies, varying methodologies of assessing Sheath blight resistance or the use of different marker densities.

Two other QTLs, *qSBR9-1* and *qSBR12-1* also identified on chromosomes 9 and 12 on the basis of individual lesion area for Sheath blight resistance have been reported at similar chromosome in previous studies (Li *et al*., 1995; Zou *et al*., 2000; Han *et al*., 2002; Sato *et al*., 2004; Tan *et al*., 2005; Pinson *et al*., 2005; Sharma *et al*., 2009; Liu *et al*., 2009 and Channamallikarjuna *et al*., 2010).

In other studies, six QTLs on chromosomes 2, 3, 4, 8, 9 and 12 were reported using 255 F_4 bulk population of Lemont 9 and Teqing (Li *et al.*, 1995). Zou *et al*., 2000 also identified six QTLs on chromosomes 2, 3, 7, 9 and 11 in 128 F_2 clonal populations of Jasmine 9 and Lemont. Two QTLs on chromosomes 9 and 11 were identified in F_2 clonal population of Teqing and Lemont 9 (Tan *et al*., 2005). Two QTLs have been identified for Sheath blight resistance on chromosome 5 and 9 in rice 240 RILs which was derived from Minghui 63 and Zhenshan 97 (Han *et al*., 2002). Two QTLs have been identified for Sheath blight resistance on chromosome 3 and 12 in rice line WSS2 which was derived from Tetep (Sato *et al*., 2004).

Many investigations have been conducted to know the nature of QTLs for correlated traits. There are many examples of phenotypic correlation between agronomic traits in rice. In order to explain the true relationship between Sheath blight resistance and other agronomic traits, here QTLs for plant height and tiller per plant was also mapped in the same population. By comparing the location of different

QTLs on chromosome 1, it was found that QTL for agronomic traits such as plant height and tiller per plant located side by side and plant height QTL shared with Sheath blight resistance QTL.

The QTL, *qSBR1-1* was mapped on the chromosome 1 between markers RM3825 and HvSSR1-87 with LOD score 7.3 and accounted 26.64% of phenotypic variation for the trait individual lesion length cover percentage of sheath length. On the other hand QTL, *qPH1.1* for plant height was also mapped between markers RM3825 to HvSSR1-87. The same region of chromosome 1 shared by two traits. There was no relation worked out with tiller number per plant. In a Teqing/Lemont F_4 population, Li *et al*., 1995 reported that a large proportion of the phenotypic variation in ShB resistance was explained by the morpho-developmental traits (mainly HD, 42%, and PH, 4%). They were identified three QTLs for heading date and four QTLs for plant height in the resistance loci interval and thus suggested that the QTLs for sheath blight resistance were closely associated with the QTLs for heading date or plant height. Similarly, PH and HD explained 43% of the ShB reaction in a mapping population derived from Pecos, a tropical japonica reported to be ShB resistant (Sharma *et al*., 2009).

5.3 Leaf blast resistance

The Blast resistance in the cultivar Dagad deshi was found to have very complex inheritance. Blast resistance in rice is generally classified into two types, complete and partial resistances (Bonman and Mackill, 1988). The partial resistance reduces the extent of pathogen reproduction in the compatible interaction (Jonson, 1983).

In this experiment, compatible interaction between pathogen and resistant lines were worked out as marked by very small lesions or absence of lesion in

resistant lines (Fig. 4.17) and the normal distribution in graph (Fig. 4.16) suggested that the resistance in these lines might be a partial resistance. The differences in the frequency distributions of resistance were observed in RILs for blast disease. Among 122 RILs, the scores for disease severity in the blast test ranged from 0 to 9 SES scale of IRRI, 2002. Some lines had a higher or nearly equal resistance to Dagad deshi and few lines were more susceptible than Danteshwari. This suggests multi-genic inheritance of QTLs for resistance to natural local races in the field.

In similar study, the distributions of the degree of incidence in four out of nine races screened by inoculation method in 190 RILs population of two parents Suweon365 and Chucheong. The degree of incidence of race KI-197 showed normal distribution. The RIL population exhibited transgressive segregations in both directions for all traits and the population showed approximately normal distributions at all stages (Li *et al*., 2008). In the other study, the frequency distribution of resistance levels of 112 $F_{2:3}$ segregating progenies exhibited continuous distribution, which indicated that blast resistance in IR71033-121-15 against two isolates (KI307 and KI209) was controlled by QTLs(Rahman *et al*., 2011). The frequency distribution for the phenotypic traits in 261 BC_2F_3 and 31 BC_2F_5 families shown normal curve for disease lesion against pathotype P7.2 and P5.0 (Rahim *et al*., 2012). Most of partial resistance is non-race specific, quantitative and polygenic (Maruyama *et al*., 1983; Higashi, 1978).

In the present study, a major effect high significant QTL, *qLB-10.1* was mapped on chromosome 10 in the region between markers HvSSR10-17 and HvSSR10-34. The QTL worked out through QTL Cartographer 2.5 with a high LOD value 3.65 and 16.57% of phenotypic variance. The presence of this QTL was

confirmed by presence of similar *qBL10.1* on chromosome 10 near the marker

RM228 with 2% trait variance (Rahim *et al*., 2012). A QTL, *qLS10* position near the marker RG241B identified on chromosome 10 with 6.4% phenotypic variance, using 304 recombinant inbred lines of indica rice cross Zhong 156/Gumei 2 (Wu *et al.,* 2005). All these study, confirmed its presence on chromosome 10. Similarly, on chromosome 10, *Pi28*(t) a *R* gene in one of QTL identified through double haploid (DH) population derived from an IR64 by Azucena cross (Sallaud *et al.,* 2003). Liu *et al*., 2004 also identified a QTL contain a *R* gene, *PiGD-2*(t) on chromosome 10 from original donor Sanhuangzhan 2.

In other studies, nine QTLs were also identified in a double haploid (DH) population derived from an IR64 by Azucena cross (Sallaud *et al.,* 2003). Eighteen main effect QTLs were detected on chromosomes 1, 4, 5, 6, 7, 8 and 12 for blast resistance through RIL derived from a Bala × Azucena cross (Talukder *et al.,* 2004). The Quantitative trait loci (QTLs) for broad resistance spectrum (BRS) to leaf blast were located on chromosomes 7 and 9. In particular, the *QTLch9* was mapped near the *Pi5(t)* locus. Two neck-blast QTLs were mapped on chromosomes 5 and 6 (Sirithunya *et al*., 2002). Fourteen QTLs were identified and mapped on three chromosomes 1, 11 and 12 for resistance against leaf and neck blast **(**Noenplab *et al*., 2006**)**. There were four QTLs (*qBL5.1, qBL5.2, qBL5.3* and *qBL5.4*) were identified on Chromosomes 5, two (*qBL8.1*, *qBL8.2*) were identified on Chromosomes 8 and three QTLs (*qBL6.1, qBL7.1* and *qBL10.1*) were identified on Chromosomes 6, 7 and 10 using of advanced backcross families BC_2F_3 derived from *Oryza sativa* cv MR219/*O. rufipogon* IRGC105491(Rahim *et al*., 2012**)**. By using 148 Sequence Tagged Site (STS) and Single Sequence Repeat (SSR) markers, five QTLs on chromosomes 6, 7, 9 and 11 and seven epistatic QTLs were identified against two blast isolates (KI307 and KI209) (Rahman *et al.,*2011*).*

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Two QTLs were detected on Chromosome 4 and one each on Chromosome 9 and 12. The phenotypic variation explained by each QTL ranged from 7.9 to 45.7% and the 4 QTLs explained 66.3% of the total phenotypic variation (Fukuoka and Okuno, 2001). Ten putative QTLs for blast resistance on 12 rice Chromosomes have been mapped (McCouch *et al*., 1994). Two QTLs have been mapped on Chromosome 2 and 6 and one each on Chromosome 3, 5, 7, 8, 11 and 12. Furthermore, 9 QTLs have been mapped using RFLP markers on Chromosome 1-4, 6, 7 and 9 with 2 loci on Chromosome 12 (Tabien *et al*., 2002). Two QTLs on Chromosome 3 and 11 (*qBFR3* and *qBFR11*) were identified from an F_3 population derived from URN12 (resistant) and Koshihikari (susceptible) (Sato *et al*., 2006). Five QTLs relating to leaf blast resistance have been detected on Chromosomes 4, 6, 8, 11 and 12 from the BC_2F_2 population derived from the backcross of Koshihikari/*O. rufipogon* (Hirabayasi *et al*., 2005). The QTL on Chromosome 4 was found to be most effective and considered a complete resistance gene. Two main-effect quantitative trait loci (*r11a* and *r11b*) mapped both on chromosome 11 within the two regions RG103-CDO534 and RM229-RM209 (Li *et al*., 2008).

5.4 Brown spot tolerance

Little is known about the mechanisms responsible for partial resistance to Brown spot. Conventional genetic studies have provided little information on the inheritance of genes controlling the resistance. No major genes with resistance to Brown spot have yet been identified. However, varieties with partial resistance and three quantitative trait loci (QTL) for disease resistance have been identified. The use of resistant varieties would be the most economical means of controlling Brown spot. The varieties Tadukan and Tetep, offered sufficient quantitative resistance to Brown spot and are agriculturally useful (Oohata and Kubo, 1974).

The Danteshwari × Dagad deshi derived 122 RILs population were screened for Brown spot tolerance. The frequency distribution could not be classified into discrete classes of resistance and susceptibility that indicated neither parent contained all the alleles for resistance or susceptibility, nearly normal distribution was followed, the trait Brown spot tolerance be a quantitative as resistance lines showed only few small spots (Fig. 4.19 and 4.20). The frequency distribution of disease score of 110 F_5 lines derived by crossing Tadukan and Hinohikari and their parents for Brown spot shown continuous variation (Sato *et al*., 2008). A certain number of lines showed segregate transgressively over their parent indicating disease score is a typical quantitative trait. The DH lines exhibited transgressive segregation in both directions, which indicated that neither parent contained all the alleles for resistance or susceptibility. Reaction of DH lines, for brown spot, could not be classified into discrete classes of resistance and susceptibility as they showed continuous variation and skewed distribution that suggested the inheritance is quantitative (Dudhare *et al*., 2008).

In the present study, the QTL*s, qSB1, qSB11* and *qSB12* were identified at the different positions and chromosome by using 122 RIL population derived from a cross Danteshwari × Dagad deshi for Brown spot tolerance on chromosomes 1, 11 and 12 respectively. The QTL, *qBS1* between markers HvSSR1-55 to RM5 with LOD score of 3.14 explained 14.35% of the phenotypic variation was considered a major QTL related to Brown spot tolerance. The other *qSB11* with LOD score of 2.71 and 13.99% of the phenotypic variation identified by QTL IciMapping 3.2 under heavy infection condition. Previously, the *qSB11* with a LOD equal to 5.11 was considered a major QTL related to brown spot tolerance also mapped on chromosome 11 with a different position (Sato *et al*., 2008) confirmed its presence on that chromosome. The

naval major effect QTL, *qBS1* identified on chromosome 1 for brown spot tolerance which was not reported earlier.

The QTL, *qBS12* also first time mapped on chromosome 12 between markers RM20 and RM511 with LOD score 3.17. The three QTLs, *qBS2, qBS9* and *qBS11* for Brown spot resistance were detected on chromosome 2, 9 and 11, respectively by using 110 F_5 RIL population derived from a cross Tadukan × Hinohikari for Brown spot tolerance (Sato *et al*., 2008). In other study, in drought conditions, three QTLs were identified one each on chromosome # 4, 6 and 8 between molecular markers EMP2_2-ME10_11, R2171-R2123 and G187-ME2_11, explained 15.5, 9.5 and 12.2% of total phenotypic variation, with LOD score of 4.84, 3.33 and 4.33 respectively (Dudhare *et al*., 2008).

5.5 Chromosomal regions with multiple diseases resistance

The rice cultivars Moroberekan, Teqing, Lemont and Minghui 63 of several chromosomal segments were associated with quantitative resistance to two or three diseases. Integrating the QTLs data onto a single map-based format allowed us to tell chromosomal segments contain colocalizing QTLs for multiple diseases resistance. The quantitative resistance is putatively race non-specific, as indicated by evidence that resistance QTLs for different rice diseases caused by various pathogens are frequently mapped to the same or overlapping loci (Xiong *et al.*, 2002; Ramalingam *et al*., 2003; Wen *et al*., 2003; Chu *et al*., 2004; Zhang *et al*., 2005 and Li *et al*., 2006).

In the present study, the region of chromosome between markers RM499 to RM1 found common on chromosome 1 for both the disease such as Sheath blight and Bacterial leaf blight diseases resistance. The chromosomal segments also contain colocalizing QTLs for multiple diseases. The region of chromosome 12 between markers RM20 and RM511 found common for both the disease *i.e.* Brown spot and

bacterial blight. The QTLs of both diseases overlapped in this region. Similarly, previously reported on chromosome 12 a interval (40.2–64.4 cM) found QTLs for disease resistance to Bacterial blight, rice Blast, RYMV, Rice yellow mottle virus and Sheath blight. Colocalization of multiple resistance QTLs has been observed (Wisser *et al.,* 2005). The results of the study are preliminary and need to be confirmed. These flanking markers are useful for developing of multiple diseases resistance in cultivars through molecular marker-assisted selection and similar studies done by many researchers.

The rice cultivar 'Improved Pusa Basmati 1' developed with inbuilt resistance to BB, blast and ShB through molecular marker-assisted selection using flanking markers. The Basmati cultivar (carrying the BB resistance genes *xa13* and *Xa21*) was used as the recurrent parent and cultivar 'Tetep' (carrying the blast resistance gene *Pi54* and ShB resistance quantitative trait loci (QTL), *qSBR11-1*) was the donor (Singh *et al*., 2012). Molecular markers have made it possible to identify and pyramid valuable genes of agronomic importance genes that confer broad-spectrum bacterial blight resistance three resistance genes (*Xa4* + *xa5* + *Xa21*) were transferred from an indica donor (IRBB57), using a marker-assisted backcrossing (MAB) breeding strategy(Suh *et al*., 2013).

Summary, Conclusion and Suggestions for Future Research Work

CHAPTER - VI

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FUTURE RESEARCH WORK

Rice (*Oryza sativa* L.) feeds more than half of the world's population and genetic improvement of this food crop can serve as a major component of sustainable food production. It serves as a model monocot crop for plant genome analysis due to the availability of whole genome sequence information, high throughput molecular biological tools and large number of targeted mutant collections. Diseases are among the most important limiting factors that affect rice production, causing annual yield loss conservatively. Resistant cultivars and application of pesticides have been used for disease control. However, the useful life-span of many resistant cultivars is only a few years, due to the breakdown of the resistance in the face of high pathogenic variability of the pathogen population. The identification of genes that affect complexly inherited traits is often difficult so, the best approached through developing a genetic linkage map to identify quantitative trait loci (QTLs). Identification and mapping of QTL is a valuable starting point for positional cloning of genes present in the QTL region of the genomes. Once the tightly linked markers have been identified, the quantitative trait loci (QTLs) can be selected for breeding programs using marker-assisted selection (MAS) strategy.

Summary

The phenotypic evaluation of F_{14} RILs population for diseases

- The analysis indicated that the Sheath blight and Blast disease index of RILs were continuously distributed as expected for a quantitative trait. The trait Bacterial blight resistance and Brown spot tolerance for RILs population

showed nearly continuous variation with normal distribution as in quantitative trait.

- Among all 122 F_{14} RIL lines used for screening of four diseases, the line number such as “71” and “77” found resistance for all four diseases e.g. Bacterial leaf blight, Sheath blight, leaf Blast and Brown spot. There were 8 lines which showed resistance to three disease such as line number 46 for Sheath blight, leaf Blast and Brown spot; line number 70 and 88 for BLB, leaf Blast and Brown spot; line number 18, 73 and 78 for BLB, Sheath blight and leaf Blast and lastly 28 and 94 line for BLB, Sheath blight and Brown spot. At least 17 lines found for two disease resistance and rest for single disease resistance.
- There were many RM and HvSSR markers found significance at 0.01% level with BLB traits on chromosomes 1, 3, 4, 8, 9, 11 and 12. The markers HvSSR4-35 and HvSSR4-38 on chromosome 4 and RM-459 on chromosome 5 were showed significant at 1% level and the only marker RM273 on chromosome 4 was found significant at 0.1% level for the trait total lesion area for Sheath blight resistance. Similarly, markers RM3825 and HvSSR1-87 were found significance at 0.1% and 0.01% respectively for trait individual lesion length cover % of sheath length. The markers such as HvSSR2-12 on chromosome 2; RM410, RM242 and RM553 on chromosome 9; RM277 and HvSSR12-51 on chromosome 12 were significance at the 1% level with the trait leaf Blast. Similarly, the highly associated marker for Brown spot tolerance was HvSSR1-55 on chromosome 1 significance at 0.1% level.

QTL mapping through Composite Interval Mapping

- A total of 10 and 3 QTLs were identified for Bacterial blight resistance under different conditions such as mean lesion length and BLB reaction qualitative (0-9) scale (SES IRRI, 2002) using QTL cartographer 2.5 while 3 and 1 QTLs were identified using QTL IciMapping 3.2 respectively. These QTLs were found to be present on chromosome #1, 3, 4, 5, 6, 9, 10 and 12.
- Three significant major QTLs, *qBbr1a, qBbr1b* and *qBbr1c* were mapped on chromosome 1 for mean lesion length; another 3 significant major effect QTLs, *qBbr12a, qBbr12b* and *qBbr12c* were identified on chromosome 12 by QTL cartographer 2.5 and QTL IciMapping 3.2 and others 9 minor QTLs showed on 3, 4, 5, 6, 9 and 10 chromosomes.
- A total of 7 QTLs were identified for Sheath blight disease resistance using QTL cartographer 2.5 using composite interval mapping while 2 QTLs were identified using QTL IciMapping 3.2, out of which 2 QTLs were common with both the software. The QTLs were found to be present on chromosomes 1, 2, 3, 4, 9 & 12.
- One major significant QTL, *qSBR4-1* for total lesion area for Sheath blight identified on chromosome 4; one major significant QTL, *qSBR1-1* and one minor QTL, *qSBR1-2* mapped on chromosome 1 for individual lesion length cover % of sheath length respectively. There were four small effect QTLs also found on chromosomes 2, 3, 9 and 12.
- The QTL, *qSBR1-1* was mapped on the chromosome 1 between markers RM3825 and HvSSR1-87 with LOD score 7.3 and accounted 26.64% of phenotypic variation for the trait individual lesion length cover percentage of sheath length. The same region of chromosome 1 shared one QTL such as

qPH1.1 for plant height. This major effect QTL, *qSBR1-1* found share QTL of plant height.

- A QTL, *qLB10.1* was identified for Blast resistance using RILs population using QTL cartographer 2.5 while no QTLs were identified using QTL IciMapping 3.2. The QTL was found to be present region between markers HvSSR-10-17 and HvSSR10-34 on chromosomes 10. The LOD score of the QTL was 3.65 and explained 16.57% of the phenotypic variation.
- In the present study, a total of one QTL, *qBS12* was identified for Brown spot tolerance using QTL cartographer 2.5 on chromosome 12 while 2 QTLs were identified such as *qBS1* and *qBS11* on chromosome 1 and 11 respectively using QTL IciMapping 3.2.
- The three chromosomes which showed multiple disease resistance area in this experiment. The region of chromosome 1 between markers RM499 to RM1 found common for both diseases BLB and Sheath blight resistance. Similarly, the region of chromosome 12 between markers RM20 and RM511 found common for both the disease i.e. Brown spot and Bacterial leaf blight resistance. Another region of this chromosome between markers RM511 and RM260 coincided for BLB and Sheath blight. On chromosome 9 between markers HvSSR9-7 and RM444 common for Sheath blight and BLB resistance.

Conclusion

- The parent Dagad deshi found resistance toward Sheath blight and leaf Blast. Similarly Danteshwari toward Bacterial leaf blight resistance and slightly Brown spot tolerance. The phenotypic variation of RILs for these diseases found continuous variation as quantitative trait.

- In conclusion, small effect QTLs were found prevalence in this experiment for BLB. Few major effect QTLs worked out in this experiment such as *qBbr1a, qBbr1b* and *qBbr12a, qBbr12b* on chromosome 1 and 12 respectively of similar chromosomes of previously mapped QTLs. This lending credibility to their existence in parents. The QTLs, *qBbr12a* and *qBbr12b* high LOD and PVE% may be useful for crop improvement programmes.
- The large numbers of QTLs identified conferring Sheath blight disease resistance demonstrate the durability of Dagad deshi is due to many genes with small effects. The 7 QTLs were identified for Sheath blight under heavy infection condition in this experiment corresponded to similar chromosomes of previously mapped QTLs, lending credibility to their existence in parents.
- Identification of two QTLs for Sheath blight resistance is valuable finding for rice geneticists, breeders and pathologists. The ShB resistance QTLs, *qSBR4-1* for total lesion area on chromosome 4 and *qSBR1-2* on chromosome 1 appear to be independent of such associations with other trait, explaining 12.1 and 3.62% of phenotypic variation for Sheath blight resistance, respectively offer as a breeding target for partial resistance in Indian rice cultivars and a starting point for gene isolation. The QTL, *qSBR9-1* that has been mapped to region between markers HvSSR9-7 and RM444 may be useful for crop improvement by marker-assisted transfer. The major effect QTLs, *qSBR1-1* and *qPH1.1* of plant height found on same position may not be useful.
- The QTL, *qLB10.1* mapped between markers HvSSR-10-17 and HvSSR10-34 on chromosomes 10 with high LOD value 3.65 and 16.57% of phenotypic variance found experiment specific QTL may be useful for crop improvement by marker-assisted transfer as *Pi28*(t) a *R* gene and *PiGD-2*(t) a *R* gene were previously reported on this chromosome.

- The QTL for Brown spot tolerance *qSB11* with LOD score of 2.71 and 13.99% of the phenotypic variation identified previously mapped QTL; *qSB11* with a LOD equal to 5.11 was on chromosome 11 with a different position lending credibility to their existence in parent. The major effect QTL, *qBS1* identified on chromosome 1 for brown spot tolerance which was not reported earlier.
- The multiple disease resistance regions of chromosomes 1, 9 and 12 can be use as resistance source for non-race specific.

Suggestions for Future Research Work

- To carry out map-based cloning of each QTL to elucidate the resistant mechanisms.
- In future could use the SSR markers that were tightly linked to the QTLs to promote breeding process of rice disease resistance.
- Validation of more number of SSR markers can provide better genome coverage and greater arsenal of tools for QTL mapping and marker assisted selection.
- The QTLs very often exhibits high QTL × E interaction, the detected QTLs need to be cross validated across the different environment and crosses along with the cross validation of linked molecular markers.
- *R* genes and defense-associated transcription factors are attractive classes of candidate genes for investigation of QTLs need to identify.
- Once genes conditioning QTL are identified for crop plants, natural variation can be better characterized and exploited.
- Identification of positional candidate genes is a step toward isolation of the genetic factors controlling quantitative traits.

Abstract

"QTL MAPPING FOR BACTERIAL LEAF BLIGHT, SHEATH BLIGHT, BLAST AND BROWN SPOT TOLERANCE USING RIL POPULATION OF RICE (*Oryza sativa* L.)."

By

LINCOLN MANDAL

ABSTRACT

Rice (*Oryza sativa* L.) is a high economic and staple food of more than half of the world's human population. Diseases are among the most important limiting factors that affect rice production, causing annual yield loss conservatively. Due to the breakdown of resistant cultivars, in the face of high pathogenic variability of the pathogen population, identified quantitative trait loci (QTLs) can be use for disease control. Identification and mapping of QTL is a valuable starting point for positional cloning of genes present in the QTL region of the genome. Once the tightly linked markers have been identified, the quantitative trait loci (QTLs) can be selected for breeding programs using marker-assisted selection (MAS) strategy.

Two potential *indica* genotypes, Danteshwari: a high yielding popular rice cultivar but resistance to few diseases and Dagad deshi: a tall deep rooted poor yielder and susceptible to few diseases, along with their derived F_{14} RILs were used for the phenotypic evaluation of four diseases such as Bacterial leaf blight, Sheath blight, leaf Blast and Brown spot tolerance. The analysis indicated that the Sheath blight and Blast disease index of RILs were continuously distributed as expected for a quantitative trait but Bacterial blight resistance and Brown spot tolerance for RIL population showed continuous variation with nearly normal distribution. Among all 122 lines used for screening of four diseases, the line numbers "71" and "77" were found to be resistant for all four diseases such as BLB, Sheath blight, leaf Blast and Brown spot. Only eight lines showed resistance against all three diseases.

The Single Marker Analysis tests the association between marker genotypes and trait values using F-test in QTL cartographer 2.5. There were many RM and HvSSR markers found significance at 0.01% level with BLB trait on chromosomes 1, 3, 4, 8, 9, 11 and 12. The only marker RM273 on chromosome 4 was found significant at 0.1% for total lesion area for Sheath blight resistance. Similarly, markers RM3825 and HvSSR1-87 were found significant at 0.1% and 0.01% respectively for individual lesion length cover % of sheath length. HvSSR2-12 on chromosome 2; RM410, RM242 and RM553 on chromosome 9; RM277 and HvSSR12-51 on chromosome 12 were significant at the 1% level with leaf Blast. Similarly, the highly associated marker for Brown spot tolerance was HvSSR1-55 on chromosome 1 significance at 0.1% .

A total of 162 well distributed rice SSR (RM and HvSSR) markers polymorphic between Danteshwari and Dagad deshi were used to construct a linkage map. The genotypic data and phenotypic data of field condition of diseases reaction were analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2. A total of 10 and 3 QTLs were identified for Bacterial blight resistance under different conditions

2002), respectively using QTL cartographer 2.5 while 3 and 1 QTLs were identified using QTL IciMapping 3.2. These QTLs were found to be present on chromosome #1, 3, 4, 5, 6, 9, 10 & 12. Three significant major QTLs, *QBbr1a, QBbr1b* and *QBbr1c* on chromosome 1 and *QBbr12a, QBbr12b and QBbr12c* on chromosome 12 identified for mean lesion length similar chromosomes of previously mapped QTLs, lending credibility to their existence in parents.

A total of 7 QTLs were identified for Sheath blight disease resistance present on chromosomes 1, 2, 3, 4, 9 and 12. One major significant QTL, *qSBR4-1* for total lesion area for sheath blight identified on chromosome 4; one major significant QTL *qSBR1-1* and one minor QTL, *qSBR1-2* mapped on chromosome 1 for individual lesion length cover % of sheath length respectively. The major effect QTL *qSBR1-1* found share QTL of plant height.

A QTL *qLB10.1* was identified for Blast resistance found to be present region between markers HvSSR10-17and HvSSR10-34 on chromosomes 10. The QTL *qLB10.1* with high LOD value 3.65 and 16.57% of phenotypic variance found experiment specific QTL may be useful for crop improvement as *Pi28*(t) a *R* gene and *PiGD-2*(t) a *R* gene were previously reported on this chromosome.

In the present study, a total of 3 QTLs such as *qBS1, qBS11 and qBS12* on chromosome 1, 11 and *12* respectively were identified for Brown spot resistance. The QTL for Brown spot *qSB11* with LOD score of 2.71 and 13.99% of the phenotypic variation identified as previously mapped QTL *qSB11* with a LOD equal to 5.11 was on chromosome 11 with a different position lending credibility to their existence.

The three chromosomes which showed multiple disease resistance area in this experiment. The region of chromosome 1 between markers RM499 to RM1 found common for both diseases BLB and Sheath blight toward resistance. Similarly, the region of chromosome 12 between markers RM20 and RM511 found common for both the disease i.e. Brown spot and Bacterial leaf blight resistance. Another region of this chromosome between marker RM511 and RM260 coincided for BLB and Sheath blight. On chromosome 9 between marker HvSSR9-7 and RM444 common for sheath blight and BLB resistance.

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Appendices

Appendix I: Primers used for developing genotypic data

S.No.	Markers	Forward Sequence (5' --------->3')	Reverse Sequence (5' --------->3')
1	**RM - 499**	TACCAAACACCAACACTGCG	ACCTGCAGTATCCAAGTGTACG
2	**HvSSR 1-24**	ACGCAGTTACACCCACTACT	ATAAGTGCCTATGCATGGTT
3	**HvSSR 1-33**	AACTTGGGCTCTTTAATTCC	CAGAGTTCGAGAGAGACCAG
4	**HvSSR 1-34**	AAACTGGAGATGAACTCGAA	GTAACGAACTAGAGCATGGG
5	**HvSSR 1-49**	TCCTAAAGTTCACACCAACC	TGTCGATTCTCCTTCACTTT
6	**RM - 428**	AACAGATGGCATCGTCTTCC	CGCTGCATCCACTACTGTTG
7	**HvSSR 1-55**	ACACCATACCAATACGAAGG	ACACCGTACTGTTTATTGGG
8	**RM - 84**	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
9	**RM - 1**	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
10	**HvSSR 1-80**	TTTGAGCAAATAAACTTGAGG	GCTTCTACTTCCACAAGGC
11	**HvSSR 1-87**	TTGGTACACGACCATGATTA	ATGGATCTGTGTGTGCGT
12	**HvSSR 1-89**	TGCGACGGATAGAGTACATA	GGATGCAAAGAAAGAACAAG
13	**RM - 259**	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
14	**RM - 243**	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
15	**RM - 572**	CGGTTAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC
16	**RM - 24**	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG
17	**RM - 449**	TTGGGAGGTGTTGATAAGGC	ACCACCAGCGTCTCTCTCTC
18	**RM - 5**	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
19	**RM - 212**	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
20	**RM - 3825**	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
21	**RM - 302***	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
22	**RM - 486**	CCCCCCTCTCTCTCTCTCTC	TAGCCACATCAACAGCTTGC
23	**RM - 14**	CCGAGGAGAGGAGTTCGAC	GTGCCAATTTCCTCGAAAAA
24	**RM - 109**	GCCGCCGGAGAGGGAGAGAGAG	CCCCGACGGGATCTCCATCGTC
25	**RM - 485***	CACACTTTCCAGTCCTCTCC	CATCTTCCTCTCTTCGGCAC
26	**HvSSR 2-1**	AAGAGATGAGAAGAGCAATGA	CAACTTAGAGGAAGAAGGAGG
27	**HvSSR 2-12**	TCTCCAATTCTCCATCAAAC	CTTGCTTGAGCGAGTCTAAT
28	**HvSSR 2-23**	AGCTAGCTACACACTTTCCG	ATAGATGCATGGCGATATTT
29	**HvSSR 2-27**	GGTGCAATTCTTATTCCTTG	AATTTGGATTCAGATGTTGC
30	**HvSSR 2-78**	GTTTCCTTGCAAACAGACAT	AGTCATTCTAGCATTTCCCA
31	**RM - 174**	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG
32	**RM - 492**	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTCAGGC
33	**RM - 475**	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
34	**RM - 341**	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
35	**RM - 221**	ACATGTCAGCATGCCACATC	TGCAAGAATCTGACCCGG
36	**HvSSR 3-6**	AGATGAGCTTCAGTGCTAGG	TTCACCACAAAGTTCACAAA
37	**HvSSR 3-9**	TGTATTCAAGGAGGGCTAGA	CAACTGTTTCCTGGAATGAT
38	**HvSSR 3-35**	TTGATTACGTGAATAGCTCG	CATAGCTAACTTGTGCGTTG
39	**HvSSR 3-40**	CGAGAGGTTCAGAGAGAATG	GCATTCACCCTAAGGATACA
40	**HvSSR 3-41**	ATGCAATTACTTGTTGCCTT	AAGTTCTGAACAACCACCAC
41	**RM - 231**	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
42	**HvSSR 3-56**	GCCTATCAGGCTATCATCAC	GTGATCGACATTGAGGAGTT
43	**HvSSR 3-71**	CACACCAACTCACTCTTGAA	CCGTTTCGTCTATGTTCATT
44	**HvSSR 3-85**	GCAAACGACACAAGTCATTA	ATAGTGCCCTTTCTTTCACA
45	**RM - 517**	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC
46	**RM - 7**	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT
47	**RM - 232**	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
48	**RM - 411**	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG
49	**RM - 135**	CTCTGTCTCCTCCCCCGCGTCG	TCAGCTTCTGGCCGGCCTCCTC
50	**RM - 55**	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG
51	**RM - 85**	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
52	**RM- 307**	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
53	**HvSSR 4-26**	GAGGAATTCATTCATCATGC	ATTTCGTTATTTGCATTGGT
54	**HvSSR 4-35**	ACCAACCTAATACCGATGTG	CGCGAGTGTTGTAACTTTAAC
55	**HvSSR 4-38**	CCAAGCACCTCTTAACTTGA	CCGTTCTTATTAGGTTGTGG

56	**HvSSR 4-39**	CAAATAAGATCGCTGAAACC	TTCGGAGTAAATTGGACATC
57	**HvSSR 4-42**	GATGGTGAATCTCGGTCTAA	TGTCCCATCATCACAAACTA
58	**RM - 564**	CATGGCCTTGTGTATGCATC	ATGCAGAGGATTGGCTTGAG
59	**RM - 273**	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC
60	**RM - 348**	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
61	**RM - 317**	CATACTTACCAGTTCACCGCC	CTGGAGAGTGTCAGCTAGTTGA
62	**RM - 559**	ACGTACACTTGGCCCTATGC	ATGGGTGTCAGTTTGCTTCC
63	**HvSSR 5-13**	TCCTCTACAGTTGTCTGCCT	CATTCCTCTCCACTTTCTTG
64	**HvSSR 5-23**	GCAGCCATCTATCATCTAGC	CTAGCTGCACCAGTTTGATT
65	**HvSSR 5-31**	TGGAGCTGTGTTGTTGATTA	ATTGTGACATGCTGATGTTG
66	**HvSSR 5-39**	TGAGAGGATACTTGGGACTG	CCAGCATGCAACTGTAACTA
67	**HvSSR 5-48**	GAATTGAAGGTGGGACATAA	GAAGATGGCATGTAAACGAT
68	**HvSSR 5-51**	CCATGAAATAGTTCTAGGGAA	TAATTAATGCCTTCGTGGAT
69	**HvSSR 5-52**	GCTTAGTACTTGCGGCTAAA	CCATCTTACATGTCCTCACC
70	**HvSSR 5-56**	AAACTATCCGCTTGTGAAAT	CCGGTTAAGGACTCCTATCT
71	**HvSSR 5-65**	ATTAAACGCACACTGGAAGT	AAACGGAGGGAGTAGTTAGC
72	**HvSSR 5-66**	GTTATGCGCTTCTGCTTATT	AGTTGGCTTCTGGATTACAA
73	**RM - 163**	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
74	**RM - 440**	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
75	**RM - 459**	CTGCAATGCTGCATGACC	CACTTTCTCTGCAGCACCAG
76	**RM - 188**	TCCGCCTCTCCTCTCGCTTCCC	GCAACGCACAACCGAACCGAGC
77	**RM - 421**	AGCTCAGGTGAAACATCCAC	ATCCAGAATCCATTGACCCC
78	**RM - 178**	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC
79	**RM - 26**	GAGTCGACGAGCGGCAGA	CTGCGAGCGACGGTAACA
80	**RM - 274**	CCTCGCTTATGAGAGCTTCG	CTTCTCCATCACTCCCATGG
81	**RM - 87**	CCTCTCCGATACACCGTATG	GCGAAGGTACGAAAGGAAAG
82	**HvSSR 6-35**	GAAAGGAAATCAGGTTGTGA	CCCATTAGACATTTCGGATA
83	**HvSSR 6-44**	GGAGCATCCATCACAATATC	GTAATTTCAGTCAGCCAAGC
84	**HvSSR 6-56**	AGCATTTGTGTGTGCAATAG	ATGCTTGCCTCATCAGTAGT
85	**RM - 225**	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
86	**HvSSR 6-65**	GTGTGGCAATTTAACATCCT	TTGTTGCTTGTTCTTCACTG
87	**RM - 217**	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
88	**RM - 136**	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC
89	**RM - 340**	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC
90	**RM - 400**	ACACCAGGCTACCCAAACTC	CGGAGAGATCTGACATGTGG
91	**RM - 481**	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG
92	**HvSSR 7-40**	GATTTACTCGCAAGTTACCG	TGTTTCAGGTTCGTCTATCC
93	**HvSSR 7-43**	CAACTCAGTTCCAATCCCTA	TTGTGTGTTTCATATACGGC
94	**HvSSR 7-46**	ACAGCTGTAGAGGATGAGGA	TCCCTAATTCGAATCACAAC
95	**RM - 125**	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
96	**HvSSR 7-53**	CGAGCATGTCTGTCAAGTAA	GTTCGAATGTAATGTTGGCT
97	**RM - 2**	ACGTGTCACCGCTTCCT	ATGTCCGGGATCTCATCG
98	**RM - 11**	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
99	**RM - 234**	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG
100	**RM - 248**	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
101	**RM - 337**	GTAGGAAAGGAAGGGCAGAG	CGATAGATAGCTAGATGTGGCC
102	**RM - 152**	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG
103	**HvSSR 8-29**	AACTGAGAGGCTGCTTGTAT	TAAAGGGTTCACTCATGGAC
104	**RM - 310**	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC
105	**RM - 44**	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
106	**RM - 483**	CTTCCACCATAAAACCGGAG	ACACCGGTGATCTTGTAGCC
107	**RM - 72**	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
108	**RM - 515**	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
109	**RM - 256**	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
110	**RM - 230**	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTTCAAG
111	**RM - 433**	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
112	**RM - 281**	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG
113	**HvSSR 9-5**	GAACGGAGGGAGGTTGTT	AAAGTGTCCTAAAGCCAAGTC

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114	**RM - 444**	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG
115	**HvSSR 9-7**	CATCTCAGCAAACAAGAACA	GTAAAGACTCCAGCTTTCTCC
116	**HvSSR 9-19**	TCGAATTTAGTCCAGGGTAA	GGTGAGAGATCTTGAGTTCG
117	**HvSSR 9-25**	GATCGATCTCATCATCACCT	TAGCTTCCTACTGGGAGTGA
118	**HvSSR 9-27**	TGGGCATCTGGTACTATCTT	AGCTCATTCCACAGGTTAGA
119	**HvSSR 9-37**	AATCTCAACTGCTCGGATTA	TTGATTGATTGATTGAACGA
120	**HvSSR 9-57**	GGAGGTTGTTGTTACGTTGT	GGGAGGGTAATTCAGGTAAG
121	**RM - 296**	CACATGGCACCAACCTCC	GCCAAGTCATTCACTACTCTGG
122	**RM - 434**	GCCTCATCCCTCTAACCCTC	CAAGAAAGATCAGTGCGTGG
123	**RM - 410**	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG
124	**RM - 108**	TCTCTTGCGCGCACACTGGCAC	CGTGCACCACCACCACCACCAC
125	**RM - 242**	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
126	**RM - 288**	CCGGTCAGTTCAAGCTCTG	ACGTACGGACGTGACGAC
127	**RM - 553**	AACTCCACATGATTCCACCC	GAGAAGGTGGTTGCAGAAGC
128	**RM - 278**	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
129	**RM - 201**	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
130	**RM - 245**	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG
131	**HvSSR 10-1**	ATGTATCGCTCGACAGATTT	CCGATTCATTGATGATTTCT
132	**HvSSR 10-5**	TCTCGCTCACTACCAGACTT	AATTTCGCTTCACATCACTT
133	**HvSSR 10-17**	CGTCTTGAATCAATTTCCAT	GATTGCCCGTAGAACTATTG
134	**RM - 222**	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG
135	**HvSSR 10-34**	TAGACCGAGGAATTGAAAGA	TTTGGGCTTATTGTCAGTTT
136	**RM - 171**	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
137	**RM - 228**	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
138	**RM - 484**	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
139	**HvSSR 11-1**	TGTGTGTCCGCATACTTAAA	ATGTCAAAGTCCGAAAGTGT
140	**HvSSR 11-2**	TAGATTGGGTGATGGATAGC	CTACTTGATCCAGGGAAATG
141	**HvSSR 11-3**	GGTTGACACCGTTAACATTT	TGGAACTACCTACCTAGCCA
142	**HvSSR 11-13**	TGAAACCACAATGAGTCAAA	GCCCTAAACCCAAATAGAAG
143	**RM - 202**	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
144	**RM - 229**	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT
145	**RM - 21**	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
146	**RM - 26334**	GACTCCCTACTAGTGGTTCTGATTCG	CCTTTGACGATTGTGATGCTACG
147	**RM - 206**	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
148	**RM - 254**	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
149	**RM - 224**	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
150	**RM - 20**	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
151	**HvSSR 12-35**	ATGACCATAATCCCAACAAA	GTCGTGGTGTTATTCTTGGT
152	**HvSSR 12-36**	ATCAGCGACTAAGGATCTCA	CTAATGTTGCCACATACGAA
153	**HvSSR 12-40**	ATCTAACAACAACAATCCCG	CATCTTCATCCCTCGTGTAT
154	**HvSSR 12-48**	AAACTCGATCAGACTTAGAGAAG	TCTCTGATGGCAATACAACA
155	**HvSSR 12-51**	AATCATCATATTGCCGAAAG	ATCACCATCTATCATTGCAC
156	**RM - 277**	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG
157	**RM - 511**	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC
158	**RM - 260**	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG
159	**RM - 519**	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
160	**RM - 28305**	GTCATCTTCGCAAATGGTGATGG	GGTCGTCGTGGTGTTATTCTTGG
161	**RM - 270**	GGCCGTTGGTTCTAAAATC	TGCGCAGTATCATCGGCGAG
162	**RM - 17**	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA

Appendix II: SSR markers used for parental polymorphism (Danteshwari and Dagad deshi)

S. No.	SSR marker	Forward primer	Reverse primer
1	RM6464	CGAGGAGAATACTCGTTCGGTAGC	CCCTTCTCCATCTCATCTCACTCC
2	RM10018	ACTAGTACACCTCAACTTCACTCC	CCTTTAGTTTGCTTGTGACC
3	**RM3252**	ATGCAAGCATCTGCTTATGG	GTTGGTAACTTTGTTCCCATGC
4	RM6470	AGTGTCAGAACCAGGATGAAATGC	AGTGCAGCTTCAGCCATGAGG
5	RM8068	GTGTCATATGCAAGCAACAACTCC	AGTATGTACGTCTCCTCCGTTGC
6	RM10218	AGCTAGCCCTCTTGTCTCCATGC	CACACTAGCACAGTGGCATACTGG
7	**RM3746**	CCTTGATCGGAAGTAGCTCAACG	GCCCTCAGAGCAGTAAGGAGAGG
8	RM8131	ACCGGCGGTCCTAATAACTATGG	GGTCCGATTAGATGAGAATCAGTTGG
9	RM600	CCTGTTAGGTGGCACATTTATGG	TCGGCCCTGTATATCATTACTCG
10	**RM8071**	GGACAAGAGGTTCCTCAATCTCG	AAACGGGATTATCGCCACTACC
11	RM140	CTTGCACAAGAGATGATGATGAGC	CATGCTGAGAAATAGTACGCTTGG
12	RM594	TCGAGAGAGGGAGAGTGAGAACATGG	GCCTTCGCACATAAAGGATGAACC
13	**RM129**	CACTCCACGCCATCTCTCTCG	CTCAGACTCGGAGCTCATCTTCC
14	**RM8139**	GGAACAAACGAAGCCTTAGTGG	GGACCCATTGTATCTGGTATTACGG
15	**RM3341**	GTCGTCGCCATCATTGGTATCG	CACCTTTCCCGGATCAAGTACG
16	**RM7405**	CCCATATATAGGAGGAGGAAGG	AGTCAGTCATCACTGGTAGTCG
17	**RM11307**	AAAGCTCTGCAATCTTCTCTCC	GAATACGACATCAGAACAGTGC
18	RM6738	GCAAGTCGATAGGCTAATCAATCC	CATGTGTAGTGGGAAGATGTGTGG
19	RM128	TGATTTCTTGGAAGCGAAGAGTGAGG	CCTCCTTGTGCTCAGCCATGC
20	**RM1095**	CCCATTCAGTTGATCCTGTCTGC	AGCTGGGATGCAGAAGAGTATGG
21	RM11739	TCTTCAGGGAGAAGATGAGG	TTCACAAACGAACTCCAAGG
22	RM3285	AAGGAACGCGAGAGAAGAGAACC	ATTCTGAGCAGGAGAAGGGAAGG
23	RM3738	GTGATGGGAGAGCAGGAGAAGG	CGCAAGTGGACCTCTTATTGTGC
24	**RM5794**	AATCCGTCAACCACCATGAACG	TTGCAGACTCATGGACACATGG
25	RM5310	GGGACCAAGACCTTTCCAATGC	GCGGAAGCAGGAGAATCGTAGC
26	RM3362	ATTGCGTGGGATATGGACTATGC	CCTCTTCAATTCCCTTCCTCTGC
27	RM12276	GTCGACGGCTTCCTCAAGATTGG	TGAGACCTCTGTGAAGGCACTCG
28	RM6840	CGACTGGAAGAAGGGATCATGG	CACACTACCAAGACTCCGCTATGG
29	**RM6842**	CCGTGCATCTCGCTACCTAACC	TGCACACACAACTTAGAGGAAGAAGG
30	RM6367	GCAACCACGACATCAAAGAAACC	GGAGGTTAGTGCTTCGGAGTGG
31	**RM12368**	GAGATAAGTGCCACGATTGATTGC	GGAGCCGTACGAGTAATCTCTGC
32	RM12478	CTAGGGTTTGCGGGCGATGG	CCGCGGTTGACGTAGATGATGG

33	RM6616	TCAAGATCAACGCACTCCTCTCC	TCGTACGAGCAACAGGTGGTAGC
34	**RM4355**	GGGATGAGAGTAGAAGGCACAAGG	GCTTAATGCCTTTGATCGTTGC
35	RM555	TTGACATGCGAAATGGAGATGG	TTGGATCAGCCAAAGGAGACC
36	RM12923	AAATGCACAGGCATTCGTAGACC	GAAGAAGTGGATGGAGGACATGG
37	**RM6375**	CGAATGGAACGACAAGAGATGC	ATAGATCGACAACGTAGATCCACAGG
38	RM452	GTGGACTTGGCGAGATGCTACG	GTTAAGGGCAGCCACCAGATCG
39	RM550	GCTCTGGTCCGAAGTCTTCTTCC	GGCTAAGCCCACATGAGACAGG
40	RM13069	GGAGGGTTATTCTCGCGTAAAGG	GACCCTTCACATTTCGGTCAGC
41	RM13170	GCCTCTTCACCCTCATCCTTCTCG	CGCCTGCCACCTTTATCTTGAACC
42	**RM6509**	GTAAGACGGACAGCCATGGAAGC	GACAGCACCTGCTCGAACTGC
43	**RM13541**	CTCCTCGCTTCGTCCACTTCC	CCATGTGTCACCGACTCAACG
44	RM13542	CTTGCTTCAAGCGGTGGTGACG	CACGTCGCTCCACTCCAGATCC
45	RM6617	CTCCTCCTCCCACCTCTACTCC	TAATAGATGGCGATGGACGAAGG
46	RM13611	CTCTTGAACGGCTGCACGAAAGG	CGCGAGAATGGTAGGTGGATCG
47	RM573	TCATGTTGACGCACACATACACG	CTCTTCTTCCCTGGACCACACC
48	RM425	ACCACAGCAGGTGGAACAGG	GCTAGCTAAGCCAACACCAACG
49	RM14001	TGTGGCTGGGCTCCGATACC	ACCCTGCAGGATCATCAGAACG
50	RM6307	GCCTCACCTCATCATCACAACG	TGGTCCTTCCTACTGTTGGTTCG
51	RM1063	TTGATCCACTATCGTGGCCTACC	TGGCAAGTGCAACTGCAAGG
52	RM208	AGTACCACCACCATTCTCTGCAAGC	TCGATTGGCCATGAGTTCTCG
53	RM3850	GCCTCGAGAAAGAAGCATGTCC	AAGCGACCTGTACCTCAACTTCG
54	**RM7485**	TCATGGAAATGCCAGTTTCTCC	TTCTTCGATTAACTAGCCTCGACAGC
55	RM5807	CGCCATGCCTAACCTATTATTGTGC	ATCGTCATCCATCCAGCAAGC
56	RM3654	AGGCGAGAGAGATGGAGATGG	GCGGCGGATATAAAGAGAATGG
57	RM3265	CCGTCTCTCTGTTGTTGTTCTGC	TGGTTCGCCAACAATTAAGACG
58	RM60	CAAGTTCACCCGCCTTCTCG	TTTCCATCATTAGCAGGCAGTAGC
59	RM14303	GTCTGTGCGCTCCTTGTTCTAGC	CCTGGACCAATTTGTATGGTTGG
60	RM14327	GATGCAGTAGGAACACCAAACAGC	ATCGAGTACCAAGTGCCTGTGC
61	**RM5474**	GTGGGTTTGTGTTTGGAGAGACG	GTGTTGGTGAGCATAGCAGTTGG
62	**RM3392**	AGCAACCAACCCAGTAGTTAGCC	GCTCATTTGCATGCTGTGTTAGC
63	RM175	GACGGAGGAGTTCGAGAGGAAGC	GTGAAGCGACTAGGCGGAGAAGG
64	RM14593	GCACCACCGCTCACACTTGC	GCTCCGGTTTCTCGAGGAGTTTACC
65	RM1256	TTCTTCTTCGTCTTCTGGTTCC	CAAAGCCATACTAAACCCTAGCC
66	RM5748	TATCCAAGCCTCCATACACATAGTGC	ACAAGAGGTGAAGAACTGGATGAGC
67	RM3204	CTTACACACATGGCCCACATGC	TCCTCTCTTCTCACTCTCCCAACC

68	RM15064	AACGTAACACAGCTGAGCAGCTACC	ATCTCCTGCAGCCTCCACACC
69	RM15065	ATAGAGGTCGTCGGGAGGAAGG	ACTGAGTTCCAATGGCTCAACG
70	RM7642	TTAGATCACGATACTCAGGGATGC	CGAAGAAAGAGAGCACGAAACG
71	RM15281	CGGGCTTATATCTTTGGCAAATGG	GCCTCCTCCCTCCTTTCTCG
72	**RM5626**	GGACGCCACCTTCCTCTTCTGC	CGGTCATAAACGCCATTAGACCAAGC
73	RM15567	TGCATCGATCCTTTCTTTGTCC	CCGGGACGCTATATGTTCTTCC
74	RM426	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTCATTGTAGAGTGC
75	RM15721	CAGCCAAGCTCTCCAATTAAACC	TGTGCCTTCTGATCTGATGTTGC
76	RM15741	AGCTGTTAGCCGACTTGTCAACC	TCTCCAACAGTTCTTCGATTGTCC
77	RM3525	CGCAAACGACACAAGTCATTACC	CTCAGCTCATCAAGACCTCAAGC
78	RM1373	TGCTATACCCAAATGTCCAAGC	ATCTTCTGAGTGCTGCCAAGC
79	RM16140	CGAGATGGCGTCAGCGAAGG	AAATGGCGAACAAGGCTGTCACC
80	RM3684	CGACGCAACCTATACTTGACAAAGG	GGTGTCCCAGCAAGTATTTCACC
81	**RM16147**	GAGATCATCCTGAACAACCACTGC	TGTCCACCCAAACCCTCTTTACC
82	**RM7389**	ACGGATGCATGATCGAGATGG	TTTGAGCCGGAGGTAGTCTTGG
83	**RM7585**	TGCAATCGCGTAGTTGGTAGAATAGG	CTCGACTACCTCGCCATCATCC
84	**RM16368**	TGTCCAGAGAATGACAAAGTACGC	GGATGTATATCTGCCACCAAATGC
85	RM16382	ACTCTGGTATATCCAAGCGATCC	TCACACTCCGTTTCTGTTCAGG
86	RM16493	TCGGCAGCAACAACTCTTAAACC	TCAACAAGATGACTCCCTGTAGCC
87	RM6659	TGTGGAGGCTTAGGAAATTCTGG	TGTGAAACATGCCACGATACTGC
88	**RM16559**	CCTGGAACCTGGAGGTGTTCTCG	GTCGTGGACGATTTCTTCGTCAGC
89	RM401	GCATGAGCTGCTCTCATTATTGTCC	GAAACGAACCAAACGTTCATCG
90	RM16717	ACAGCACTCATCATTCCATGTCC	GATAACTGATCGACGCGACACC
91	RM6314	CATGTCTGATATTGCGGTTCAGG	TCAAGCCCTGCCCAACTACG
92	RM16825	AACTAAACACACCCTGAACAGC	ATGTAGTACCCAAAGCACAACG
93	**RM5979**	GTTTGAACTCTGGTCTCAATGC	ACAGTATACGTGGCTCAATCAGG
94	RM3708	GCGTAAGACGACGAGACCTTACC	TGATGACGTGGCTTTCATTTGG
95	RM3839	ATGCATGTGATGCCAAGAGTGG	GAAAGCACACTGCACACATACCC
96	**RM17303**	TAGCGATTGGATGGAGGCTGAGG	GGTCGCCTCGCCATTAGTTACG
97	**RM17388**	CGAGCCACGCACTCAACTCTATCC	GCTATGTTGACGGGCCTTGTGG
98	RM349	GCTCGTCTTTCGTCTCTGTGTGC	AAGTACGCGCTGTCCATCATCC
99	RM8217	TTGCCAGATGGAGGTTCTAATGC	TTATCAACATGAGACTGCGGAGTAGG
100	RM17604	TCCTCTCCTCTCCGATCCTTAGC	CACATCACAACCACAAACCATGC
101	RM17616	TCGTCGTCCTCTTCTCTTCATCG	GCTAACACCGAAAGAGGCAAAGC
102	RM507	TGCCCATGTATGTGAGGTACTCC	GCCTAATCCAGGACAAGCTACGG

103	RM1024	AACTGCCATCTCTGAAACTCTGC	CATCTCACTTCAGAAGGATCATAGCC
104	RM17804	GTCACCACCACAATCTCTCTCC	GTCAATCTCCTCAAGAACTGACG
105	RM5374	TTAGGGTTAGGTCTGTTGTGTACTGG	CGCTGGATCACAACACTGTAGC
106	RM17863	GGACGTCATGATTTGTGCTTGG	GTCATTGGCTTGCAGGATACACC
107	RM17886	AAGAGATGTGTGGTTCAACTCC	CCTAGGTATTCCCAAGAGAGTCC
108	RM17962	CAGCAAATCTCTATCACTGCAACC	GCTAGATGACCACCTGCTGTACG
109	RM7588	GCAATTTCCGAAGCCCATGACG	GCCCATGGGTACGTGCTATGATCC
110	**RM3381**	ACGAACGCGAGCTGACAGAGG	AATAGCTGCCAGCAACTGCAACG
111	RM5140	GGCACTCGTATTTCTCAACTTCTCC	GGGTGTATCAGGAGTACAGGTTGC
112	RM6229	TTCACACTGCTCCACAGCCATCC	ATTCGATCGACGCCGGTAGGG
113	RM18451	ATATACAGCGCGGACATTGTGG	CATGTCATCTTCACGCGAATCC
114	RM18614	TGGCGCAATATCTCTCTCATTCC	TGCCACTTGTGTGTTGTTCTGC
115	RM6054	AGGCTCTTCGGCTTCATCTCC	GGTCTCTGATCAGTTTGCTTTGG
116	RM3486	GGAGGTCGGCACGTAGTAGAGG	GTCGGTACTATTCCTGCCATCG
117	RM538	CAGTTTGACACAAGCAGCAAGC	CTCCACAGCTTGAGGGAATGC
118	RM3790	CGATCAACCACCTCAACTACTGC	TGTGCATGTGGACACGTAATGC
119	RM3170	GCAGTGTCATTCTCATGAAACCTACC	CAGACTCCAAAGCACCCATAACC
120	RM19218	CGGAGGGAGTAGGTACGTAGGG	CCCATTCCATTCTACACTGACG
121	RM6775	AATTGATGCAGGTTCAGCAAGC	GGAAATGTGGTTGAGAGTTGAGAGC
122	RM7158	CATGGACTTGTTAGCAGCGTACC	ATTTCATCGATGGCCTCTACACC
123	**RM6467**	TGTTGGATTGGAATCGGAAAGC	CTCTGCTGTGCTGTGCTGCTAGG
124	RM1369	CATCGATTAGCTTACATGGCAACG	ACTAGTGCGACCGTCTTCAATGG
125	RM3805	ACACCACCATCAACGTACCAACC	AAGTCGAGAGGAAGAAGCCAAGG
126	RM19623	CATTCTGCAGTGTCCATTGTGACC	GTGGTTGGTCTGCTCCAGTTCG
127	RM3431	AAGGGAACATTCTGGAAGACACG	ACACATTGCGTGTAGTGTGAAGC
128	RM5963	TCAAGTTACGGGAAATGTGTGG	CTGCCTAGCTTCCGTTTCTCC
129	RM3183	GTGGTGCTAGTATGGACGAGAGG	CGGTTGGTAGACTGTAAACAAAGTGC
130	RM7583	ACTGAGCCAGCCACTGACAAGC	CCGTAAAGCAGTCAGTCATTCAGC
131	RM19976	AATGGAGTCATCGCACCAAGG	TTTAACTGGTCCCTACGATCCTATCC
132	RM20069	GCGAGCGAGAGGAGAGATAGACG	CGAATTCGGCACGAGTAATAGGG
133	RM20071	GGAGGCGCCTTGTTGGAACC	ATGTCATCGGAATCGGATCAAGC
134	RM20152	GGGTGGGCGTATTATAGATTCAAGG	TTAGGCCCAATAGTGGAGGAAGG
135	RM20155	GTCTCGTCTCGTCGTCTTCTCC	CCTCTAACCTCGTCGGAATCG
136	RM7434	AGGCTTCTTGGAATGGAACTGC	GGGAATATACGTGGATGTGAGAGG
137	RM1370	GGAGGGAGGAATGGGTACACG	TTGAGAGTGAAACGAGAACCAACC

138	**RM3765**	ACACCATGACACCAAAGGAAGG	GGATGCTTCCAATCCTCTCACC
139	RM5753	GCACCATCCGTCAGAAACAGC	TCCGAGAAAGAAGAACCGCTAGG
140	RM5463	AGTGCCTGTTTGTTTCCTCTTCG	CATGGTCAGAGCAAGTTAGTGTGG
141	RM6652	AAGCTCGTCGAGAGTAAGAAGACC	ACCAGTCCAATCTTCGTCATCG
142	RM3394	GAGAGGGAAGGAGTTTCTTAGC	TAGTTTACACGTACCCATGTGC
143	RM20891	GTGGATGTACGTATAAGCATGTGTGC	GTACGGCTGACGGCTTTATTCC
144	RM20973	CGTGATGGTACAGGGAAAGAAGG	CGCTACACAACACAACACAAATCG
145	RM3224	CGCGAGGAAGAAGAAGAGATCC	ATTCATCGAGTGACGGAGAGAGG
146	RM3484	TCCGGTCGTCCTCATCGTATCC	GCCCTCTTGCTCCCACATCG
147	RM21238	GAGCTTCTCCTCACCCATCACC	CTTCTGCAGAGGGTGTTCAACG
148	RM21242	GAGAGGAATGGAATGGAATGAGG	GAACAGGCATGGTGAAGAGTGC
149	RM21323	ATTGCGATGTTTGGATGTACCG	AGCAACTCGTCAATGAACATGC
150	RM3456	TTCTCCTTGCGACACAGATGAAAGG	GCCTCCTATATAAACCGGCGAACC
151	RM214	GAACATGCTTTCAACCATCAGG	GATCCTCTCAGTTCAGTGCAAGC
152	RM5543	GGTGCTAGGTACATGGCTGACG	AGAGCAAATTCTGGGCTATCTGC
153	RM500	AGAAGTGCAGGTTGGCTCTGC	CACGAATCTCGGAGTGTCTAGGG
154	RM5875	AATAAAGCGAGATGGACGAACC	TTTCCCACCAGAGGAAGATGG
155	RM2752	GATCCGCTTGCACATGGATAGG	TACACGTGTGAGCGAGCTTAGCC
156	RM22105	AAGTGGAGGCGTACCTTGTTCC	CGTATCGCGTTCGTAATGTTGC
157	RM6389	ACGAGGAGTTCGTCGCTACTGC	AGTCCTCGAACACGTCGACTTCC
158	RM420	CCTCTCACTCTGCCTGCTCTACC	TCTCTAACTCTTGAGTGACAGCAACC
159	RM7034	GTGACCAATCCTTTGACAACTCG	TGATACATGAAACGTCCGTCTAGC
160	RM3702	CCAGCACCATCTGAAATAGAAGC	CCATTGATTAGGACGGTCAAAGG
161	RM3309	GCCTACTCAGCTTCCTCTCCTTCG	CGCCATTTACGGCAGCAACC
162	RM22418	GCAGTATCACGCAGTAGCACACC	CCATCCTCTTCCTCATCACACG
163	RM547	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC
164	RM8243	TCTACCTTAGCTGTCCTGAATTGG	CACATACCTGTTCCGTTTGATCC
165	RM22694	TTAGCTGTATTTAGCCCGACATAGCC	CGCCGGTTCTTCTCCTCTTAGG
166	RM22696	AGGTGAGACTGTGAGAGTCATGC	AGAGTACGAAGCCTCTATCTGACC
167	RM22837	ACCTGGGTCAGATGTCTGTTTGG	GGTAGAGCTCCATCCATCTTAGTGC
168	RM22883	TTGCCTCCCTCAACTCATGTCC	ACAACGTGGACAAAGATGACACG
169	RM6990	ACTGGGTGTGATCCTTTCTGATGC	GTGATCCCAGATACACGATGTAGGG
170	RM6193	ACAGCTTCACGATGTTCTTGTGC	GTCAAGAAGCTCTGGGCTAACG
171	RM22997	AGTCATGGTGTTGGACTGTTGG	CAAGATGGATGTGTGAACATGG
172	RM23001	CAGTTCCTCTCCTCCACCACTTCG	TGGTGGACTGGAGGGCTACTGC

173	RM23175	CGTGAAACACAAGAACCAGATGC	AGCAATGTCTGCCTGCTACTGC
174	**RM23251**	TCCGATACTCCCATAGTTAGACC	ATGTGGGTTGGCTATAGTCTAGG
175	RM3845	TCGGATGAGTCCTTCGGTTTCC	TCTCAGCTCGATCTCCTCTCTAGACC
176	RM3452	TGGACTTGGTCTCTCCAAACTCC	CAGTATGTGTTGGTGGGTCAAGC
177	RM502	CATCTCTGTTCCACTTGCTTTGC	CTACCAACAACCCAACAAGAAGG
178	RM23502	GGCTAAAGCCTCAGAGCCAACC	ATTTCGTTCGTTCGTTCGTTCG
179	RM7631	TGAGACAGGCAACATATGCTGAGACC	GCATGTTCATCTTTGGTCACTCATGG
180	RM23654	CTCCGATGCCTTCTTCCTCTTGC	AAAGGGAGTAGCAAGCCGAGTGG
181	RM23662	GAGAGGACGATGGCACTATTGG	CGAGGAACTTGATTCGCATGG
182	**RM23736**	GGCGATACCTGCCATAGTTTCC	CCGAAAGCAATCTATGAGACACC
183	RM23742	GTGGCATGGAGAAAGGAGGAAGC	GGCCCAATATGTCACTCCCTACCC
184	RM23743	AGGTTTGCAGAAGCCAGAAAGAGC	GGCCGATGGTATGATGTGACC
185	RM5799	CTTGCACAAGAGGCAACACTCC	GTTTGGTAGGTCGCATTGTTTGG
186	RM23916	GATGGGTTGGGTGGGTTAGG	GTCCAGTGATAAGCCATGCTTGC
187	RM24085	CGACGAACTCCTCTACCGTTTACC	CTGCGTGTATCCAATCCCAAGG
188	RM3912	CACTCAGATTTGGCCGATCC	GCTGATCCAGATCTACCTGACACC
189	RM6845	CGGCAAACTCTACGAGGTAATGC	CACACGTTCTTCTCCTTCATCTCG
190	RM24271	TCTTGCATTGACACCTCTTGAGC	AGTCCCAACAACTGGAAGAGAGG
191	**RM257**	CCGTGCAACTTAAATCCAAACAGG	GGAATCCTATATGAGCCAGTGATGG
192	RM6491	GGTGTTCCTGCGTGGCTTCG	GTTCCCGCGCTTTGTCTGTCC
193	**RM24718**	TGACGTGGCAAGTTGACTGTGG	TAGCCGATGGAGCCACTAGAAGG
194	RM24740	AGTCAACACATGAGCGTGAGAGG	TGCTTTGGATGTACGACTTGTCC
195	RM1026	TCTGAGCTTACAGCTGCTTGACC	TGCTGCCTAGGCTAGAAAGTCC
196	RM6797	CTCCTCCATCAGGATCATCTCG	GTGAAGCCAGCTAGGTTGAATGC
197	RM7492	CAAGGATTTGAGGGAACATGG	TTTAGAGATGGTTGCCAAGAGC
198	RM24952	AGTGGAGGAATATGACTTCAGAGG	AGAAGATGGTAGCTGGCTTGC
199	RM6179	GCGCCGCCGAGATGATAAGC	AGCGGCATCTCGTCCATCTCC
200	RM3882	AGTATTGCCACCAGATGGCTACC	CCTTCCTCAAACTGGTGATTTCC
201	RM216	GATGGTAAAGGAAGAACGTGTGC	CACTCATAGACGCATCACATAGCC
202	RM6646	GGCAGTTTGATGTTGGAGAACTGC	ATTCAGCGACGACATGCACACC
203	**RM25149**	CATGCCTATTGCCTCTGATGACC	TTGCCCGTAGAACTATTGGATCG
204	**RM1126**	TATTCTACTCGCAAGTCGCAAGC	GTGGATCACAATGCGAACTTGG
205	RM25366	TCGGTCTCTGTGCCGTGATTAGG	CACCAGCGCAGCAACTAACATCC
206	RM5689	TTAAGTCCTGTAGTAGGTCACACC	CGATGAGTCAGATTGAAGTAGC
207	**RM1375**	GCTTGTTGGCAGTTTGTATTGTGG	GGTACACTAAGTGGGCAAATCAGG

208	RM25663	GCTTGAGCTCGTCGTGGAAGG	CTCCTGCGTTCTCCATCGAAGC
209	RM3510	ATAGAATGCCATGGAGACCAACC	AGACGACGAAGGCAGATAAGTCG
210	RM3563	TGGTACGGAAAGACGAGAGATGC	TGAGGCGAGTGTCTGATAACTGC
211	**RM6673**	CCACCCGTCTCATGTTCTACTCG	ATGGAGATGATACACTCGCATCG
212	**RM4771**	AACTGAGAAACATGGGACAGAAGC	CTCAACCACACCCTCATTCACC
213	RM590	GAGATCGAGGAGGAGGTGAGG	AGTACTGCCGATCATATGGAAGC
214	RM6327	GGAGCTGATACAACAATCAGACAGC	CGCTACGCTGCTCCAGATTAGG
215	RM7173	TTTCTGTGGGTCCTCATCTTCTCTCC	CAATGTGTGATGTCGGATTCTTGG
216	**RM26063**	GATCCATATGCCTCTTCGATTGG	AACTCCAGCAGTGAGAGCGTAGC
217	**RM26105**	AGCATCAATTCAGCTTGCTTGC	TTCTGGTTCTTTGAGAGAGTGTGC
218	**RM332**	GAAGGCGAAGGTGAAGAAGAAGC	CCTCCCTTGCATGATACCTTGG
219	RM26108	TTGTGCCTGTGTCAGAAGAACTAGG	GCAAAGGTAGCTAACACACATACGG
220	RM26249	AGAAACAGCTGGAACTCACAATGG	CATGCTCGGCTCCTCTACCC
221	RM536	TACCAGGATCATGTTTCTCTCC	ACTGTGAGATTGACTGACAGTGG
222	RM5590	GAGGGAGGGAGTACATATCTGATCG	AGCAACTGGATAAGCGATTGAGG
223	**RM26643**	CTCCCTTCTTCTGCTGAGACACC	CAAGCTACTCATTGGGCTCATCC
224	RM26646	GTTCATCGGATTCTCGGTTCAGG	GCGGAGGAACAAGACACGAAGC
225	**RM26652**	CAATCCATTGCTGGTTGATGC	CAAGATCTCCAAGGTGCTGAGG
226	RM26796	TTCAAGACATGGTGTGGATCTGG	GCCACTGTGCCAACATTATAACC
227	**RM26998**	ACGCACGCACATCCTCTTCC	CGGTTCTCCATCTGAAATCCCTAGC
228	RM27167	GCGATCTCTCGCTTCTTGTTTCTCG	GCTCCGCGTCGACATGATGG
229	**RM27318**	TGAACTGGACATGCCTTTATCC	ATGCGCCTTACTTTGTTGTAGG
230	**RM27326**	ATCAACGAGTACGCAACAGTCC	TCCTGTCTCTTCACATCCTAATCG
231	**RM3577**	GTGACGGCAGAGGAGAGAAAGC	CAAATCTTTCTGCATGCCACACC
232	RM7443	ATTAACCCTTCATCAGGCTACGC	AAAGTGCTGCGTGTTACTTTGG
233	RM5568	GATCCAAATGGAAAGACCTGTGC	TTATTGCTTGCGCCTTTAGTCC
234	**RM3323**	CATCTTGGCGTTGATACGAAGG	ACGAGTCGAGCACTACATCTCTCG
235	**RM5927**	TGTATAGCCCGGAAGTATGATCC	TCTGGTCTCGTCTCTCATGTGC
236	RM3747	CTTGGTTTGGTTGTGTCCAAGC	GTTCTGATGAACAGGCCGTAGC
237	**RM27542**	GCCATCTTCTCGTACCTCTTCTTCC	CCACCTACACCACCACGTACTCC
238	RM27564	CTTCTTCGAGGGTTTCTCGTTGC	CTCCTCTTATGCCCACCACTTCC
239	RM27706	ACTCCCTCCGACACCATCATCC	ATACGGGAACCCTCACGCTACC
240	RM1036	CTTCCCTGTCCATAGATCTGCTTGG	GAATGACCCAACCTGTCATTCTTAGC
241	**RM27877**	GGAAGCCATGAAAGATGTGTTGC	AATTTCTCCGAGCACCTGAAACG
242	RM27962	GGGAGTCGTGGATTCTGAGACG	ATCCCACGCCAGGAGATAATAAGG

243	RM27970	TCCACCACTCTGACGTCTACTAACC	CTGCGGGAAGTGTAGGAGAAGC
244	RM27973	CCACACTGCCCAGGATTTAAGC	CTGTTCCCATCATCCAAATGACC
245	RM28130	CAGCAGACGTTCCGGTTCTACTCG	AGGACGGTGGTGGTGATCTGG
246	RM28404	GTGGGAGTCGAGAGGCGATAAGG	AAAGGACGGCTCATAGGTGATGG
247	RM7018	TTTCTCTCCACCCGTTCCTCACG	CAAGCATGGCCAAGTCATGTCG
248	**RM28607**	AGCATTACAGTGTCCAGGTAGGG	CCTCCCTTCTTATATGCCTTTCC
249	RM6265	TTCTTCTCCAGAGACACCAAGAGC	TCTTCTTCTCTTCCCTCCTCTCG
250	RM6396	ATCATCGTCGTCATCCTCTCTCC	CGTCCAGTTCGTAGGCGTATAAGG
251	**RM1159**	TGTGAATGCCCGTATGGATGG	TCTGAAACCATATCGTCGCATACC
252	**RM6411**	GTGAAGGCTGTACCATCCAACG	CGGGTATTGTCGGTGTTCAGG
253	RM5313	TCTTCTACTCCTCGTCTTCGTTCG	CATGCAGAGCAGAGACTTCTTGG
254	**RM1227**	CATCGACATGTGGACCACTCC	GCCTGAGACAAGTCCATGGTAGC

Appendix III: **Mean phenotypic value of diseases symptom in RIL population**

Mean phenotypic value of Bacterial blight resistance in RIL population

Ori. No.	Line No.	Mean Symp. Length (cm)	BLB qualitative (0-9) scale Score
10	1	6.85	5.00
11	2	2.03	1.00
12	3	3.93	3.00
14	4	3.55	5.00
15	5	4.19	1.00
16	6	3.81	1.00
17	7	4.35	5.00
19	8	6.38	5.00
20	9	4.52	1.00
22	10	3.89	1.00
23	11	5.83	3.00
25	12	3.08	1.00
26	13	4.91	3.00
27	14	20.05	9.00
28	15	3.97	3.00
30	16	2.11	1.00
31	17	3.81	1.00
32	18	2.03	1.00
33	19	9.18	7.00
35	20	8.88	5.00
39	21	5.4733	5.00
40	22	4.7067	1.00
42	23	6.14	7.00
44	24	6.4867	5.00
45	25	3.2867	1.00
55	26	5.7067	1.00
58	27	8.7867	7.00
59	28	2.8	1.00
60	29	4.4267	5.00
65	30	6.5733	1.00
70	31	2.6133	1.00
72	32	3.76	3.00
74	33	3.9133	7.00
76	34	2.4667	1.00
78	35	2.1333	1.00
79	36	2.74	1.00
80	37	2.0733	1.00
84	38	11.26	7.00
85	39	21.02	7.00
89	40	2.7733	1.00
91	41	3.0333	3.00
92	42	7.2867	7.00
93	43	4.34	1.00
95	44	3.44	3.00
98	45	3.5133	3.00
102	46	4.6533	5.00
104	47	11.447	7.00
105	48	15.633	7.00

106	49	13.6	7.00
109	50	3.9667	1.00
114	51	7.16	7.00
118	52	6.66	7.00
119	53	2.58	1.00
121	54	5.8933	5.00
124	55	10.653	5.00
125	56	5.3667	5.00
126	57	2.5867	1.00
128	58	4.02	5.00
130	59	6.84	7.00
133	60	8.2333	5.00
139	61	9.3867	5.00
140	62	2.18	1.00
143	63	6.5667	5.00
148	64	6.5933	5.00
149	65	3.9733	5.00
155	66	4.7133	3.00
156	67	2.66	1.00
157	68	4.88	1.00
159	69	4.2733	3.00
164	70	2.8	1.00
165	71	1.6733	1.00
168	72	2.8267	1.00
171	73	1.9733	1.00
172	74	3.02	5.00
174	75	18.807	9.00
175	76	2.6933	1.00
177	77	2.0667	1.00
179	78	2.0333	1.00
180	79	17.493	5.00
186	80	14.667	7.00
191	81	2.7	1.00
192	82	2.76	1.00
193	83	1.9067	1.00
197	84	3.3333	5.00
198	85	2.3933	1.00
201	86	2.8533	5.00
202	87	4.7667	5.00
210	88	1.8333	1.00
211	89	3.2267	5.00
212	90	2.42	3.00
213	91	4.2	5.00
215	92	19.433	7.00
218	93	2.9867	5.00
220	94	1.5133	1.00
224	95	13.667	5.00
225	96	5.5467	5.00
226	97	8.0267	5.00
227	98	8.8467	7.00
229	99	2.2733	3.00
230	100	5.2133	5.00
231	101	6.74	5.00

237	102	5.28	5.00
239	103	6.5733	5.00
240	104	9.7133	7.00
241	105	3.26	1.00
242	106	2.62	1.00
244	107	1.9	1.00
245	108	2.64	3.00
246	109	0.8933	1.00
251	110	7.46	5.00
252	111	6.0133	7.00
254	112	4.5467	3.00
255	113	4.3933	5.00
256	114	5.8067	5.00
257	115	1.6133	1.00
259	116	3.2867	7.00
261	117	2.9333	5.00
262	118	8.1933	7.00
268	119	14.387	7.00
269	120	2.7267	3.00
270	121	24.82	9.00
271	122	9.26	7.00
P 1	P1	2.28	3.00
P 2	P2	11.347	7.00

Mean phenotypic value of Sheath blight resistance in RIL population

Ori. No.	Line No.	Total lesion Area (cm^2)	Individual lesion Area (cm^2)	Lesion Length % of Sheath length
10	1	13.53	1.19	8.77
11	2	20.59	1.26	7.22
12	3	21.51	1.31	6.91
14	4	25.24	1.43	5.51
15	5	20.03	2.35	9.39
16	6	10.34	1.50	9.81
17	7	18.13	1.30	8.96
19	8	10.40	1.12	8.40
20	9	9.81	1.26	11.09
22	10	8.42	0.99	8.18
23	11	9.29	0.87	5.32
25	12	14.51	2.43	8.53
26	13	12.40	1.69	12.08
27	14	12.17	0.67	5.41
28	15	19.94	1.66	11.76
30	16	8.50	1.31	10.40
31	17	11.79	1.46	9.22
32	18	8.90	0.86	8.08
33	19	10.87	1.28	7.28
35	20	11.45	0.87	6.48
39	21	9.68	2.59	16.64
40	22	11.23	1.12	7.37
42	23	11.17	0.65	5.48
44	24	9.45	1.08	7.72

45	25	6.03	0.78	5.41
55	26	6.03	0.59	4.00
58	27	9.59	0.97	5.70
59	28	5.89	0.78	9.27
60	29	13.57	3.25	13.36
65	30	8.98	1.03	5.97
70	31	12.13	1.85	10.89
72	32	7.54	1.20	8.83
74	33	11.88	2.04	10.51
76	34	15.05	4.21	6.34
78	35	7.90	1.24	6.78
79	36	11.47	1.30	8.13
80	37	9.76	1.43	11.76
84	38	9.90	1.27	6.80
85	39	11.14	1.41	7.52
89	40	7.87	1.42	8.40
91	41	8.75	2.21	10.71
92	42	17.66	1.85	10.95
93	43	12.58	1.87	9.23
95	44	19.32	1.34	7.43
98	45	16.28	1.41	6.53
102	46	9.18	1.04	7.15
104	47	13.22	0.73	5.08
105	48	19.95	1.60	6.05
106	49	19.94	2.64	11.59
109	50	26.64	1.80	9.29
114	51	9.94	0.88	5.36
118	52	10.91	2.36	8.94
119	53	11.98	1.46	11.26
121	54	8.53	1.56	8.65
124	55	15.63	1.09	5.49
125	56	12.16	1.28	8.61
126	57	11.36	1.61	10.65
128	58	11.63	1.91	10.68
130	59	10.76	0.88	6.35
133	60	11.90	1.36	8.42
139	61	10.40	1.39	9.23
140	62	10.33	1.32	9.78
143	63	17.51	3.40	13.67
148	64	12.75	1.48	8.31
149	65	24.19	1.32	7.29
155	66	16.72	1.80	11.64
156	67	14.46	1.78	10.37
157	68	13.69	1.45	7.59
159	69	24.23	2.24	8.15
164	70	12.17	1.79	14.12
165	71	2.90	0.84	8.63
168	72	12.15	0.89	7.95
171	73	8.25	0.90	8.99
172	74	8.39	1.11	9.89
174	75	3.79	0.39	2.92
175	76	12.41	0.61	6.16

177	77	7.26	0.67	7.74
179	78	10.16	0.81	7.81
180	79	8.99	1.15	10.54
186	80	12.73	1.22	7.55
191	81	14.32	1.55	11.67
192	82	11.41	1.22	9.39
193	83	10.60	1.24	9.82
197	84	7.15	0.97	9.18
198	85	9.30	1.21	8.58
201	86	13.23	1.34	9.07
202	87	5.30	0.94	7.20
210	88	8.98	1.32	10.22
211	89	13.21	1.29	6.46
212	90	9.78	1.32	8.75
213	91	12.96	0.95	4.96
215	92	9.70	0.28	3.97
218	93	6.82	0.50	4.89
220	94	8.29	0.48	4.29
224	95	4.08	0.26	3.70
225	96	3.57	0.38	5.00
226	97	4.78	0.59	5.31
227	98	5.68	0.57	4.51
229	99	16.41	1.56	8.51
230	100	1.80	0.23	4.68
231	101	8.10	0.97	9.93
237	102	9.59	1.85	13.98
239	103	21.08	1.70	13.88
240	104	3.25	0.35	3.73
241	105	5.18	0.82	10.71
242	106	14.73	1.38	8.02
244	107	18.91	1.54	14.55
245	108	16.78	1.62	10.63
246	109	15.06	1.09	11.12
251	110	12.23	1.34	10.80
252	111	11.44	1.26	9.84
254	112	8.34	0.65	6.98
255	113	10.02	0.98	7.70
256	114	17.15	1.85	8.37
257	115	10.57	1.19	9.76
259	116	13.27	1.64	11.65
261	117	11.10	1.16	9.90
262	118	13.63	1.15	9.40
268	119	16.77	1.59	10.44
269	120	14.81	2.28	13.14
270	121	12.02	1.45	11.14
271	122	9.41	1.27	10.83
P 1	P1	10.28	1.62	13.72
P 2	P2	24.14	1.05	7.82

Mean phenotypic value of Blast resistance in RIL population

Ori. No.	Line No.	Blast reaction R2 13-08-2013
10	1	3
11	2	3
12	3	3
14	4	3
15	5	3
16	6	3
17	7	3
19	8	5
20	9	3
22	10	3
23	11	3
25	12	5
26	13	5
27	14	3
28	15	5
30	16	3
31	17	7
32	18	5
33	19	5
35	20	5
39	21	1
40	22	3
42	23	5
44	24	5
45	25	2
55	26	3
58	27	3
59	28	3
60	29	3
65	30	3
70	31	5
72	32	3
74	33	3
76	34	3
78	35	3
79	36	5
80	37	5
84	38	5
85	39	2
89	40	5
91	41	3
92	42	2
93	43	2
95	44	3
98	45	1
102	46	1

104	47	3
105	48	2
106	49	3
109	50	3
114	51	3
118	52	3
119	53	1
121	54	3
124	55	3
125	56	2
126	57	2
128	58	1
130	59	3
133	60	2
139	61	3
140	62	2
143	63	2
148	64	3
149	65	1
155	66	3
156	67	1
157	68	1
159	69	3
164	70	1
165	71	1
168	72	2
171	73	1
172	74	1
174	75	1
175	76	1
177	77	1
179	78	1
180	79	3
186	80	3
191	81	7
192	82	7
193	83	1
197	84	7
198	85	5
201	86	5
202	87	7
210	88	0
211	89	7
212	90	7
213	91	3
215	92	3
218	93	7
220	94	5
224	95	5
225	96	6

226	97	6
227	98	7
229	99	7
230	100	7
231	101	5
237	102	7
239	103	5
240	104	5
241	105	7
242	106	9
244	107	5
245	108	9
246	109	5
251	110	9
252	111	3
254	112	7
255	113	7
256	114	7
257	115	7
259	116	7
261	117	5
262	118	3
268	119	7
269	120	3
270	121	8
271	122	4
P 1	P1	7
P 2	P2	2

Mean phenotypic value of Brown spot resistance in RILs population

Ori. No.	Line No.	Score (0-9 scale)	Ori. No.	Line No.	Score (0-9 scale)
10	1	9	143	63	4
11	2	8	148	64	3
12	3	6	149	65	2
14	4	6	155	66	7
15	5	5	156	67	4
16	6	3	157	68	2
17	7	8	159	69	4
19	8	3	164	70	2
20	9	4	165	71	2
22	10	3	168	72	4
23	11	6	171	73	7
25	12	2	172	74	5
26	13	3	174	75	3
27	14	5	175	76	4
28	15	2	177	77	2
30	16	5	179	78	5

31	17	9	180	79	7
32	18	7	186	80	4
33	19	8	191	81	2
35	20	8	192	82	2
39	21	2	193	83	4
40	22	6	197	84	7
42	23	7	198	85	2
44	24	7	201	86	2
45	25	9	202	87	4
55	26	3	210	88	2
58	27	3	211	89	7
59	28	2	212	90	6
60	29	4	213	91	4
65	30	5	215	92	3
70	31	7	218	93	2
72	32	3	220	94	2
74	33	5	224	95	5
76	34	2	225	96	6
78	35	2	226	97	8
79	36	4	227	98	7
80	37	2	229	99	3
84	38	5	230	100	4
85	39	3	231	101	6
89	40	2	237	102	4
91	41	4	239	103	5
92	42	5	240	104	3
93	43	4	241	105	4
95	44	4	242	106	5
98	45	5	244	107	7
102	46	2	245	108	6
104	47	3	246	109	4
105	48	4	251	110	3
106	49	8	252	111	5
109	50	4	254	112	7
114	51	6	255	113	3
118	52	8	256	114	2
119	53	7	257	115	3
121	54	8	259	116	6
124	55	9	261	117	6
125	56	8	262	118	5
126	57	7	268	119	5
128	58	8	269	120	6
130	59	7	270	121	7
133	60	3	271	122	8
139	61	2	P 1	P1	4
140	62	3	P 2	P2	5