

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

Ph.D. THESIS

By

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**DEPARTMENT OF PLANT MOLECULAR BIOLOGY
AND BIOTECHNOLOGY
COLLEGE OF AGRICULTURE,
INDIRA GANDHI KRISHI VISHWAVIDYALAYA,
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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 Agriculture (Plant Molecular Biology and Biotechnology)**” of the Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) 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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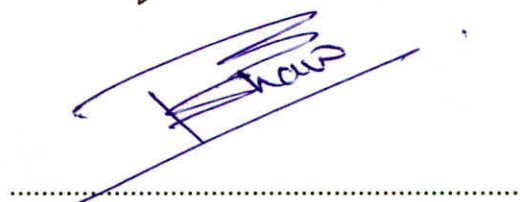
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LINCOLN MANDAL

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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 Electrophoresis
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
RGAs	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
<i>Xoo</i>	<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i>
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

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) (Arumugathan 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

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 Kyoizuka, 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). 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
<i>Xa1</i>	4	Kogyoku	(Yoshimura <i>et al.</i> , 1998)
<i>Xa2</i>	4	Tetep	(He <i>et al.</i> , 2006; Oryzabase, 2006)
<i>Xa3</i>	11	Wase Aikoku 3	(Kaku and Ogawa, 2000; Ogawa <i>et al.</i> , 1986; Qi and Mew, 1985; Sun <i>et al.</i> , 2004)
<i>Xa4</i>	11	TKM6	(Wang <i>et al.</i> , 2001)
<i>xa5</i>	5	Aus Boro lines (e.g. DZ192)	(Iyer and McCouch, 2004)
<i>Xa7</i>	6	DV85	(Lee and Khush, 2000; Porter <i>et al.</i> , 2003)
<i>xa8</i>	7	PI231129	(Sidhu <i>et al.</i> , 1978; Singh <i>et al.</i> , 2002)
<i>Xa10</i>	11	Cas 209	(Oryzabase, 2006; Yoshimura <i>et al.</i> , 1983)
<i>Xa11</i>		IR8, IR944	(Mew, 1987; Oryzabase, 2006)
<i>Xa12</i>	4	Kogyoku	(Mew, 1987; Oryzabase, 2006)
<i>xa13</i>	8	BJ1 (Aus Boro)	(Chu <i>et al.</i> , 2006)
<i>Xa14</i>	4	TN1	(Oryzabase, 2006)
<i>xa15</i>		M41, a Harebare mutant line	(Gnanamanickam <i>et al.</i> , 1999; Nakai <i>et al.</i> , 1988)
<i>Xa16</i>		Tetep	(Oryzabase, 2006)
<i>Xa17</i>		Asominori	(Oryzabase, 2006)
<i>Xa18</i>		IR24, Toyonishiki	(Liu <i>et al.</i> , 2004; Oryzabase, 2006)
<i>xa19</i>		XM5	(Lee <i>et al.</i> , 2003; Oryzabase, 2006)
<i>xa20</i>		XM6	(Lee <i>et al.</i> , 2003; Oryzabase, 2006)
<i>Xa21</i>	11	<i>O. longistaminata</i>	(Song <i>et al.</i> , 1995)
<i>Xa22</i>	11	Zhachanglong	(Oryzabase, 2006; Sun <i>et al.</i> , 2004; Wang <i>et al.</i> , 2003)
<i>Xa23</i>	11	<i>O. rufipogon</i>	(Zhang <i>et al.</i> , 1998, 2001)
<i>xa24</i>		DV86, DV85, Aus 295	(Khush and Angeles, 1999; Lee <i>et al.</i> , 2000)
<i>Xa25(a)</i>	4	HX-3, a somaclonal mutant of Minghui 63	(Gao <i>et al.</i> , 2001, 2005)
<i>Xa25(b)</i>	12	Minghui 63	(Chen <i>et al.</i> , 2002)
<i>Xa26</i>	11	Minghui 63	(Sun <i>et al.</i> , 2004; Yang <i>et al.</i> , 2003)
<i>Xa27*</i>	6	<i>O. minuta</i>	(Gu <i>et al.</i> , 2004, 2005; Lee <i>et al.</i> , 2003)
<i>xa28</i>		Lota Sail	(Lee <i>et al.</i> , 2003)
<i>Xa29(t)</i>	1	<i>O. officinalis</i>	(Tan <i>et al.</i> , 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 F₂ 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	<i>Xa-1</i>	IRBB1 Kogyoku/IR24	RFLP	Yoshimura <i>et al.</i> , 1996
2	<i>Xa-3</i>	IRBB3 (Chugoku45)/IR24	RFLP	Yoshimura <i>et al.</i> , 1992
3	<i>Xa-4</i>	IRBB4 (IR1545-339)/IR24	RFLP	Yoshimura <i>et al.</i> , 1995 Wang <i>et al.</i> , 2001
4	<i>xa-5</i>	IRBB5/IR24 (Chinsurah Boroll)/IR64	RFLP SSR	McCouch <i>et al.</i> , 1992, Blair and McCouch, 1997
5	<i>Xa-7</i>	No information	RFLP	Borines <i>et al.</i> , 2000
6	<i>Xa-10</i>	Cas209	RFLP	Yoshimura <i>et al.</i> , 1995
7	<i>xa-13</i>	IR66699-55-42/IR24	RAPD RFLP RFLP	Zhang <i>et al.</i> , 1996a Sanchez <i>et al.</i> , 1999
8	<i>Xa-14</i>	Japonica/Zhengzhuai (indica)	RFLP	Tan <i>et al.</i> , 1999
9	<i>Xa-21</i>	<i>O. longistaminata</i> /IR24	RFLP RAPD	Ronald <i>et al.</i> , 1992 Williams <i>et al.</i> , 1996
10	<i>Xa-22(t)</i>	Zhachanglong/ Zhonchu Ali	RFLP	Lin <i>et al.</i> , 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.



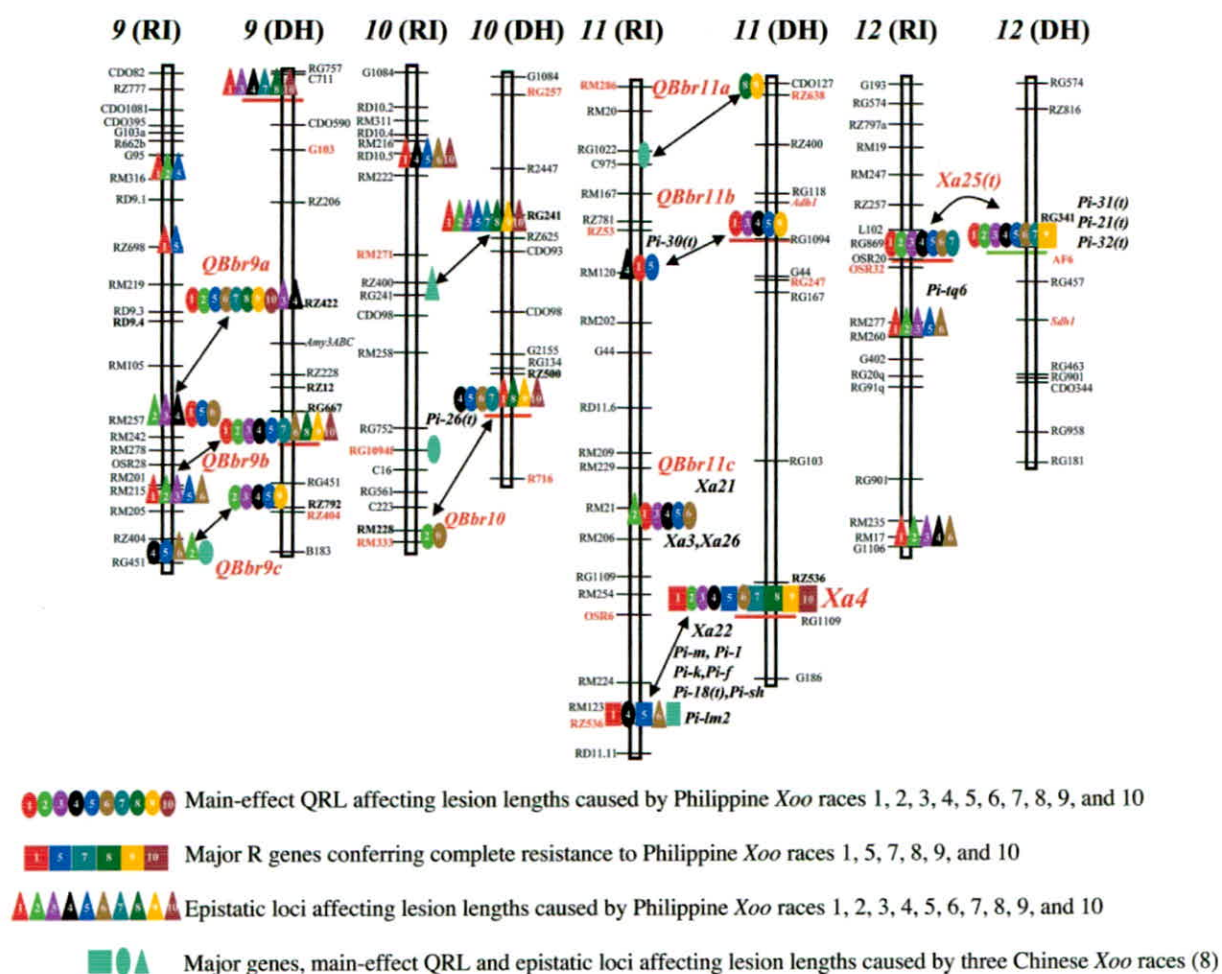


Figure 2.1 Genomic locations of *Xa4*, *Xa25(t)*, QRL (in red italics), and epistatic loci (triangles) associated with complete and partial resistance to 10 Philippine and 3 Chinese *Xoo* races detected in the Lemont × Teqing RILs (RI) and IR64 × Azucena DHLs (DH) population

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 (1993)
	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 <i>et al.</i> , 2009
	Colonized PDA plug, at 50 DAS	Greenhouse, high humidity environment	15 DAI: severity index scale	Liu <i>et al.</i> , 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 <i>et al.</i> , 2002; Prasad and Eizenga (2008) Eizenga <i>et al.</i> , 2009
	Colonized toothpick in leaf sheath, at stem elongation	Field	30 days after heading: 0—9 scale	Zou <i>et al.</i> , 2000 Zuo <i>et al.</i> , 2008 Yin <i>et al.</i> , 2009
	Sclerotia placed beneath the sheath		10 DAI: lesion length on the sheath	Chnnamallikajuna <i>et al.</i> , 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 <i>et al.</i> , 2004 IRRI, 1987
Leaf	Colonized PDA disc	Controlled conditions	3 DAI: severity on leaf; lesion length	Prasad 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 <i>et al.</i> , 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 <i>et al.</i> , 1982	Tapoochoz, Bahagia, Laka
8	Borthakur and Addy (1988)	Taraboli 1, Dholamula, Supkheru, Chidon
9	Gokulapulan and Nair (1983)	Bharati, Rohini
10	Ansari <i>et al.</i> , 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 <i>et al.</i> , 1992	LSBR-5, LSBR-33
13	Marchetti <i>et al.</i> , 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 <i>et al.</i> , 2008	TIL:455, TIL:514, TIL:642

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 <i>et al.</i> , 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 <i>et al.</i> , 1982	Tapoochoz, Bahagia, Laka
8	Borthakur and Addy (1988)	Taraboli 1, Dholamula, Supkheru, Chidon
9	Gokulapulan and Nair (1983)	Bharati, Rohini
10	Ansari <i>et al.</i> , 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 <i>et al.</i> , 1992	LSBR-5, LSBR-33
13	Marchetti <i>et al.</i> , 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 <i>et al.</i> , 2008	TIL:455, TIL:514, TIL:642

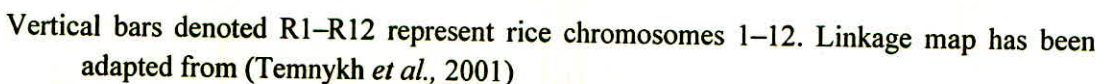
2.4.4 The QTLs for Sheath blight resistance in rice

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	<i>qSB-2</i> <i>qSB-3</i> <i>qSB-4</i> <i>qSB-8</i> <i>qSB-9</i> <i>qSB-12</i>	RG654-RZ260 R0348-RG944 RG143-RG214 RG20-RG1034 RG910b-RZ777 RG214a-RZ397	255 F ₄	Li <i>et al.</i> , 1995
2	<i>qSB-2</i> <i>qSB-3</i> <i>qSB-7</i>	G243-RM29 R250-C746 RG30-RG447	F ₂ clonal families	Pan <i>et al.</i> , 1999
3	<i>qSB-2</i> <i>qSB-3</i> <i>qSB-7</i>	RM29 R250-C746 RG30-RG477	128 F ₂ clonal families	Zou <i>et al.</i> , 2000

	<i>qSB-9-1</i> <i>qSB-9-2</i> <i>qSB-11</i>	C397-G103 RG570-C356 G44-RG118		
4	<i>qSBR-2</i> <i>qSBR-3</i> <i>qSBR-7</i> <i>qSBR-11</i>	RG171-G243A G249-G164 RG511-TCT122 CT224-CT44	DH, 127	Kunihiro <i>et al.</i> , 2002
5	<i>qSB-5</i> <i>qSB-9</i>	C624-RM26 RM242-C472	RILs 240 lines	Han <i>et al.</i> , 2002
6	<i>Rsb 1</i>	RM173	1032 F ₂	Che <i>et al.</i> , 2003
7	<i>qSB-3</i> <i>qSB-12</i>	RM13856 RM1880	60 BC ₁ F ₁	Sato <i>et al.</i> , 2004
8	<i>qSB-9</i> <i>qSB-11</i>	RM205-RM201 RM167-Y529	115 F ₂ clonal population	Tan <i>et al.</i> , 2005
9	<i>qSB-1</i> <i>qSB-2</i> <i>qSB-3-1</i> <i>qSB-3-2</i> <i>qSB-4-1</i> <i>qSB-4-2</i> <i>qSB-5</i> <i>qSB-6-1</i> <i>qSB-6-2</i> <i>qSB-7</i> <i>qSB-8-1</i> <i>qSB-8-2</i> <i>qSB-9</i> <i>qSB-10</i> <i>qSB-12</i>	RG532x C624x RG348x RZ474 RG1094e RZ590x Y1049 C RZ508 C285 G104 R662 RZ404 RG561 G1106	300 RILs F ₁₀ and F ₁₁	Pinson <i>et al.</i> , 2005
10		RM1339 RM3685 RM7072 RM3823	279 F _{2:3}	Sharma <i>et al.</i> , 2009
11	<i>qSB-1</i> <i>qSB-2-1</i> <i>qSB-2-2</i> <i>qSB-3-1</i> <i>qSB-3-3</i> <i>qSB-5</i> <i>qSB-6</i> <i>qSB-9-1</i> <i>qSB-9-2</i>	RM104 RM341 RM250 RM5626/RM426 RM85 RM13 RM190 RM434 RM245	250 F ₅ RILs	Liu <i>et al.</i> , 2009
12	<i>qSBRI-1</i> <i>qSBR3-1</i> <i>qSBR7-1</i> <i>qSBR8-1</i> <i>qSBR9-1</i> <i>qSBR3-11-1</i> <i>qSBR3-1 1-2</i> <i>qSBR3-11-3</i>	HVSSR1-68 RM251 RM336/IRM3691 RM210 RM257 RM224 RM209 RM202	127 RIL (F ₂ :10)	Channamallikarjuna <i>et al.</i> , 2010



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

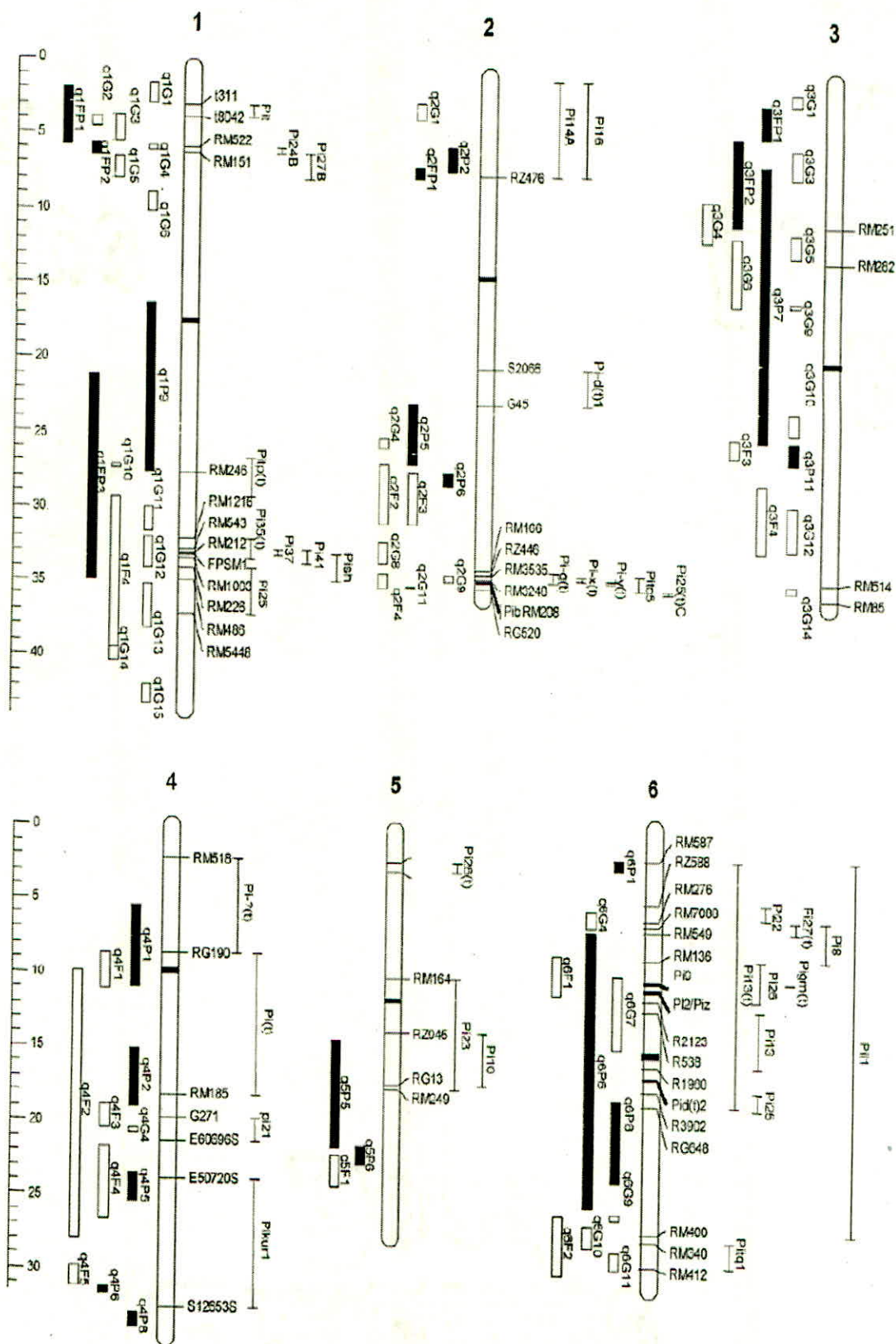
<i>Pi5(t)</i>	9	Jeon <i>et al.</i> , 2003
<i>Pi3(t)</i>	9	Inukai <i>et al.</i> , 1996
<i>Pi15</i>	9	Pan <i>et al.</i> , 1996
<i>Pii</i>	9	Ise, 1991
<i>PI28(t)</i>	10	Sallaud <i>et al.</i> , 2003
<i>PiGD2(t)</i>	10	Liu <i>et al.</i> , 2004.
<i>Pia</i>	11	Goto <i>et al.</i> , 1981
<i>PiCO39(t)</i>	11	Chauhan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

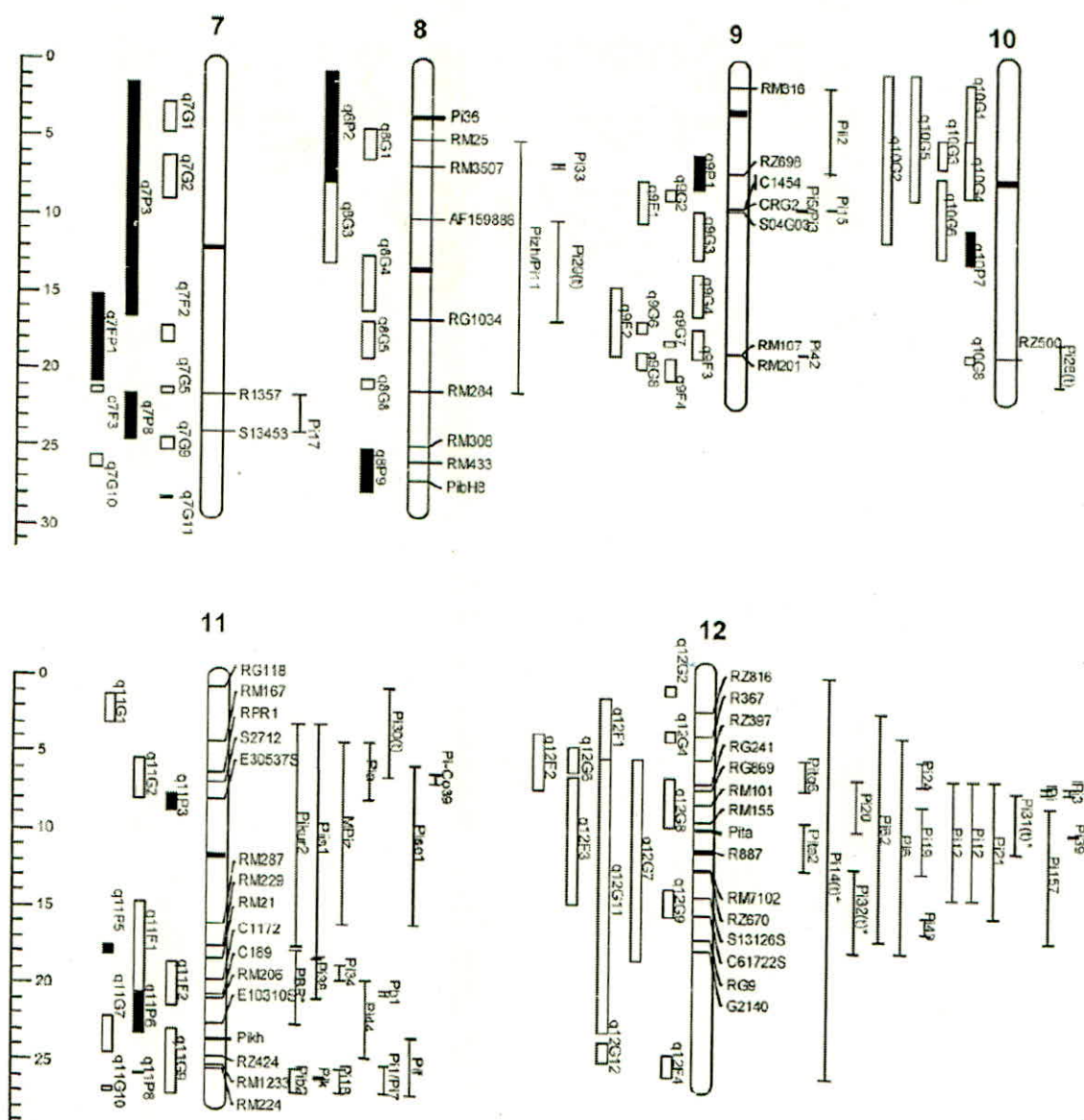
significantly the initial dataset of 347 QTL. A graphical overview of the position of these metaQTL is presented in (Figure 2.2). The average size of a metaQTL is 3.3 Mbp. In all, 11% of the metaQTL are finely mapped (<500 kb) and 5% are large (>12 Mbp).

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 <i>et al.</i> , 2002	CT9993-5-10-1-m × KDML105	G and F	Yes	Nd	6
Bagali <i>et al.</i> , 2000	IR64 × Azucena	G and F	Yes	No	13
Fukuoka and Okuno, 2001	Nipponbare × Owarihatamochi	F	Yes	No	4
Miyamoto <i>et al.</i> , 2001, 2003	Kahei × Koshihikari	F	Yes	No	2
Sallaud <i>et al.</i> , 2003	IR64 × Azucena	G	Yes	No	9
Chen <i>et al.</i> , 2003	Zhenshan 97 × Minghui 63	G	Yes	No	12
Huang <i>et al.</i> , 2004	Tainung 69 × Koshihikari	G and F	Yes	No	2
Xu <i>et al.</i> , 2004	ZYQ8 × JX17	G	Yes	No	77
Sato <i>et al.</i> , 2006	URN12 × Koshihikari	F	No	No	2
Wang <i>et al.</i> , 1994	Moroberekan × Co39	G and F	Yes	Yes	22
Tabien <i>et al.</i> , 2002	Lemont × Teqing	G and F	Yes	No	11
Zenbayashi <i>et al.</i> , 2002.	Norin29 × Chubu32	F	No	Yes	1
Loan <i>et al.</i> , 2003	Lemont × Teqing	G	Yes	Yes	14
Talukder <i>et al.</i> , 2004, 2005.	Bala × Azucena	G	No	Yes	41
Wu <i>et al.</i> , 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





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 *Bipolaris 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 teleomorphs 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 (*Pil* 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 (*Her₁*, *Her₂* and *Hes₁*). The first two were dominant for resistance and the third for susceptibility. Kernel resistance was dominant over susceptibility and controlled by two genes (*Hekr₁* and *Hekr₂*) carried by *Pil* 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 F₂ 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 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.

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. Toshya

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 \times 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 15×15 cm and 20×20 cm 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°C . The culture was grown on Wakimoto's Medium for 3 days at 30°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 multiplication. 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 \times 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 F₁₄ 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 than 1
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:

$$\bar{X} = \sum X_i / n$$

Where, $\sum X_i$ = 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

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

Where,

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

$\text{Var}(x)$ = Variance of x character

$\text{Var}(y)$ = 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 future 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°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 μ l 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°C until used.

3.4.2 Quantification and estimation of purity of DNA

The DNA was quantified using Nanodrop spectrophotometer (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 40ng/ μ l. 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 7 μl of 6X loading dye and 5 μl 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 20 μl)

S.No	Component	Stock concentration	Volume/reaction
1	DNA	40 $\mu\text{g}/\text{ml}$	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	<i>Taq</i> 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	94°C	4	1	Initial Denaturation
2	94°C	1	30	Denaturation
3	55-68°C	1	30	Annealing
4	72°C	1	30	Extension
5	72°C	5	1	Final Extension
6	4°C	∞	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 μ l of ammonium per sulphate 10 % (APS) and 70 μ l 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, 7 μ l loading dye (6 X) was added to PCR products.
- Finally, 5 μ l 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 10 μ l 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 marker analysis considered one locus at a time and fits the following regression model:

$$y = b_0 + b_1x + e,$$

Where,

y = Phenotypic value of a line

b_0 = Population mean

b_1 = 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:

$$y = b_0 + b_1x_1 + b_2x_2 + \dots + b_nx_n + b_ix_i + e$$

where,

b_1, b_2, \dots, b_n = Additive effects of the n cofactors

x_1, x_2, \dots, x_n = 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)

b_i = Additive effect of the interval position being tested

x_i = 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 distortion 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 μ l 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 μ l 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/ μ L
5% PAGE solution	65ml
APS	700 μ L
TEMED	70 μ L

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 μ l
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 ₂ O	0.5 gm
5.	Na ₂ HPO ₄ 12H ₂ O	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		

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.

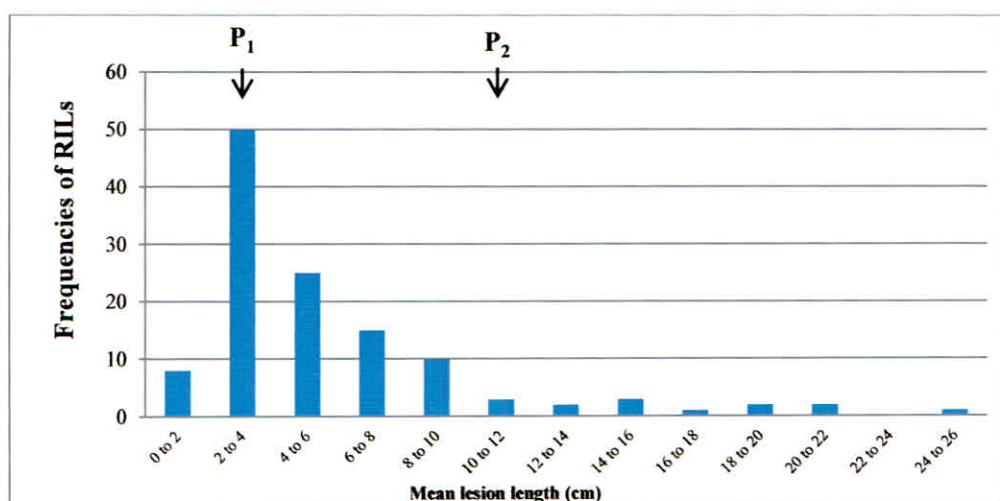
Two potential *indica* genotypes, Danteshwari: a high yielding popular rice cultivar but resistance to few diseases and Dagad deshi: a tall deep rooted, poor yielder and resistance to few diseases. The parents along with their derived F_{14} RILs were used for the phenotypic evaluation of four diseases 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

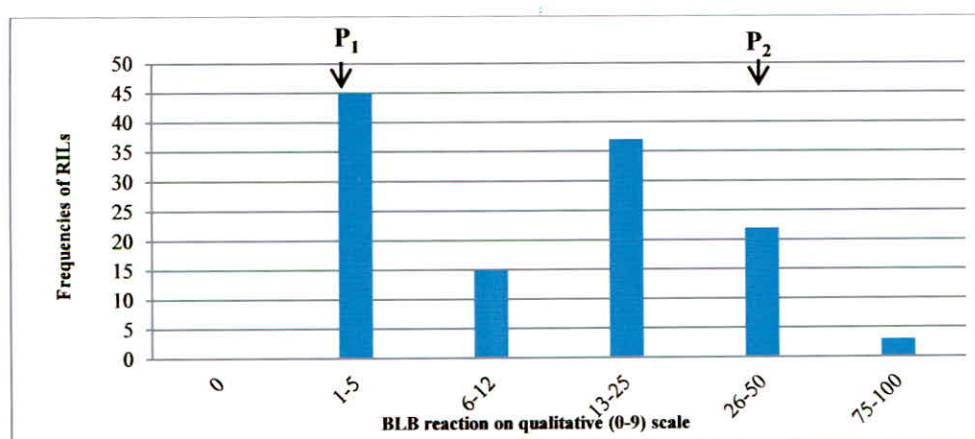
The frequency distribution of disease reaction of 122 RILs population derived from Danteshwari \times Dagad deshi was examined to determine if its normality for local *Xoo* isolate used in the experiment. The analysis indicated that nearly normal distribution was followed for RILs population (Figure 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).

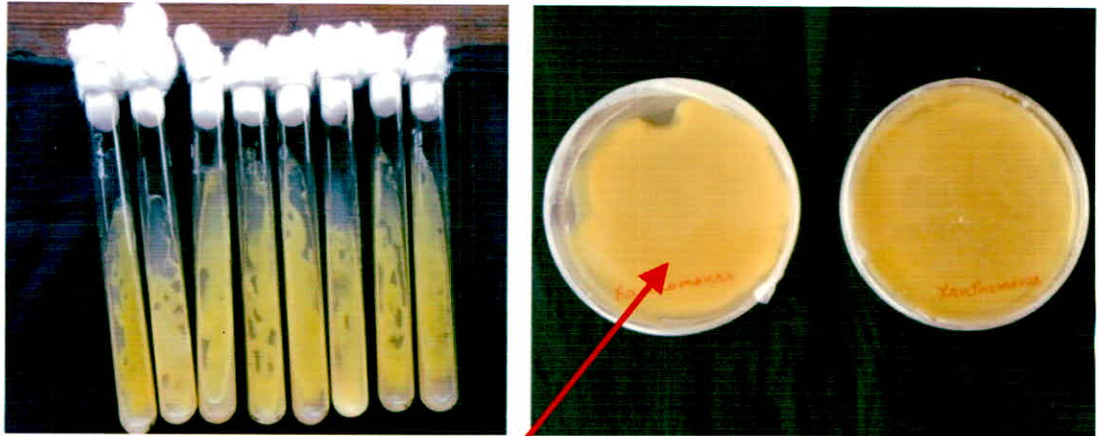


a) Mean lesion length



(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 inoculation



b) Resistant line



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

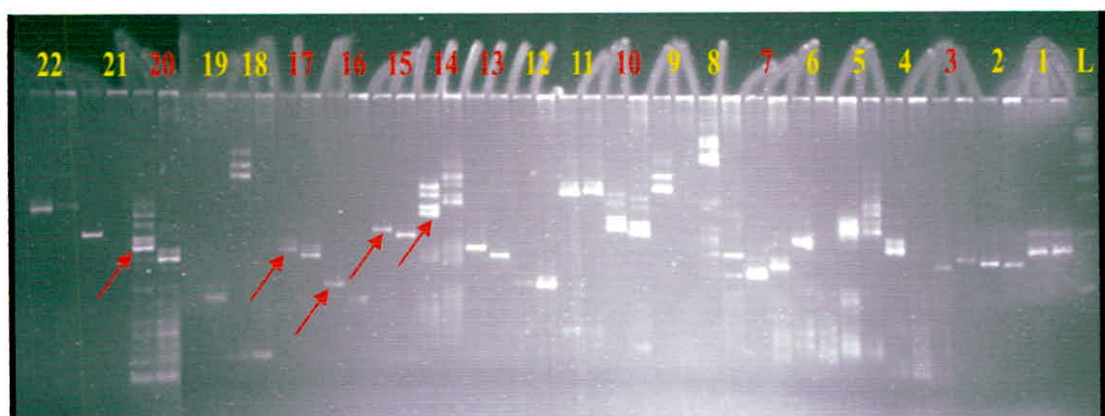


Figure 4.3 The informative SSR markers identified on the basis of parental polymorphism

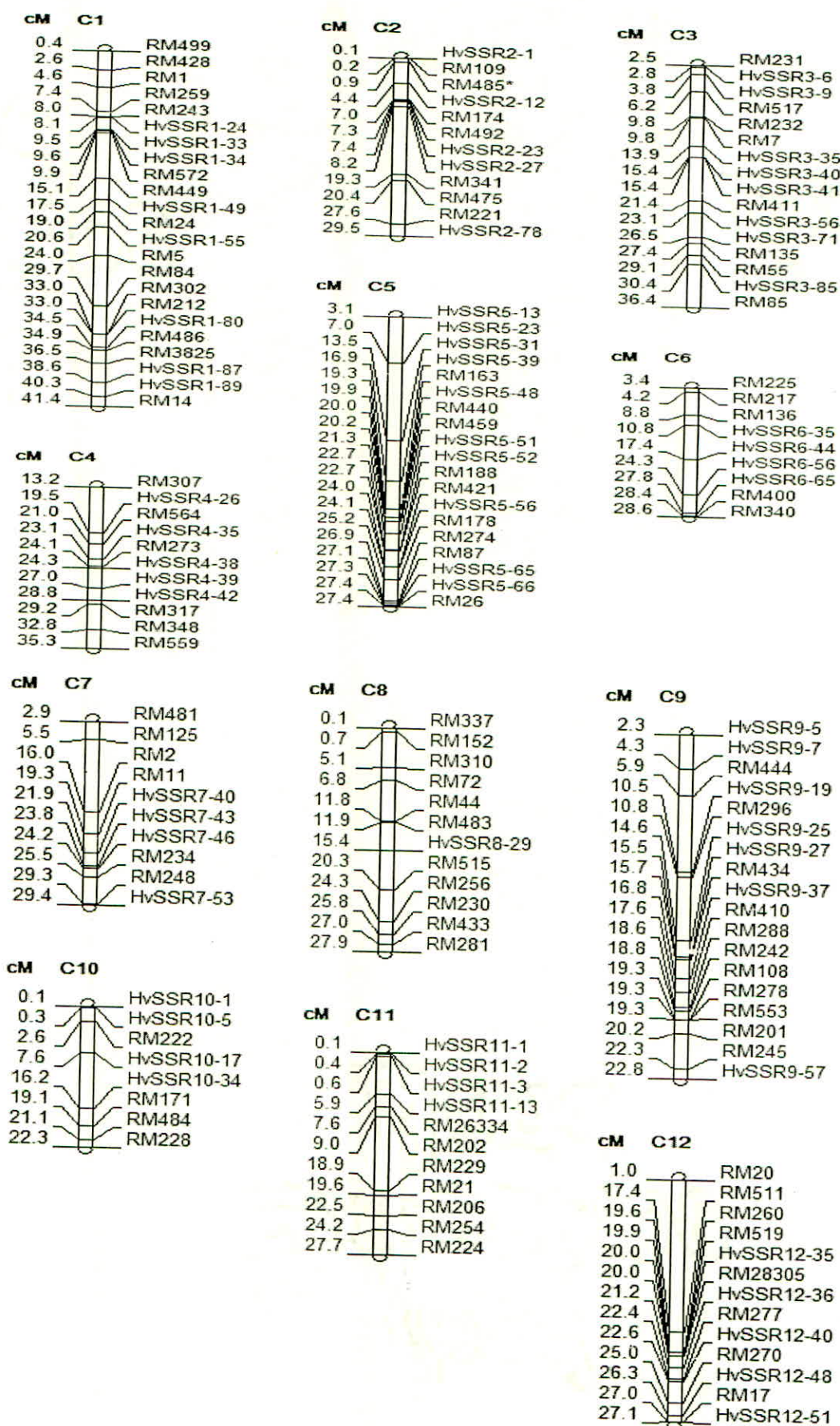


Figure 4.4 The linkage map constructed from 162 polymorphic markers

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 $y = b_0 + b_1 x + e$. The results given estimate of b_0 , b_1 and the F statistic for each marker. It was interested in whether the marker is linked to a QTL. This was determining if b_1 is significantly different from zero. The F statistic compares the hypothesis $H_0: b_1 = 0$ to an alternative $H_1: b_1 \neq 0$. The $pr(F)$ a measure of how much support there is for H_0 . A smaller $pr(F)$ indicates less support for H_0 and thus more support for H_1 . 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	<i>qBbr1a</i>	1	1.00	RM499	RM1	7.86	-1.37	13.53
	<i>qBbr1b</i>	1	39.20	HvSSR1-87	HvSSR1-89	5.33	-3.06	27.61
	<i>qBbr3a</i>	3	16.9	HvSSR3-40	RM411	4.39	-0.06	0.36
	<i>qBbr3b</i>	3	26.00	HvSSR3-56	HvSSR3-71	4.19	0.001	1.02
	<i>qBbr4a</i>	4	7.80	RM564	HvSSR4-35	4.56	-0.61	3.45
	<i>qBbr5a</i>	5	6.90	HvSSR5-23	HvSSR5-31	3.14	-0.11	0.11
	<i>qBbr5b</i>	5	15.8	HvSSR5-39	HvSSR5-48	3.03	0.01	0.02
	<i>qBbr9a</i>	9	5.60	HvSSR9-7	RM444	3.37	-0.75	1.20
	<i>qBbr10a</i>	10	16.20	HvSSR10-34	RM171	3.58	-0.46	0.37
	<i>qBbr12a</i>	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	<i>qBbr1c</i>	1	40.38	HvSSR1-89	RM14	4.43	-1.81	12.46
	<i>qBbr9b</i>	9	18.28	RM410	RM288	2.53	-1.14	6.03
	<i>qBbr12b</i>	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	<i>qBbr4b</i>	4	7.40	HvSSR4-26	RM564	5.00	-1.12	15.31
	<i>qBbr6a</i>	6	22.90	HvSSR6-44	HvSSR6-56	3.95	0.79	9.22
	<i>qBbr12c</i>	12	8.00	RM20	RM511	6.47	-1.91	52.81
	<i>qBbr12a</i>	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	<i>qBbr4c</i>	4	25.15	HvSSR4-38	HvSSR4-39	3.68	-0.88	9.43
	<i>qBbr12a</i>	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.

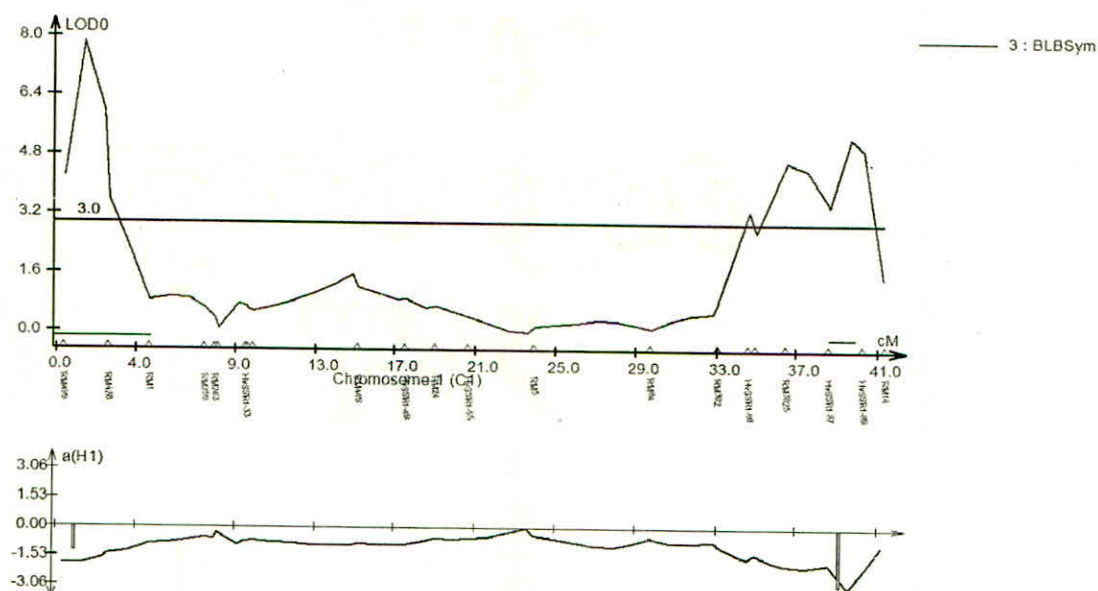


Figure 4.5 The QTLs mapped for mean lesion length trait identified on chromosome 1 for the Bacterial blight resistance using CIM. The bars indicate the most likely positions of the QTLs. The small graphic (bottom) showed the additive effect (-) value = both alleles from the susceptible parent Dagad deshi.

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 quantitative analysis result showed that this QTL was consistent 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.

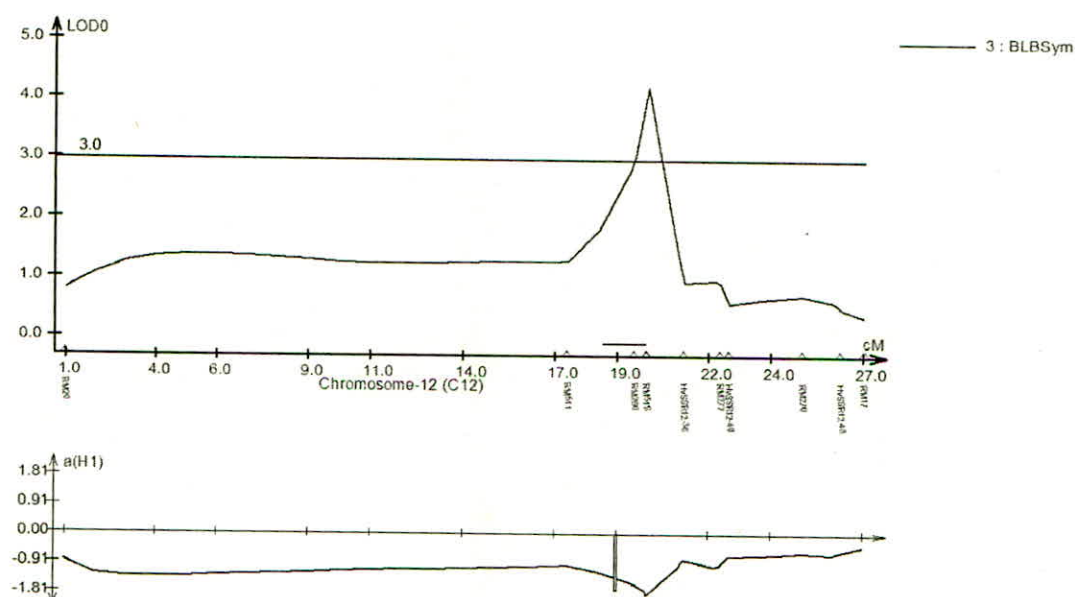


Figure 4.6 The common QTL mapped for mean lesion length trait identified on chromosome 12 for the Bacterial blight resistance using CIM. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance parent and (-) values = alleles from the susceptible parent.

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

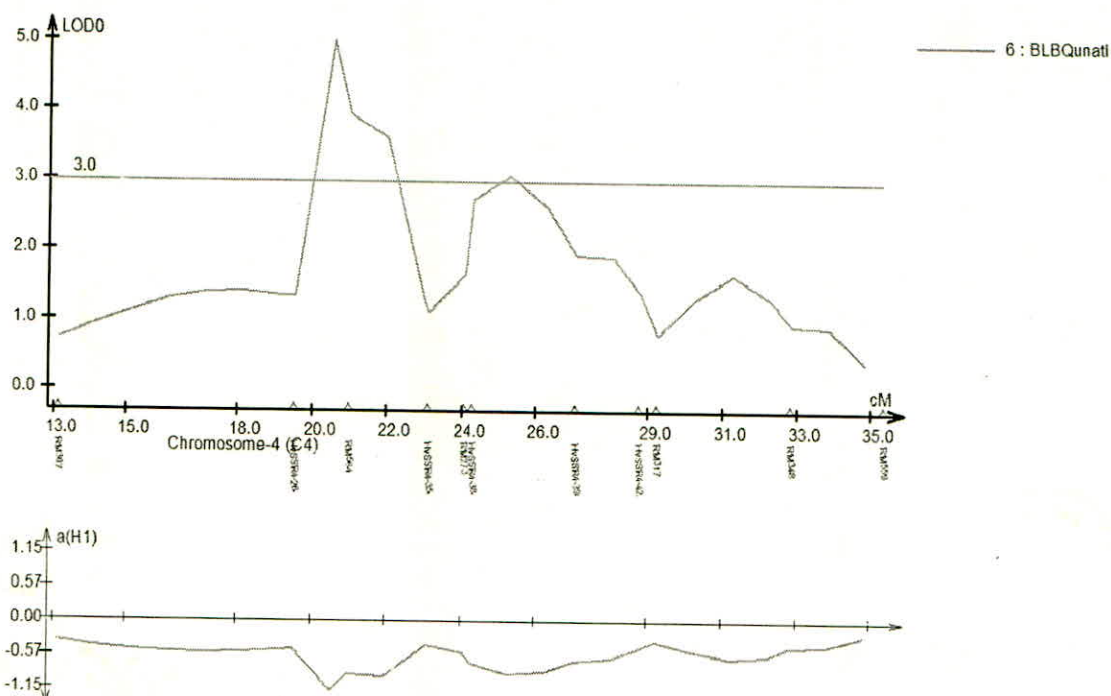


Figure 4.7 The QTL mapped on chromosome 4 for the Bacterial blight resistance trait using CIM. The bars indicate the most likely positions of the QTL.

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.

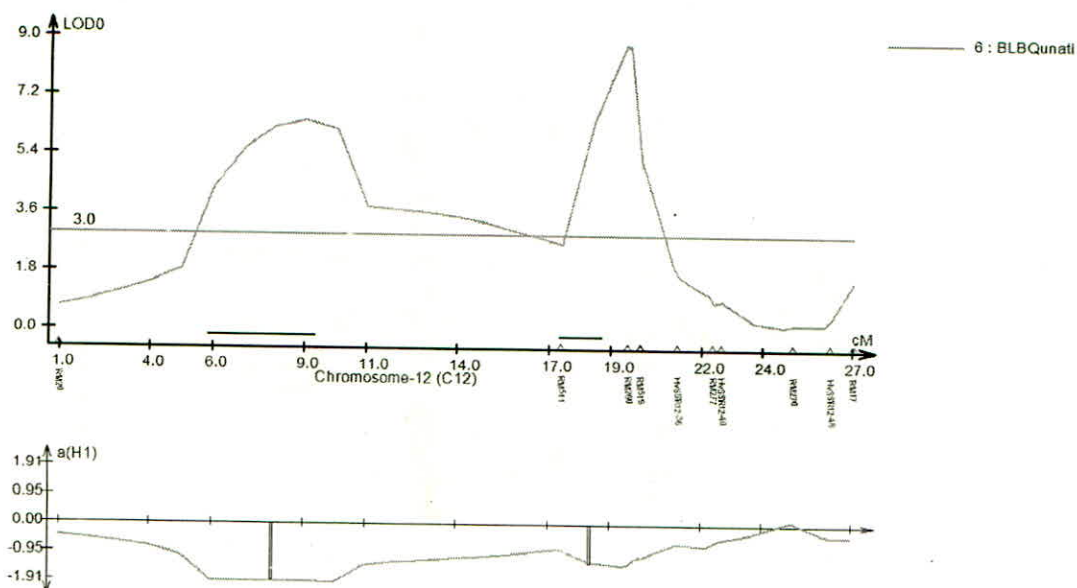


Figure 4.8 The QTLs mapped for Bacterial blight resistance trait identified on rice chromosome 12 using CIM. The bars indicate the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect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

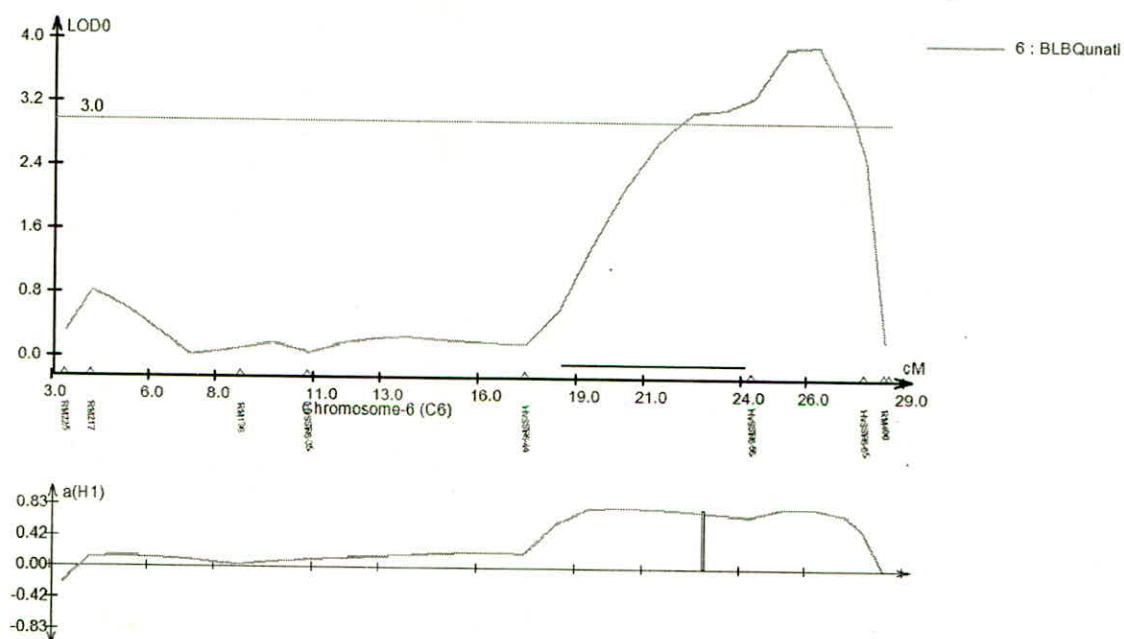


Figure 4.9 The QTL mapped for Bacterial blight resistance trait identified on rice chromosome 6 using CIM. The bar indicates the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect 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 \times 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 cm² for Danteshwari and 1.05 cm² 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.

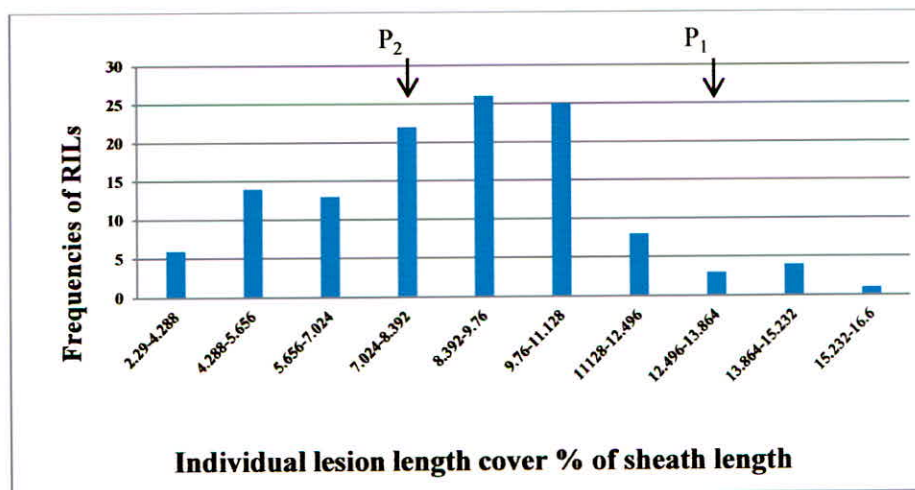
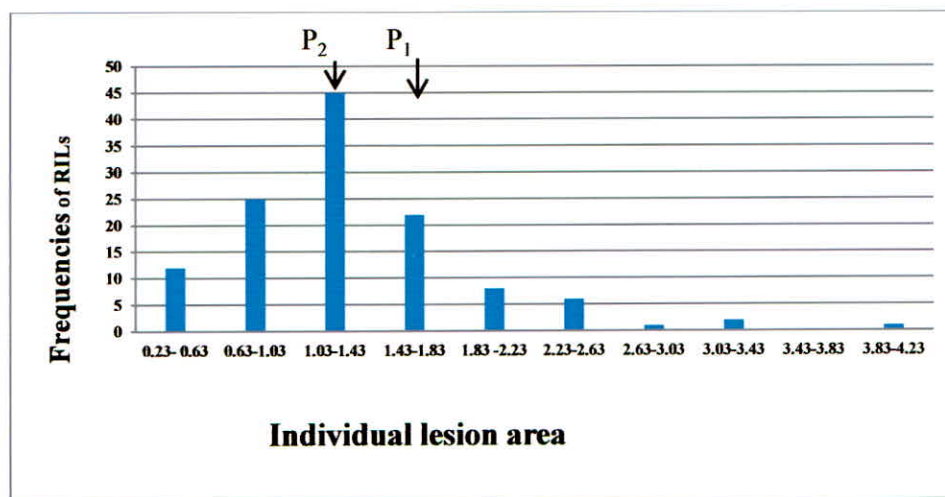
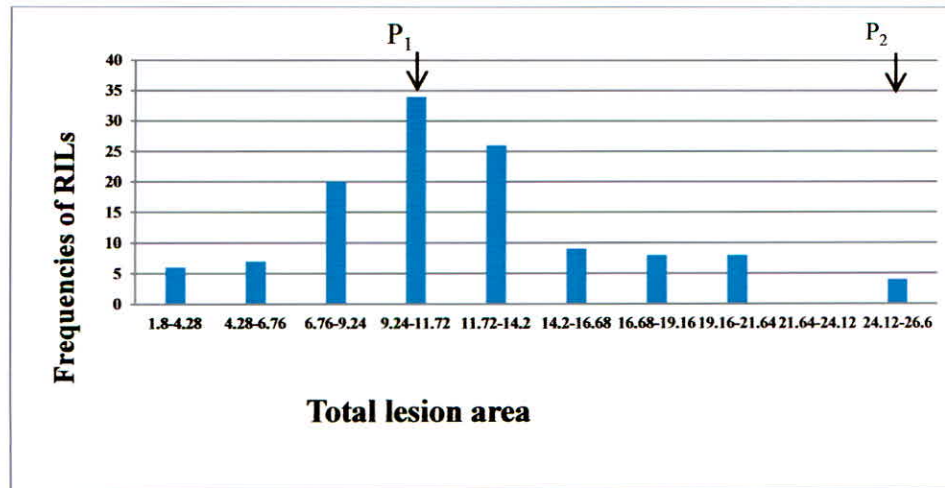


Figure 4.10 The frequency distribution of Sheath blight response of 122 F_{14} RIL lines for total and individual lesion



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 \times 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 $y = b_0 + b_1 x + e$. The results gave the estimates for b_0 , b_1 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	<i>qSBR4-1</i>	4	10.9	HvSSR4-35	HvSSR4-38	3.49	-1.9589	12.1
Individual lesion area	<i>qSBR2-1</i>	2	7.2	RM174	RM492	0.02	0.0392	0.07
	<i>qSBR3-1</i>	3	5.7	RM517	RM232	3.97	-0.0708	2.21
	<i>qSBR9-1</i>	9	3.6	HvSSR9-7	RM444	3.06	-0.1280	5.24
	<i>qSBR12-1</i>	12	17.4	RM511	RM260	5.30	-0.0476	0.11
Individual lesion length	<i>qSBR1-2</i>	1	2.2	RM499	RM1	3.19	0.3641	3.62
cover % of Sheath length	<i>qSBR1-1</i>	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	<i>qSBR4-1</i>	4	24.14	RM273	HvSSR4-38	3.04	-1.94	12.95
Individual lesion length cover % of Sheath Length	<i>qSBR1-1</i>	1	38.38	RM3825	HvSSR1-87	4.6	1.19	19.18

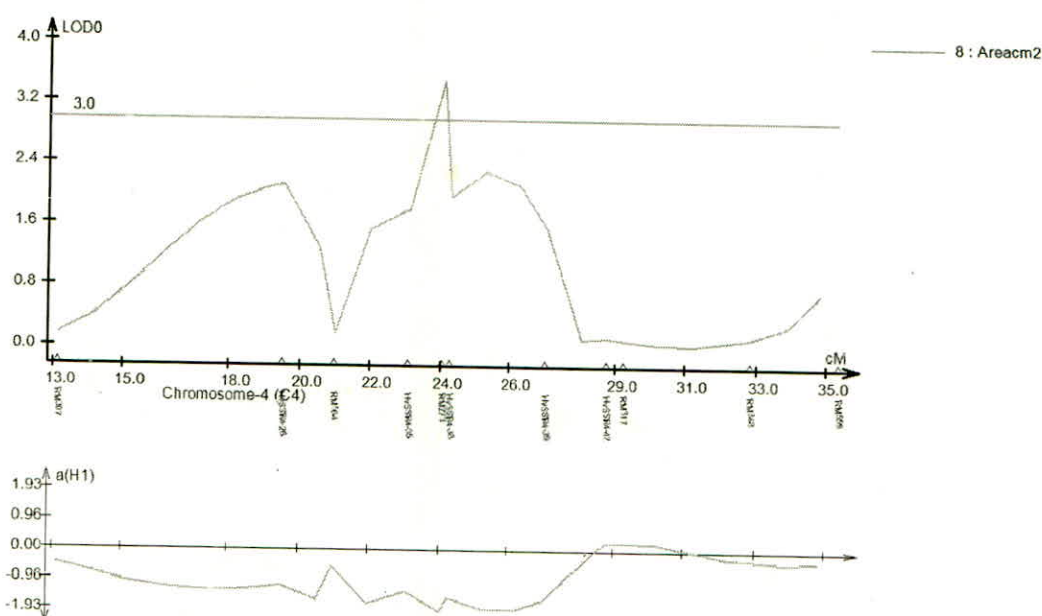


Figure 4.12 The QTL *qSBR4-1* mapped for the Sheath blight trait identified on rice chromosome 4 using CIM in experiment. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (bottom) show the additive effect where (+) values = allele from the resistance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

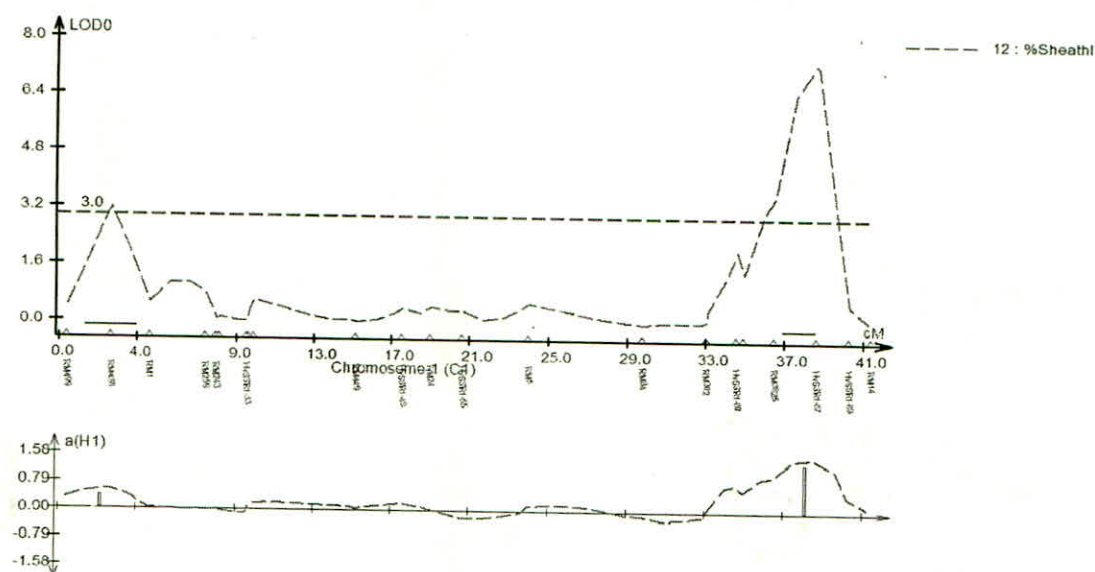


Figure 4.13 The QTLs, *qSBR1-1* and *qSBR1-2* mapped for the Sheath blight trait identified on rice chromosome 1 using CIM in experiment. The bars indicate the most likely positions of the QTLs. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance. The small graphic (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.

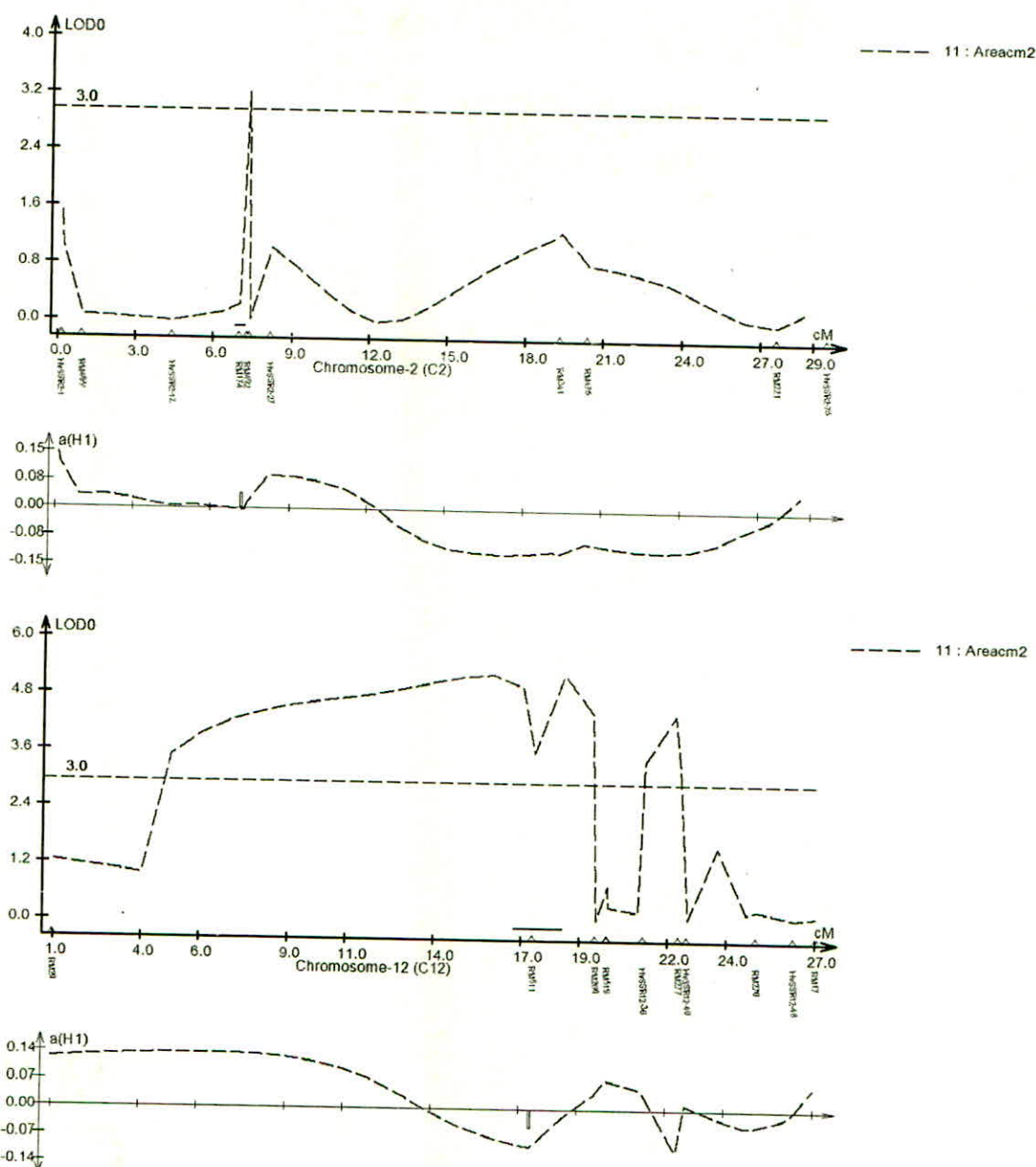


Figure 4.14 The QTL mapped for the Sheath blight trait identified on rice chromosome 2 and 12 using CIM in experiment. The bars indicate the most likely positions of the QTL.

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	<i>qTN1.1</i>	1	36.10	RM486	RM3825	4.00	0.61	11.91
	<i>qTN3.1</i>	3	0.00	RM231	HvSSR3-6	5.30	0.83	16.53
Plant height	<i>qPH1.1</i>	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	<i>qTN1.1</i>	1	36.38	RM486	RM3825	2.78	0.52	8.99
	<i>qTN3.1</i>	3	2.45	RM231	HvSSR3-6	4.21	0.77	15.28
Plant height	<i>qPH1.1</i>	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.

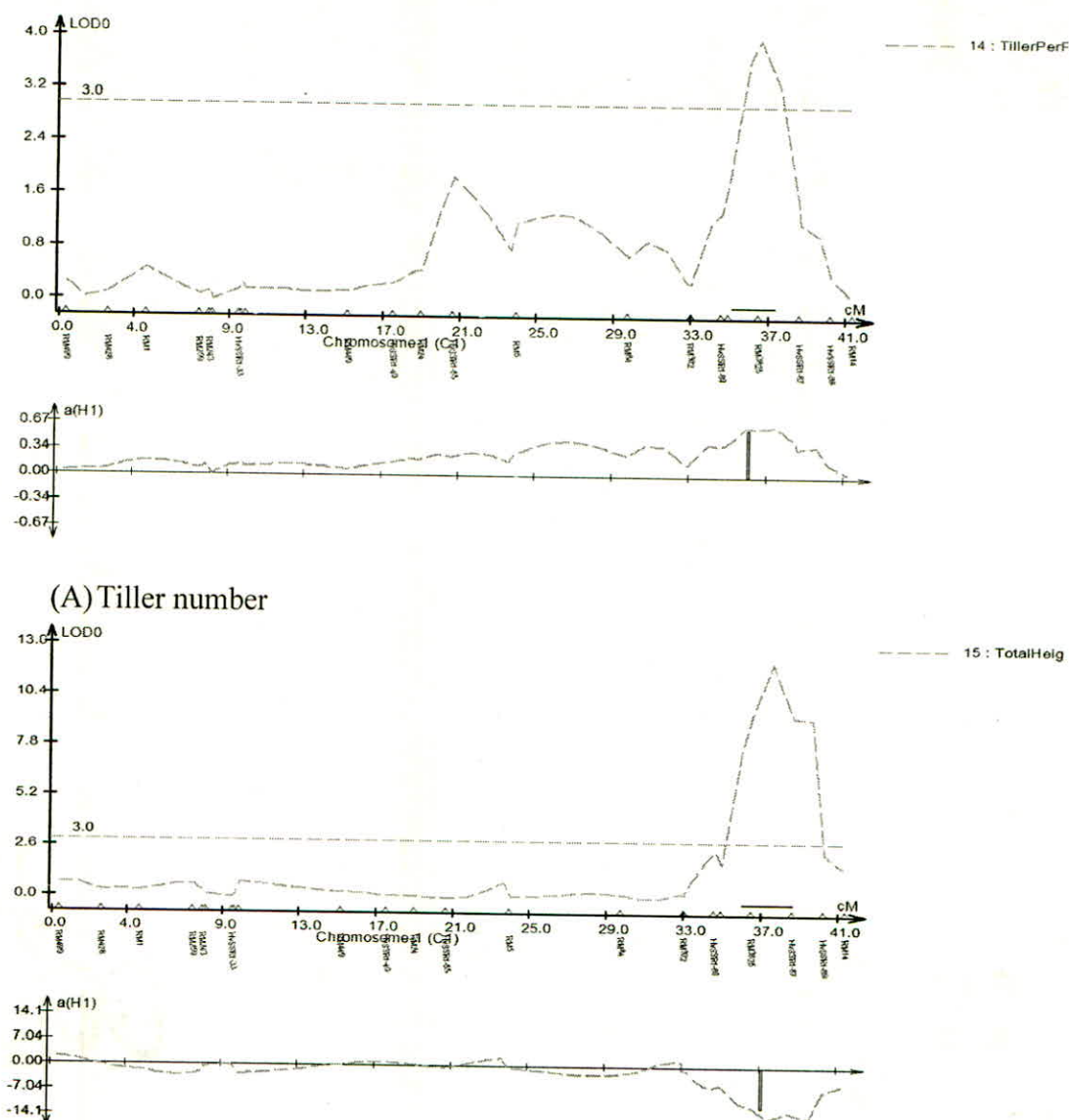


Figure 4.15 The QTL mapped for the agronomic traits plant height and tiller per plant identified on rice chromosome 1 using CIM. The bars indicate the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance.

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 \times 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.

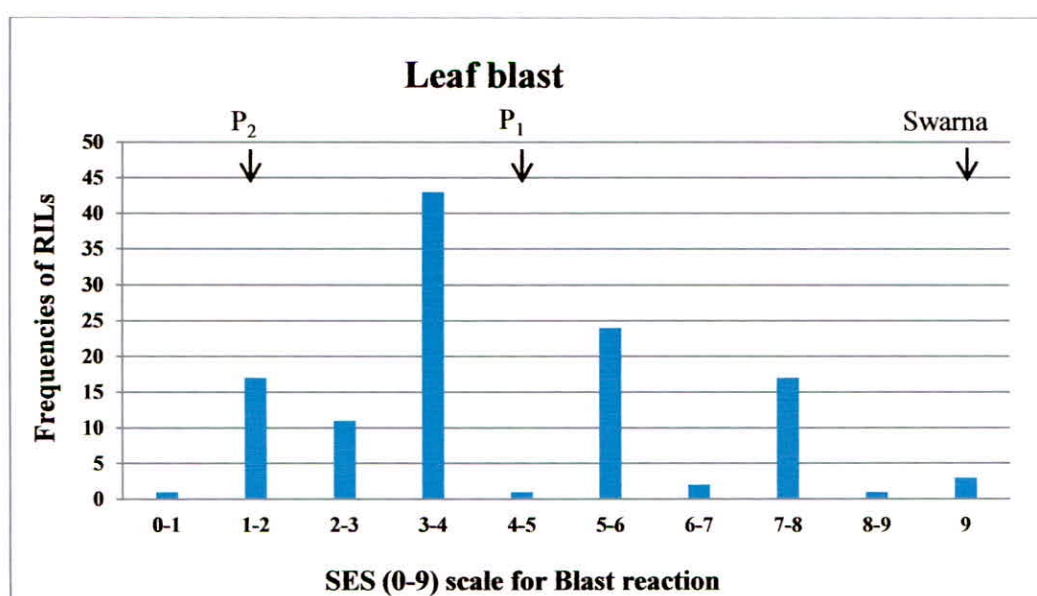


Figure 4.16 The frequency distribution of RIL for disease Blast reaction

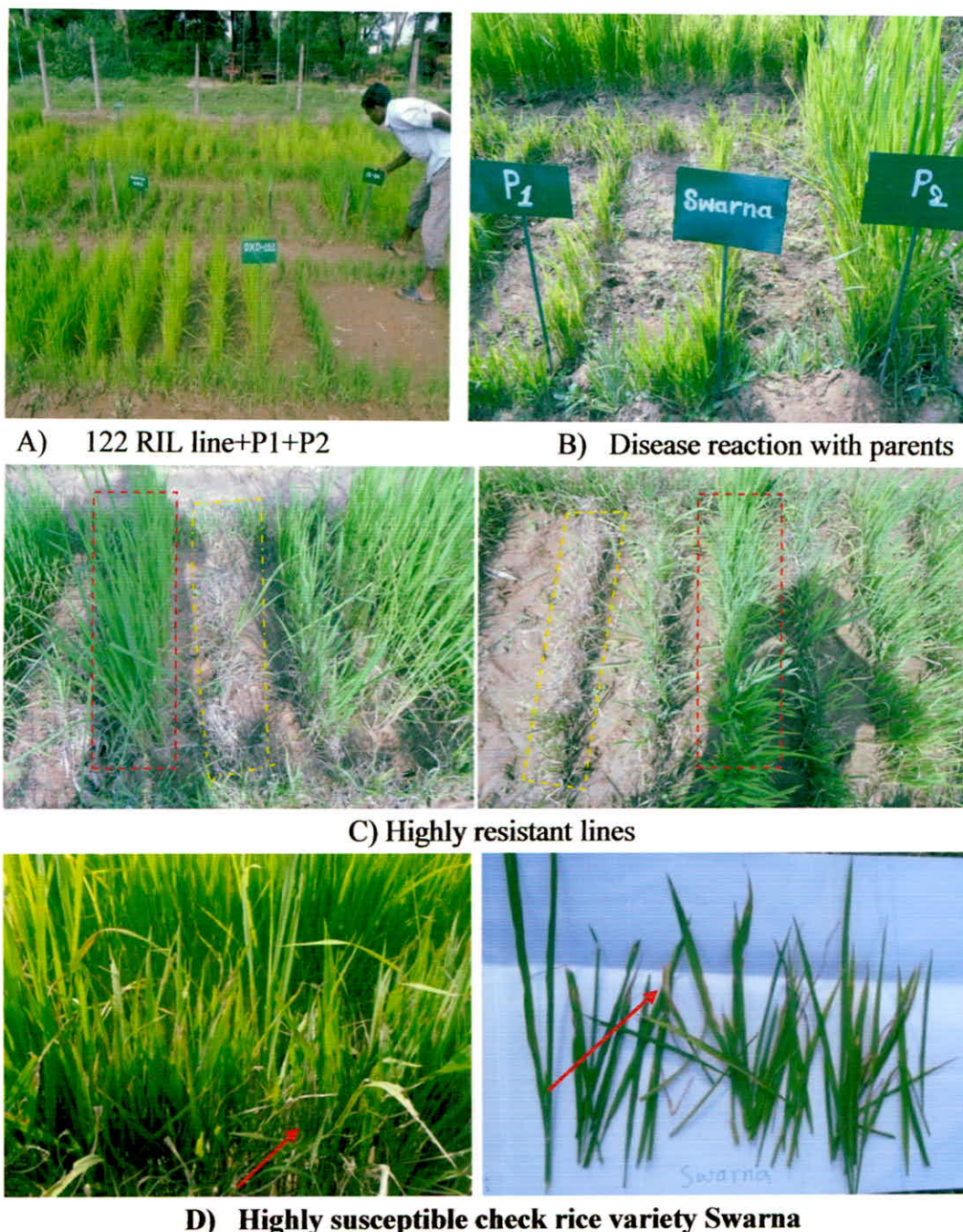


Figure 4.17 The natural occurrence of disease on RIL population used for screening (yellow arrow indicating susceptible lines)

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	<i>qLB10.1</i>	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.

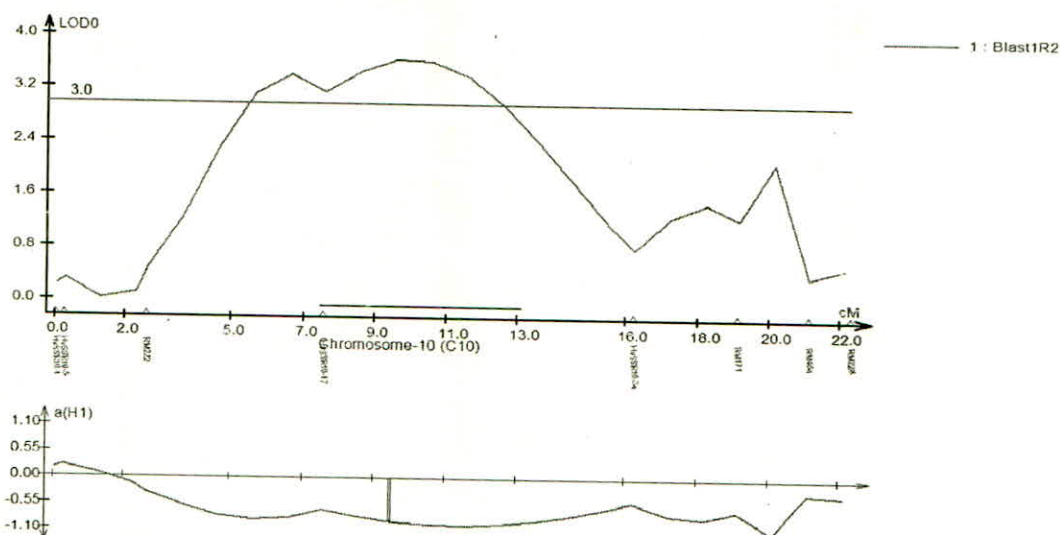


Figure 4.18 The QTL mapped for the Blast resistance trait identified on rice chromosome 10 using CIM in experiment. The bar indicates the most likely positions of the QTL. The horizontal dashed lines represent the minimum LOD i.e. 3 required for significance.

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 \times 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.

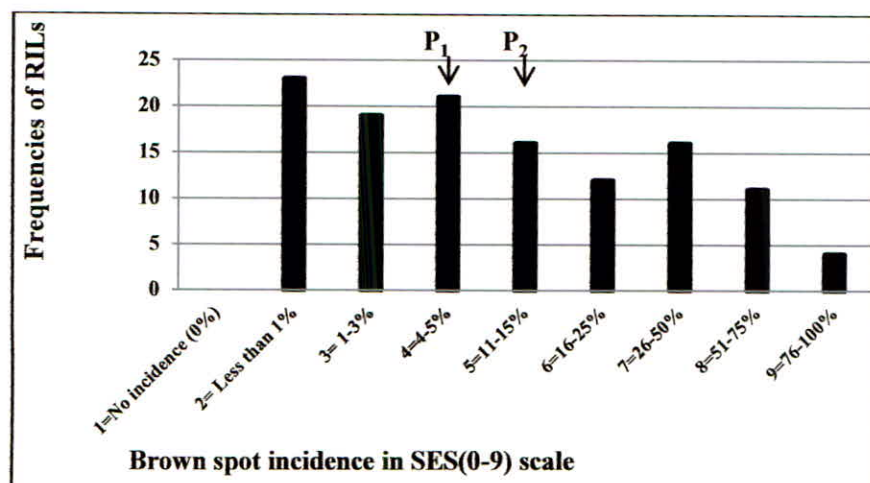


Figure 4.19 The frequency distribution of disease Brown spot resistance across 122 RIL lines in the experiment



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	<i>qBS12</i>	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	<i>qBS1</i>	1	21.38	HvSSR1-55	RM-5	3.149	-0.8198	14.3593
	<i>qBS11</i>	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.

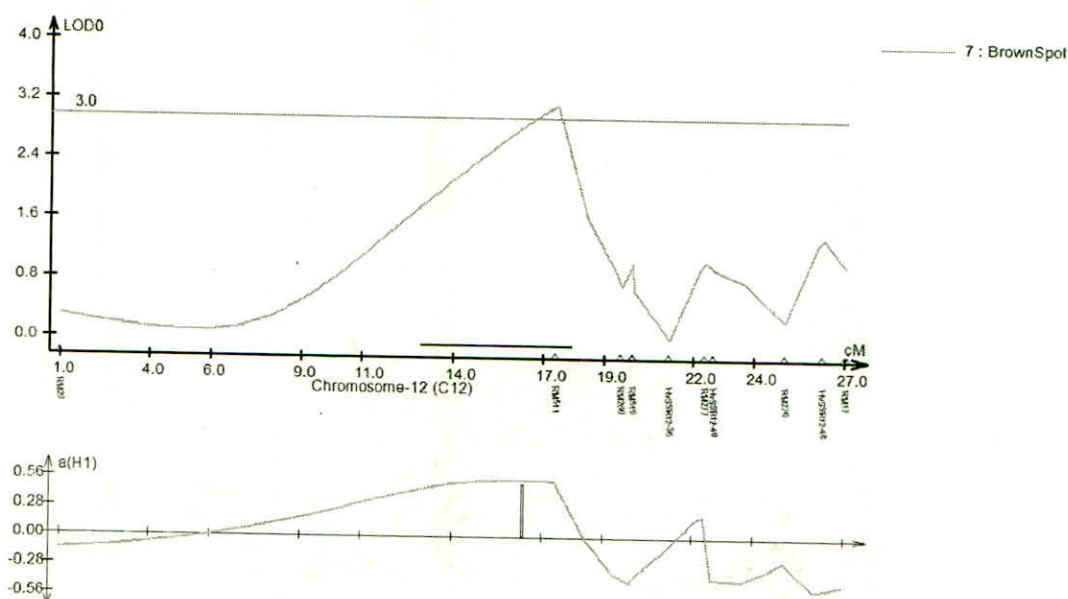


Figure 4.21 The QTL mapped for the Brown spot resistance trait identified on rice chromosome 12 using CIM in experiment. The small graphic (bottom) show the additive effect where (+) values = allele from resistance parent Dantehwari and (-) values = allele from susceptible parent Dagad deshi.

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 (Koeber 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 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.

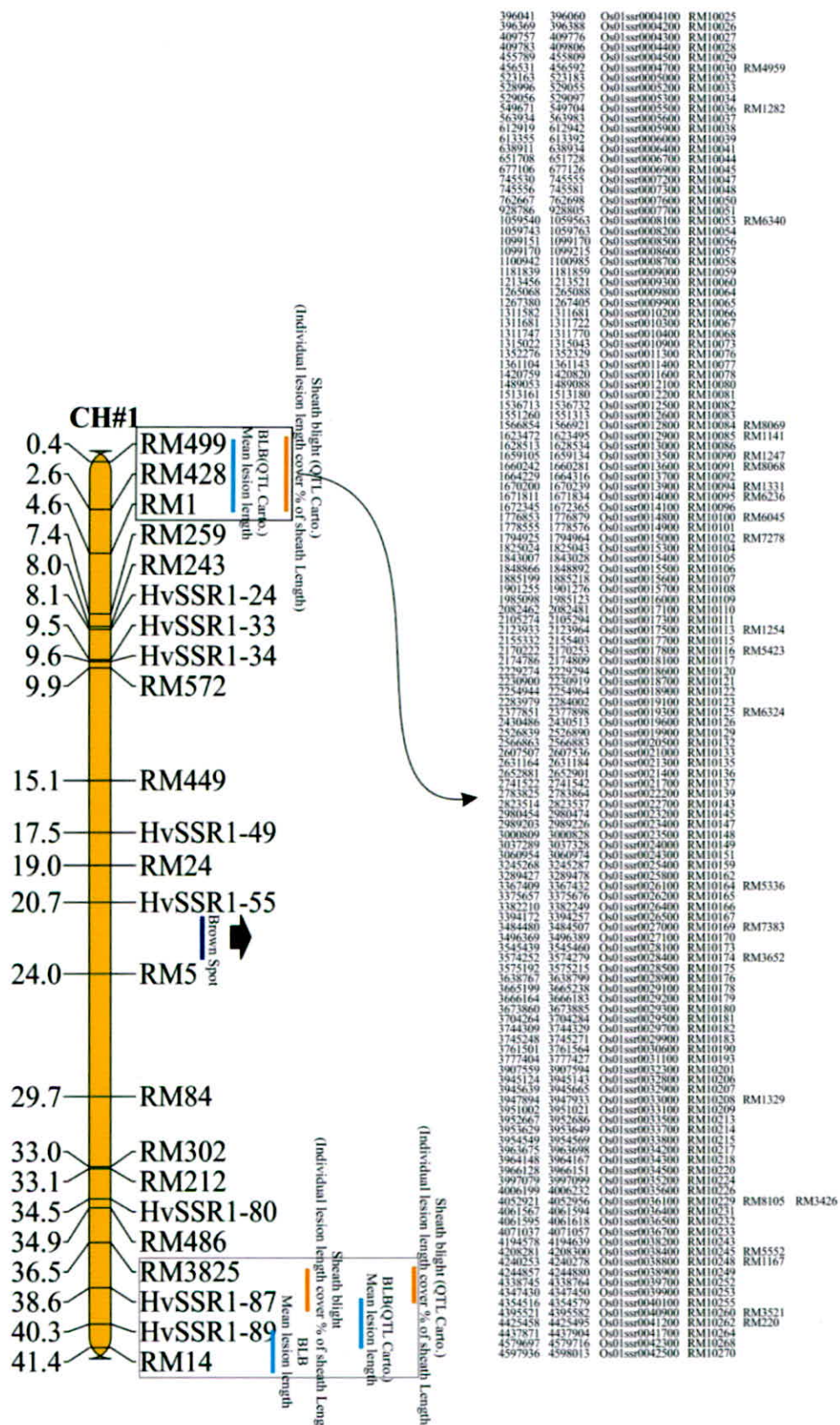


Fig. 4.22.1 Genetic locations and *in silico* analysis of QTLs for BLB trait mean lesion length and Sheath blight trait individual lesion length cover % of Sheath length on chromosome 1

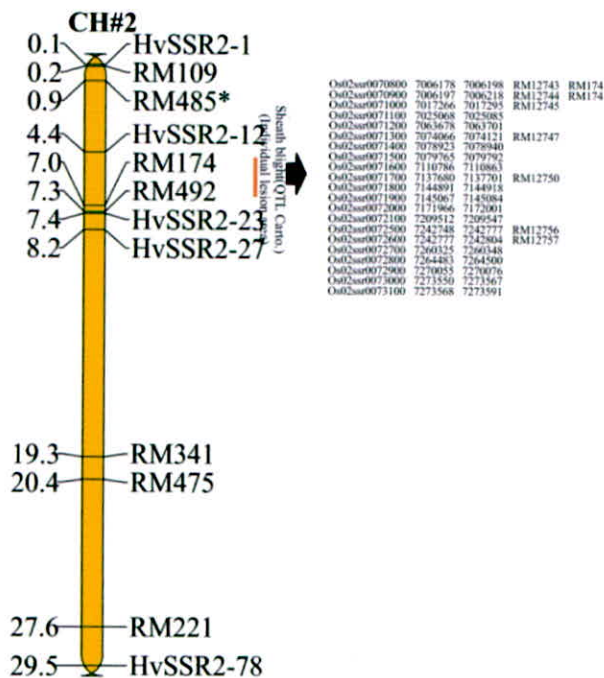


Figure 4.22.3 Genetic location and *in silico* analysis of Sheath blight QTL for trait individual lesion area on chromosome 2

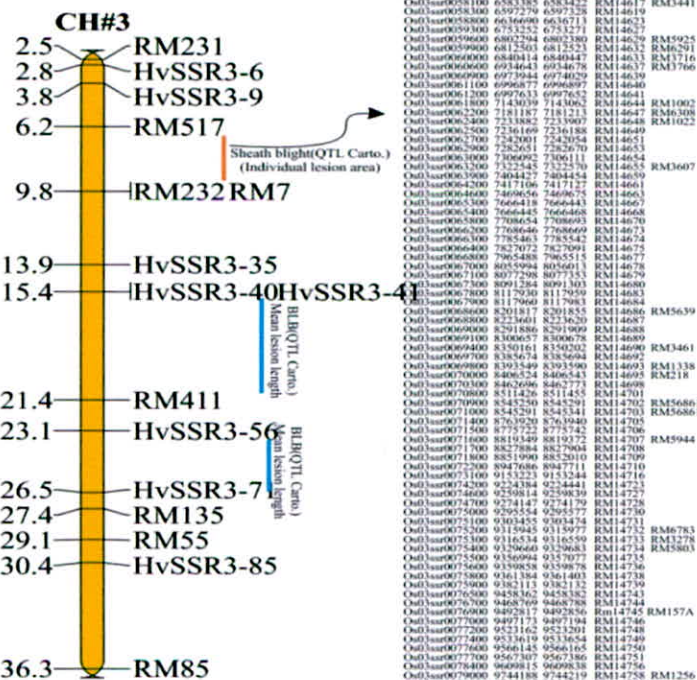


Figure 4.22.4 Genetic locations and *in silico* analysis of Sheath blight QTL for individual lesion area on chromosome 3

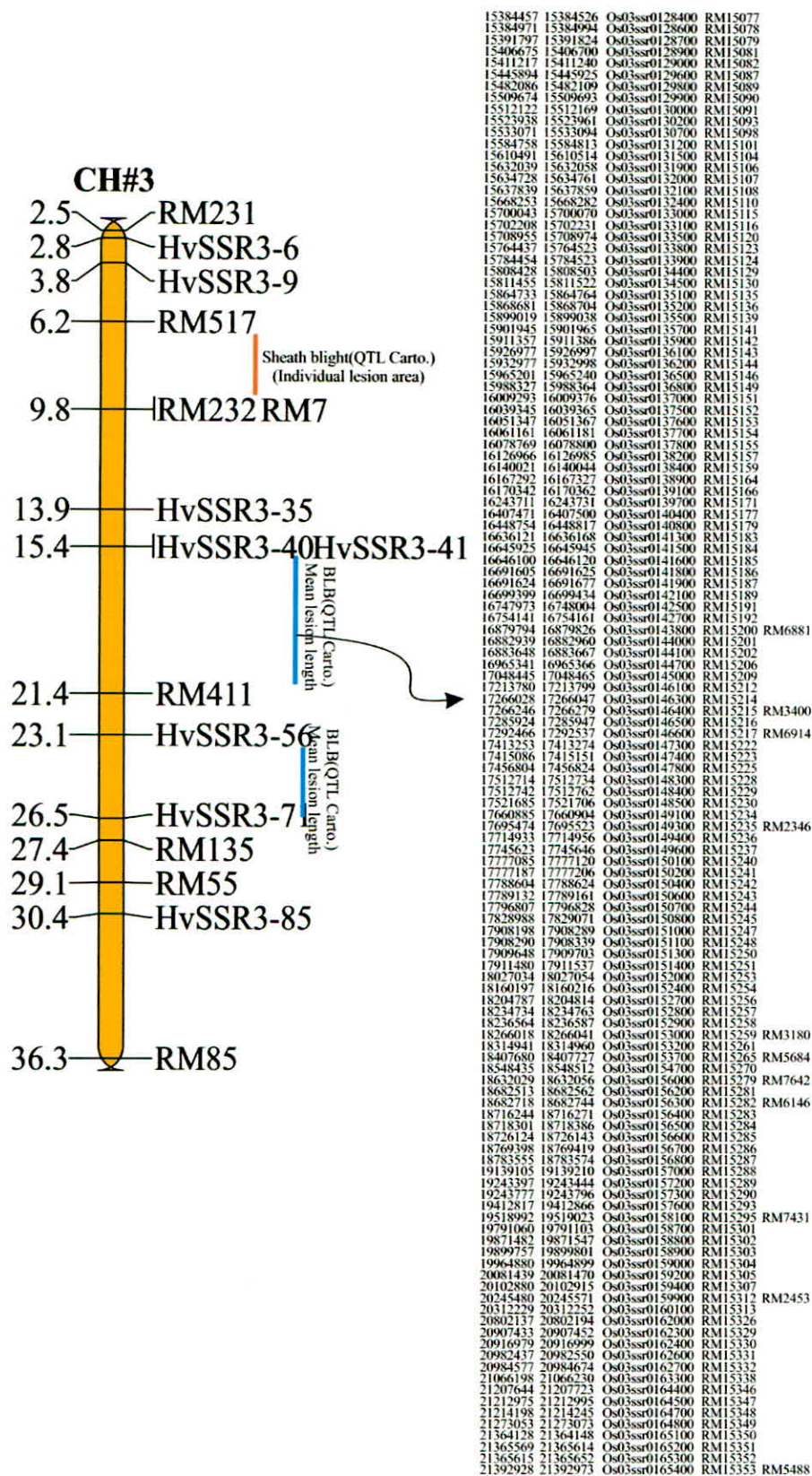


Figure 4.22.5 Genetic location and *in silico* analysis of QTLs for BLB trait mean lesion length on chromosome 3

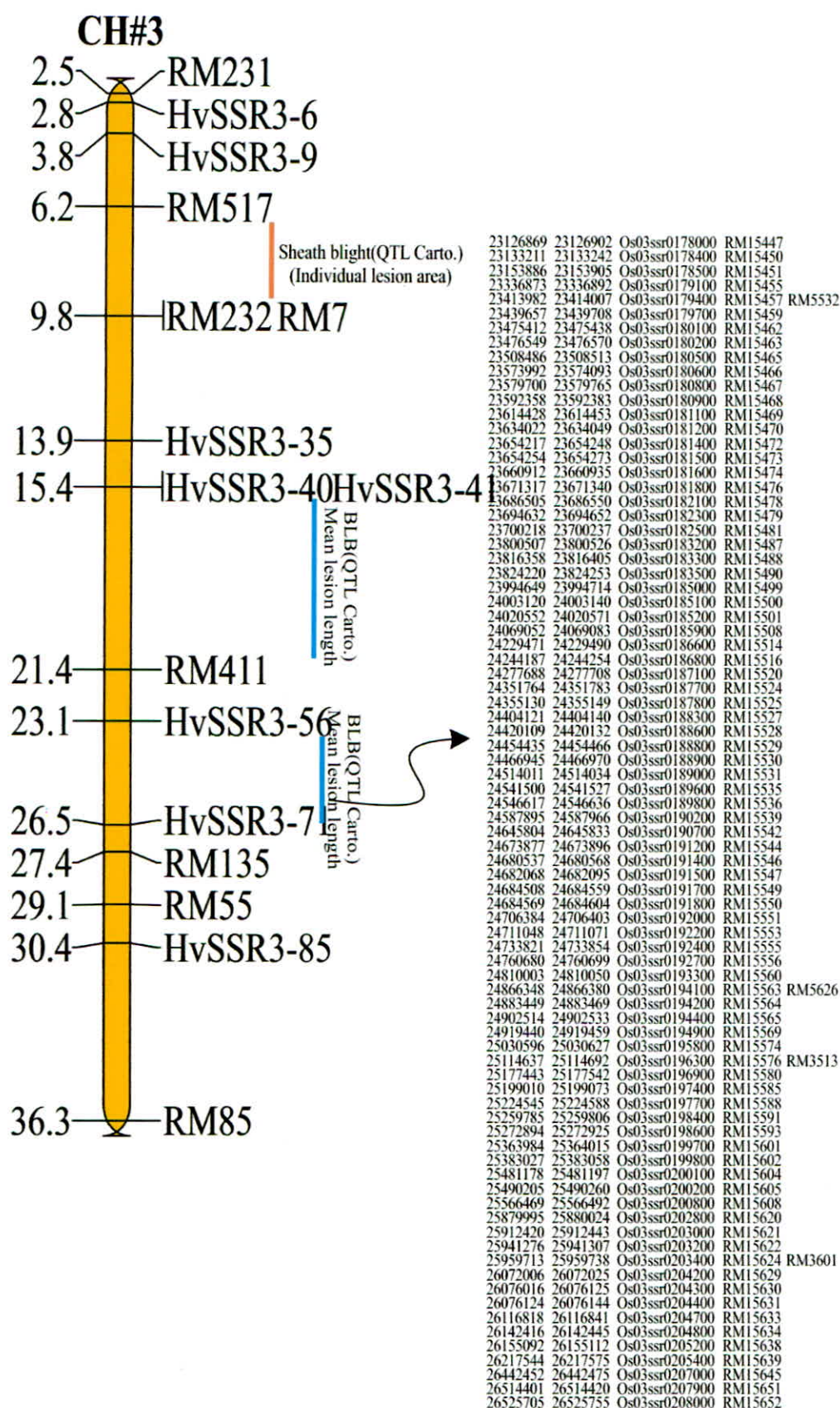
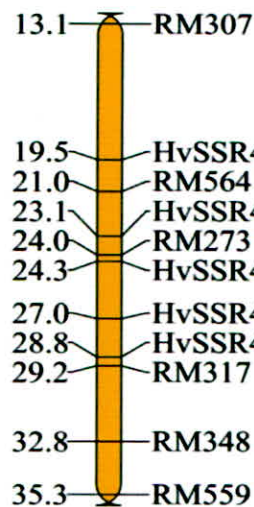


Figure 4.22.6 Genetic location and *in silico* analysis of QTL for BLB trait mean lesion length on chromosome 3



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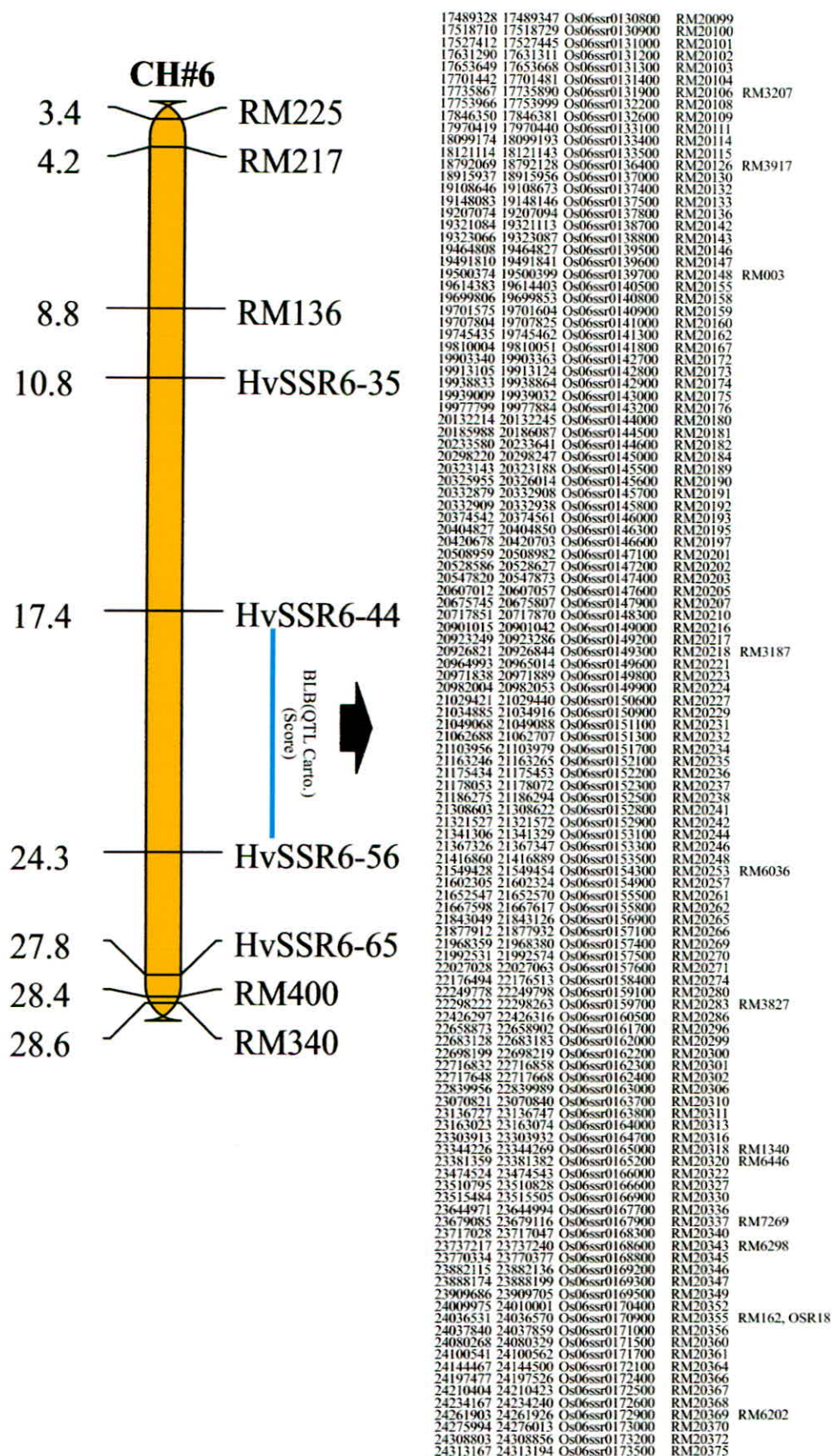


Fig. 4.22.9 Genetic location and *in silico* analysis of QTL for BLB reaction based on qualitative (0-9) score on chromosome 6

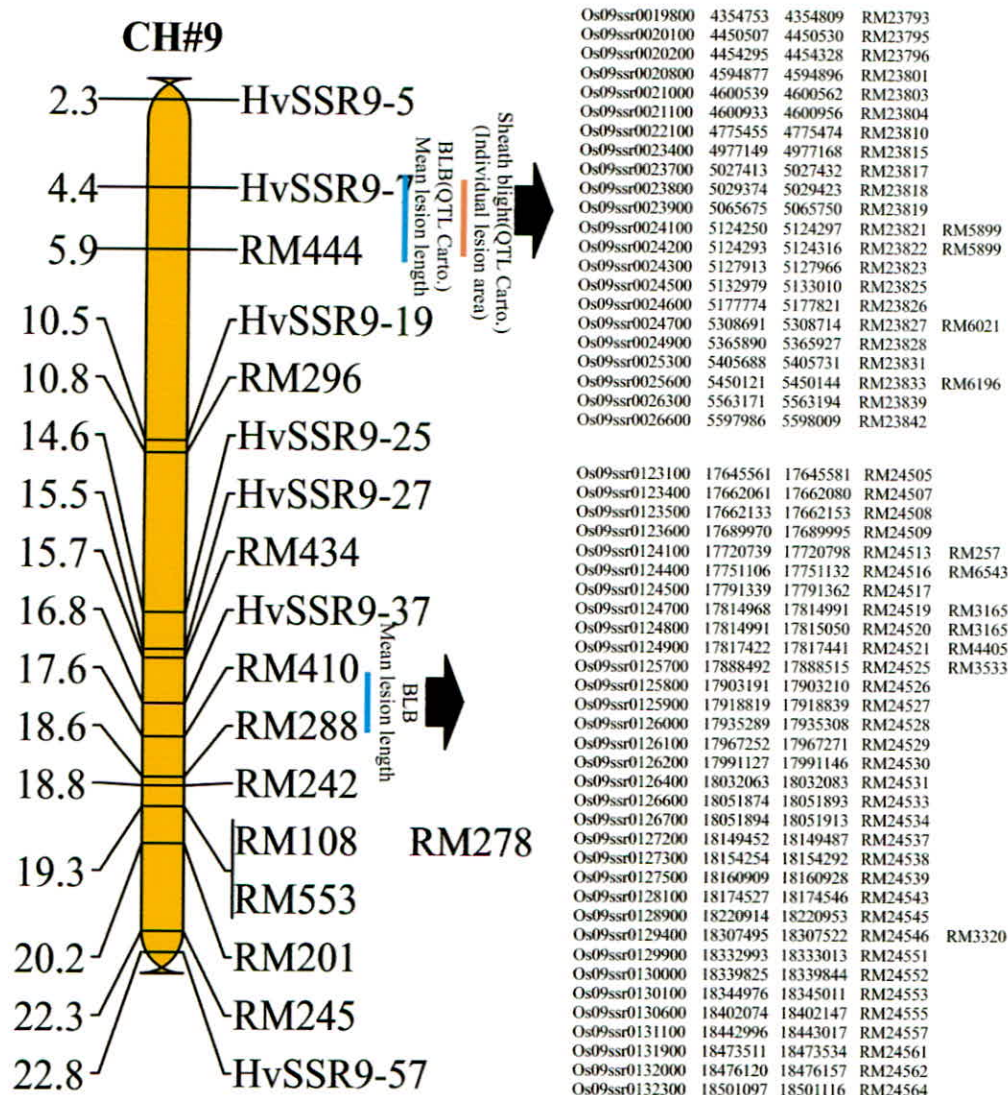
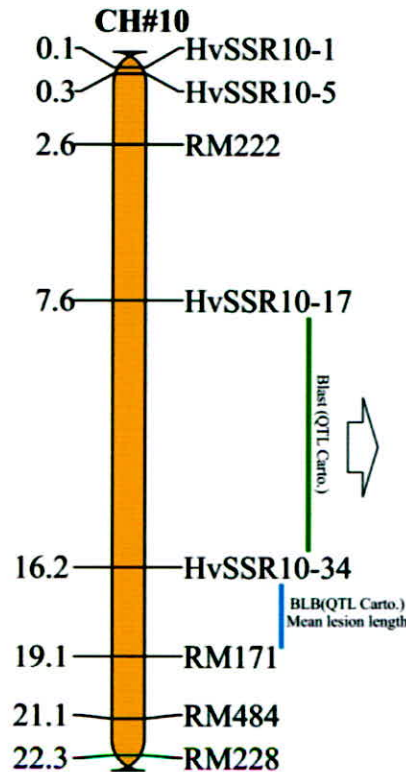


Fig. 4.22.10 Genetic location and *in silico* analysis of QTLs for BLB trait mean lesion length and sheath blight trait individual lesion area on rice chromosome 9



Os10ssr0044300	7616969	RM25151
Os10ssr0044900	7868246	RM25154
Os10ssr0045600	7939315	RM25158
Os10ssr0045900	7943131	RM25161
Os10ssr0046300	8174170	RM25162
Os10ssr0046600	8256353	RM25165
Os10ssr0047400	8405844	RM25172
Os10ssr0047500	8410514	RM25173
Os10ssr0047600	8410598	RM25174
Os10ssr0048400	8835803	RM25179
Os10ssr0049200	9078497	RM25185
Os10ssr0049400	9147854	RM25186
Os10ssr0049500	9158563	RM25187
Os10ssr0049600	9180138	RM25188
Os10ssr0049800	9188362	RM25190
Os10ssr0050000	9227459	RM25191
Os10ssr0050100	9228292	RM25192
Os10ssr0050300	9267348	RM25194
Os10ssr0050500	9288684	RM25195
Os10ssr0051400	9473961	RM25203
Os10ssr0051500	9474008	RM25204
Os10ssr0051800	9490548	RM25207
Os10ssr0052400	9600852	RM25213
Os10ssr0052800	9707493	RM25216
Os10ssr0052900	9748260	RM25217
Os10ssr0053000	9748334	RM25218
Os10ssr0053100	9770809	RM25219
Os10ssr0054000	9973420	RM25225
Os10ssr0054200	10011898	RM25226
Os10ssr0054400	10023520	RM25228
Os10ssr0054700	10114077	RM25231
Os10ssr0054900	10180789	RM25233
Os10ssr0055100	10263120	RM25235
Os10ssr0055800	10328835	RM25242
Os10ssr0056700	10645059	RM25246
Os10ssr0056800	10676955	RM25247
Os10ssr0057000	10693738	RM25249
Os10ssr0057200	10706763	RM25250
Os10ssr0057300	10715832	RM25251
Os10ssr0057800	10728472	RM25255
Os10ssr0057900	10751636	RM25256
Os10ssr0058100	10755719	RM25258
Os10ssr0058300	10788246	RM25259
Os10ssr0058400	10927062	RM25260
Os10ssr0058500	10927496	RM25261
Os10ssr0058800	10985762	RM25264
Os10ssr0058900	10985789	RM25265
Os10ssr0059200	11006019	RM25266
Os10ssr0059400	11052846	RM25267
Os10ssr0059500	11053343	RM25268
Os10ssr0059600	11058074	RM25269
Os10ssr0059800	11149495	RM25270
Os10ssr0060000	11263415	RM25271
Os10ssr0060100	11271278	RM25272
Os10ssr0060500	11306066	RM25275
Os10ssr0060800	11399164	RM25277
Os10ssr0060900	11399196	RM25278
Os10ssr0061000	11405612	RM25279
Os10ssr0061300	11500070	RM25281
Os10ssr0061600	11558184	RM25284
Os10ssr0062100	11609556	RM25287
Os10ssr0062400	11625492	RM25288
Os10ssr0062800	11739230	RM25291
Os10ssr0062900	11758371	RM25292
Os10ssr0063100	11818232	RM25293
Os10ssr0063400	11910766	RM25295
Os10ssr0064800	12035066	RM25302
Os10ssr0065300	12042141	RM25305
Os10ssr0065600	12119796	RM25306
Os10ssr0066100	12262912	RM25308
Os10ssr0066200	12298408	RM25309
Os10ssr0066300	12299623	RM25310
Os10ssr0066500	12319930	RM25312
Os10ssr0066800	12383518	RM25314
Os10ssr0067300	12761300	RM25318
Os10ssr0067400	12765859	RM25319
Os10ssr0067600	12790524	RM25320
Os10ssr0067700	12811355	RM25321
Os10ssr0067800	12813582	RM25322
Os10ssr0068100	12850286	RM25324
Os10ssr0068300	12873262	RM25325
Os10ssr0068800	12896275	RM25327
Os10ssr0069200	12977761	RM25331
Os10ssr0069300	12987447	RM25332
Os10ssr0069400	12992346	RM25333
Os10ssr0069500	13005988	RM25334
Os10ssr0069800	13019702	RM25335
Os10ssr0069900	13031786	RM25336
Os10ssr0070900	13117778	RM25343
Os10ssr0071200	13141557	RM25345
Os10ssr0071600	13258775	RM25349
Os10ssr0072000	13292367	RM25351
Os10ssr0073300	13408579	RM25359
Os10ssr0073400	13408607	RM25360
Os10ssr0073700	13497549	RM25361
Os10ssr0073900	13525459	RM25363
Os10ssr0074000	13530217	RM25364
Os10ssr0074200	13534107	RM25365
Os10ssr0074300	13543766	RM25366
Os10ssr0074500	13548099	RM25367
Os10ssr0074800	13559815	RM25369
Os10ssr0075200	13621847	RM25372
Os10ssr0076100	13779124	RM25378
Os10ssr0076200	13799854	RM25379
Os10ssr0076300	13805996	RM25380
Os10ssr0076800	13833328	RM25383
Os10ssr0078900	14017456	RM25390
Os10ssr0079300	14131829	RM25393
Os10ssr0079400	14131851	RM25394
Os10ssr0079900	14175587	RM25398
Os10ssr0080400	14312734	RM25401
Os10ssr0081100	14416021	RM25404
Os10ssr0081400	14435209	RM25406
Os10ssr0082000	14487184	RM25407
Os10ssr0082200	14527647	RM25408
Os10ssr0082400	14556470	RM25410
Os10ssr0083000	14607127	RM25412
Os10ssr0083100	14607468	RM25413
Os10ssr0083400	14640199	RM25415
Os10ssr0083500	14644679	RM25416
Os10ssr0084000	14703754	RM25419
Os10ssr0084100	14720406	RM25420
Os10ssr0084800	14786226	RM25423
Os10ssr0085500	14944132	RM25425
Os10ssr0085700	14980095	RM25426
Os10ssr0086100	15122464	RM25427
Os10ssr0086200	15170389	RM25428
Os10ssr0086300	15204443	RM25429
Os10ssr0086500	15287772	RM25431
Os10ssr0087200	15335835	RM25433
Os10ssr0087300	15336553	RM25434
Os10ssr0087400	15373554	RM25435
Os10ssr0087700	15392363	RM25437
Os10ssr0088000	15402956	RM25439
Os10ssr0088100	15435753	RM25440
Os10ssr0088200	15439971	RM25441
Os10ssr0089800	15644461	RM25452
Os10ssr0089900	15658967	RM25453
Os10ssr0090000	15659364	RM25454
Os10ssr0090600	15708543	RM25459
Os10ssr0091600	15873952	RM25462
Os10ssr0092300	15907618	RM25464
Os10ssr0092500	15916576	RM25465
Os10ssr0092800	15929074	RM25468
Os10ssr0093200	16029442	RM25470
Os10ssr0093300	16050093	RM25471
Os10ssr0093400	16050131	RM25472
Os10ssr0093700	16086674	RM25473
Os10ssr0094100	16106549	RM25475
Os10ssr0094900	16134620	RM25478
Os10ssr0095700	16220993	RM25483
Os10ssr0095800	16226520	RM25484
Os10ssr0096000	16234764	RM25486

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

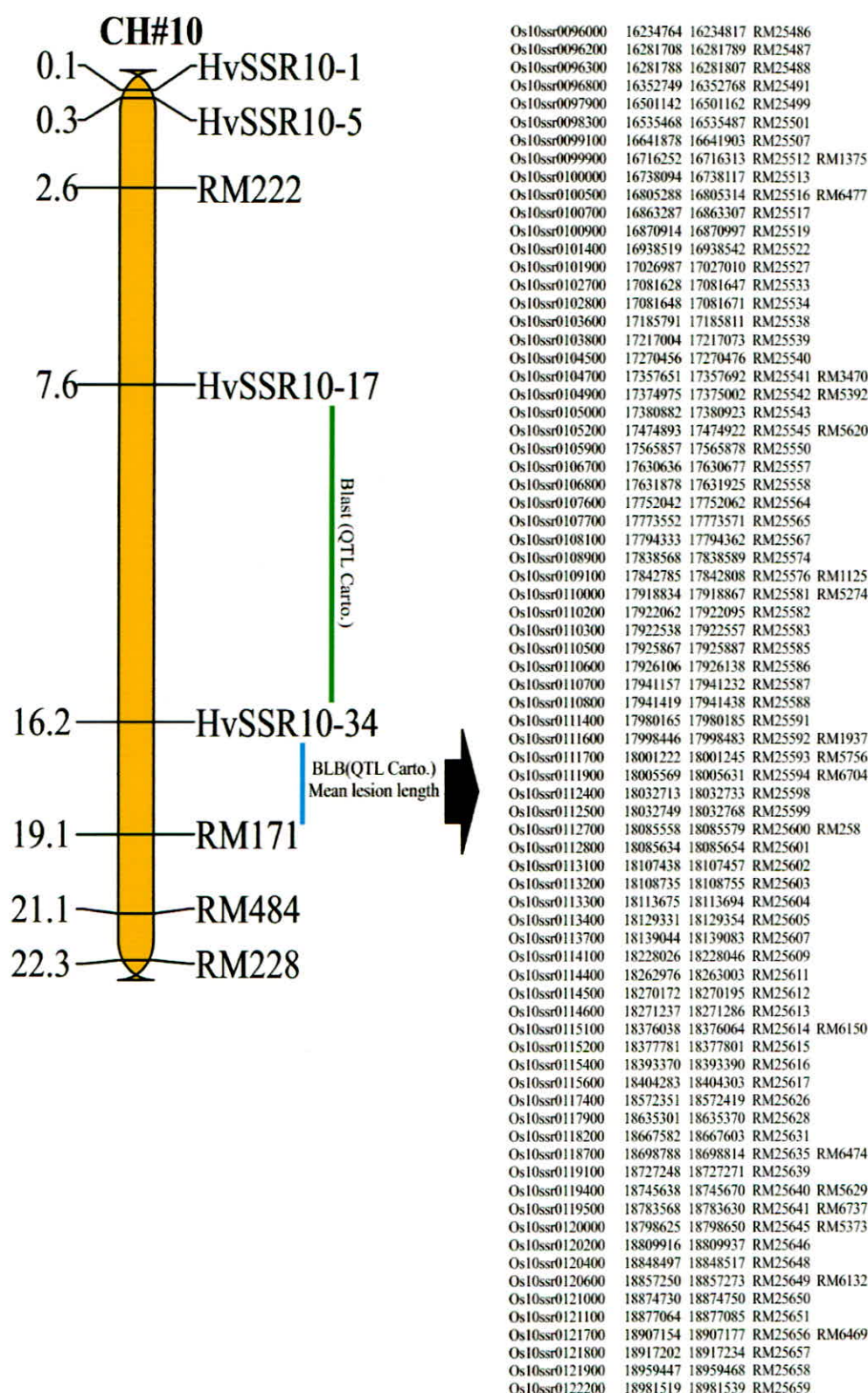


Figure 4.22.12 Genetic location and *in silico* analysis of QTL for BLB trait mean lesion length on chromosome 10

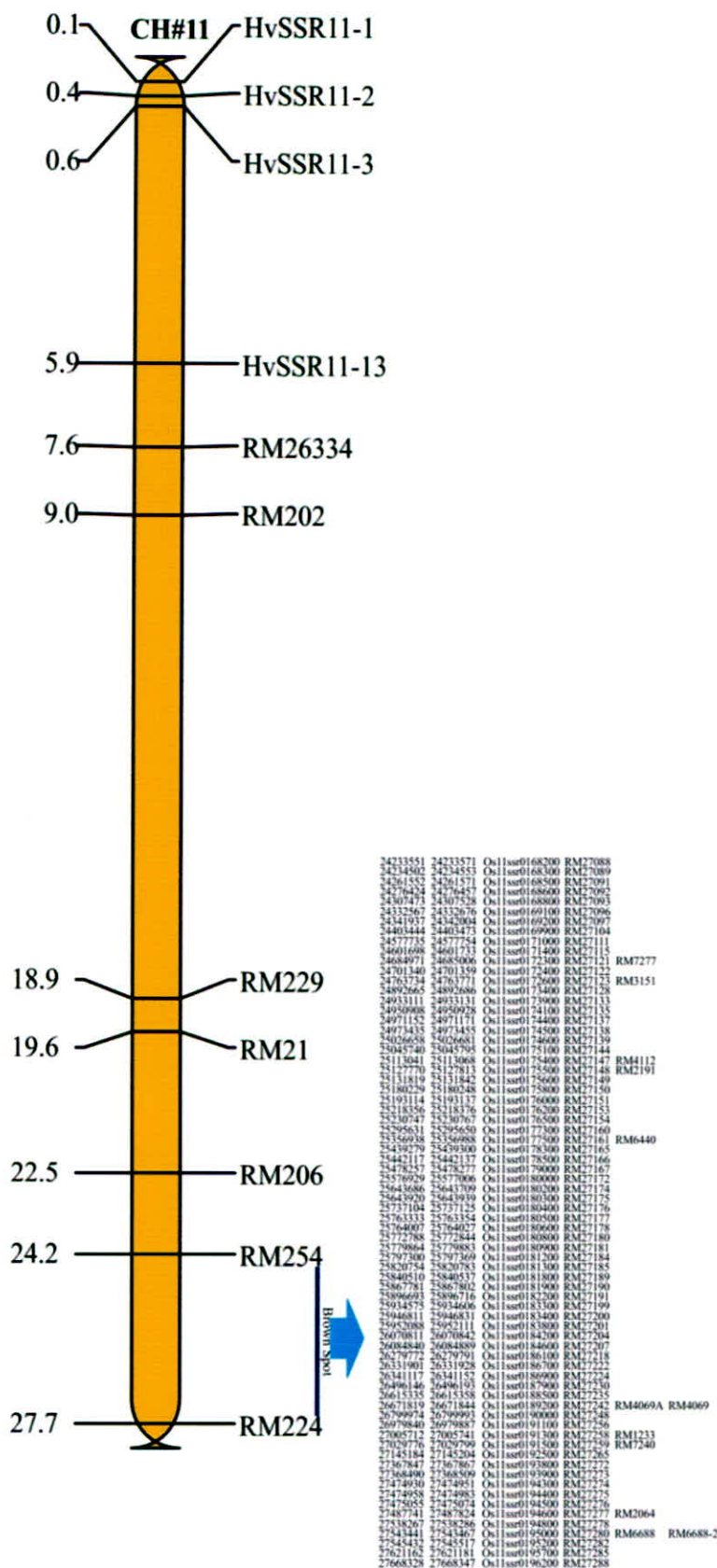
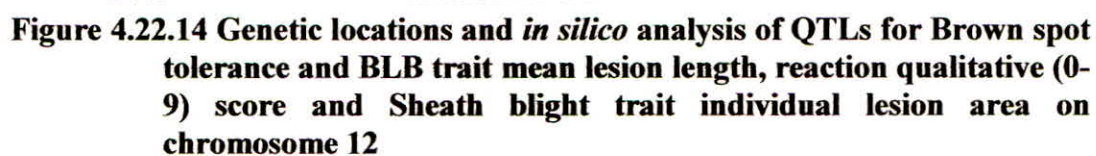


Figure 4.22.13 Genetic location of QTL for trait Brown spot tolerance on chromosome 11 and *in silico* analysis



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 F₁₄ 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 \times 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 \times 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 varieties 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 \times 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 a subset of 256 F₅ 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 (NILs) 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 F₄ 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 F₂ clonal populations of Jasmine 9 and Lemont. Two QTLs on chromosomes 9 and 11 were identified in F₂ 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 F₄ 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 \times 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 BC₂F₃ 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).

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 F₃ 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 BC₂F₂ 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 \times 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 QTLs, *qSB1*, *qSB11* and *qSB12* were identified at the different positions and chromosome by using 122 RIL population derived from a cross Danteshwari \times 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 F₅ 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 approach 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 F₁₄ 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 F₁₄ 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 \times 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.

**“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 F₁₄ 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

such as mean lesion length and BLB reaction qualitative (0-9) scale (SES IRRI, 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 colocalized with QTL of plant height.

A QTL, *qLB10.1* was identified for Blast resistance found to be present region between markers HvSSR10-17 and 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 three QTLs such as *qBS1*, *qBS11* and *qBS12* on chromosome 1, 11 and 12 respectively were identified for Brown spot tolerance. 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.

There were 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 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. On the chromosome 9 between markers HvSSR9-7 and RM444 found common for Sheath blight and BLB resistance. The results of the study are preliminary and need to be confirmed.

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Appendix I: Primers used for developing genotypic data

S.No.	Markers	Forward Sequence (5' ----->3')	Reverse Sequence (5' ----->3')
1	RM - 499	TACCAAACACCAACACTGCG	ACCTGCAGTATCCAAGTGACG
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	CGGTAAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC
16	RM - 24	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG
17	RM - 449	TTGGGAGGTGTTGATAAGGC	ACCACCAGCGTCTCTCTCTC
18	RM - 5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
19	RM - 212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
20	RM - 3825	AAAGCCCCCAAAGCAGTAC	GTGAAACTCTGGGGTGTTTCG
21	RM - 302*	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
22	RM - 486	CCCCCTCTCTCTCTCTCTC	TAGCCACATCAACAGCTTGC
23	RM - 14	CCGAGGAGAGGAGTTTCGAC	GTGCCAATTTCCTCGAAAAA
24	RM - 109	GCCGCCGGAGAGGGAGAGAGAG	CCCCGACGGGATCTCCATCGTC
25	RM - 485*	CACACTTTCAGTCTCTCTCC	CATCTTCTCTCTCTCGGCAC
26	HvSSR 2-1	AAGAGATGAGAAGAGCAATGA	CAACTTAGAGGAAGAAGGAGG
27	HvSSR 2-12	TCTCCAATTCTCCATCAAAC	CTTGCTTGAGCGAGTCTAAT
28	HvSSR 2-23	AGCTAGCTACACACTTTCCG	ATAGATGCATGGCGATATTT
29	HvSSR 2-27	GGTGCAATTCTTATTCCTTG	AATTTGGATTGAGATGTTGC
30	HvSSR 2-78	GTTTCCTTGCAAACAGACAT	AGTCATTCTAGCATTTCCCA
31	RM - 174	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG
32	RM - 492	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTCAGGC
33	RM - 475	CCTCACGATTTTCTCTCAAC	ACGGTGGGATTAGACTGTGC
34	RM - 341	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
35	RM - 221	ACATGTCAGCATGCCACATC	TGCAAGAATCTGACCCGG
36	HvSSR 3-6	AGATGAGCTTCAGTGCTAGG	TTCACCACAAAGTTCACAAA
37	HvSSR 3-9	TGTATTCAAGGAGGGCTAGA	CAACTGTTTCTGGAATGAT
38	HvSSR 3-35	TTGATTACGTGAATAGCTGG	CATAGCTAACTTGTGCGTTG
39	HvSSR 3-40	CGAGAGGTTTCAGAGAGAATG	GCATTACCCCTAAGGATACA
40	HvSSR 3-41	ATGCAATTACTTGTTCCTT	AAGTTCTGAACAACCACCAC
41	RM - 231	CCAGATTATTTCTGAGGTC	CACTTGCATAGTTCTGCATTG
42	HvSSR 3-56	GCCTATCAGGCTATCATCAC	GTGATCGACATTGAGGAGTT
43	HvSSR 3-71	CACACCAACTCACTCTTGAA	CCGTTTCGTCTATGTTTCATT
44	HvSSR 3-85	GCAAACGACACAAGTCATTA	ATAGTGCCCTTTCTTTCACA
45	RM - 517	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC
46	RM - 7	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCTGTTGTT
47	RM - 232	CCGGTATCCTTCGATATTGC	CCGACTTTTCTCCTGACG
48	RM - 411	ACACCAACTCTTGCTGCAT	TGAAGCAAAAACATGGCTAGG
49	RM - 135	CTCTGTCTCCTCCCCCGCTCG	TCAGCTTCTGGCCGGCCTCCTC
50	RM - 55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG
51	RM - 85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
52	RM - 307	GTAATACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
53	HvSSR 4-26	GAGGAATTCATTCATCATGC	ATTTCGTTATTTGCATTGGT
54	HvSSR 4-35	ACCAACCTAATACCGATGTG	CGCGAGTGTTGTAACCTTAAC
55	HvSSR 4-38	CCAAGCACCTCTTAACCTGA	CCGTTCTTATTAGGTTGTGG

56	HvSSR 4-39	CAAATAAGATCGCTGAAACC	TTCGGAGTAAATTGGACATC
57	HvSSR 4-42	GATGGTGAATCTCGGTCTAA	TGTCCTCATCATCACAACATA
58	RM - 564	CATGGCCTTGTGTATGCATC	ATGCAGAGGATTGGCTTGAG
59	RM - 273	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC
60	RM - 348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
61	RM - 317	CATACTTACCAGTTCACCGCC	CTGGAGAGTGTGAGCTAGTTGA
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	CCAGCATGCAACTGTAACATA
67	HvSSR 5-48	GAATTGAAGGTGGGACATAA	GAAGATGGCATGTAAACGAT
68	HvSSR 5-51	CCATGAAATAGTTCTAGGGAA	TAATTAATGCCTTCGTGGAT
69	HvSSR 5-52	GCTTAGTACTTGCGGCTAAA	CCATCTTACATGTCTCACC
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	GCAACGCACAACCGAACCAGC
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	CCCATTAGACATTTTCGGATA
83	HvSSR 6-44	GGAGCATCCATCACAATATC	GTAATTTCAAGTCAGCCAAGC
84	HvSSR 6-56	AGCATTTGTGTGTGCAATAG	ATGCTTGCCTCATCAGTAGT
85	RM - 225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
86	HvSSR 6-65	GTGTGGCAATTTAACATCCT	TTGTTGCTTGTCTTCACTG
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	TGTTTCAGGTTTCGTCTATCC
93	HvSSR 7-43	CAACTCAGTTCCAATCCCTA	TTGTGTGTTTCATATACGGC
94	HvSSR 7-46	ACAGCTGTAGAGGATGAGGA	TCCCTAATTGCAATCACAAC
95	RM - 125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
96	HvSSR 7-53	CGAGCATGTCTGTCAAGTAA	GTTGCAATGTAATGTTGGCT
97	RM - 2	ACGTGTCACCGCTTCTCT	ATGTCCGGGATCTCATCG
98	RM - 11	TCTCCTCTTCCCCGATC	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	TAAAGGGTTCACCTCATGGAC
104	RM - 310	CCAAAACATTTAAAATATCATG	GCTTGTGTTGTCATTACCATT
105	RM - 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
106	RM - 483	CTTCCACCATAAAACCGGAG	ACACCGGTGATCTTGTAGCC
107	RM - 72	CCGGCGATAAAACAATGAG	GCATCGGTCTTAATAAGGG
108	RM - 515	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
109	RM - 256	GACAGGGAGTGATTGAAGGC	GTTGATTTCCCAAGGGC
110	RM - 230	GCCAGACCGTGGATGTTT	CACCGCAGTCACTTTTCAAG
111	RM - 433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTAC
112	RM - 281	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG
113	HvSSR 9-5	GAACGGAGGGAGGTTGTT	AAAGTGTCTTAAAGCCAAGTC

114	RM - 444	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG
115	HvSSR 9-7	CATCTCAGCAAACAAGAACA	GTAAAGACTCCAGCTTTCTCC
116	HvSSR 9-19	TCGAATTTAGTCCAGGGTAA	GGTGAGAGATCTTGAGTTCCG
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	GCCTCATCCCTCTAACCTC	CAAGAAAGATCAGTGCGTGG
123	RM - 410	GCTCAACGTTTCGTTCTCG	GAAGATGCGTAAAGTGAACGG
124	RM - 108	TCTCTTGCGCGCACACTGGCAC	CGTGCACCACCACCACCAC
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	CCGATTTCATTGATGATTTCT
132	HvSSR 10-5	TCTCGCTCACTACCAGACTT	AATTCGCTTCACATCACTT
133	HvSSR 10-17	CGTCTTGAATCAATTTCCAT	GATTGCCCCGTAGAACTATTG
134	RM - 222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG
135	HvSSR 10-34	TAGACCGAGGAATTGAAAGA	TTTGGGCTTATTGTCAGTTT
136	RM - 171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTGT
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	TGGAACCTACCTACCTAGCCA
142	HvSSR 11-13	TGAAACCACAATGAGTCAAA	GCCCTAAACCCAAATAGAAG
143	RM - 202	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
144	RM - 229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTAATGT
145	RM - 21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
146	RM - 26334	GACTCCCTACTAGTGGTTCTGATTCG	CCTTTGACGATTGTGATGCTACG
147	RM - 206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
148	RM - 254	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTGGTAGC
149	RM - 224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
150	RM - 20	ATCTTGTCCTGCAGGTCAT	GAAACAGAGGCACATTTATTG
151	HvSSR 12-35	ATGACCATAATCCCAACAAA	GTCGTGGTGTATTCTTGGT
152	HvSSR 12-36	ATCAGCGACTAAGGATCTCA	CTAATGTTGCCACATACGAA
153	HvSSR 12-40	ATCTAACACAACAATCCCG	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	AGAGAGCCCCATAATTTCCG	AGGTACGCTCACCTGTGGAC
160	RM - 28305	GTCATCTTCGAAATGGTGATGG	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	CGAGGAGAACTACTCGTTCCGGTAGC	CCCTTCTCCATCTCATCTCACTCC
2	RM10018	ACTAGTACACCTCAACTTCACTCC	CCTTAGTTTGCTTGAGCC
3	RM3252	ATGCAAGCATCTGCTTATGG	GTTGGTAACTTTGTTCCCATGC
4	RM6470	AGTGTGAGAACCCAGGATGAAATGC	AGTGCAGCTTCAGCCCATGAGG
5	RM8068	GTGTCAATATGCAAGCAACAACCTCC	AGTATGTACGTCTCCTCCGTTGC
6	RM10218	AGTAGCCCTCTTGTCTCCATGC	CACACTAGCACAGTGGCATACTGG
7	RM3746	CCTTGATCGGAAGTAGCTCAACG	GCCCTCAGAGCAGTAAGGAGAGG
8	RM8131	ACCGCGGTCTTAATAACTATGG	GGTCCGATTAGATGAGAATCAGTTGG
9	RM600	CCTGTAGGTGGCACATTTATGG	TCGGCCCTGTATATCATTACTCG
10	RM8071	GGACAAGAGGTTCTCAATCTCG	AAACGGGATTATCGCCACTACC
11	RM140	CTTGACACAAGAGATGATGATGAGC	CATGCTGAGAAATAGTACGCTTGG
12	RM594	TCGAGAGAGGGAGAGTGAGAACATGG	GCCTTCGCACATAAAGGATGAACC
13	RM129	CACCTCCACGCCATCTCTCTCG	CTCAGACTCGGAGCTCATCTTCC
14	RM8139	GGAACAACGGAAGCCTTAGTGG	GGACCCATTGTATCTGGTATTACGG
15	RM3341	GTCGTGCGCATCATTTGGTATCG	CACCTTCCCGGATCAAGTACG
16	RM7405	CCCATATATAGGAGGAGGAAGG	AGTCAGTCATCACTGGTAGTGC
17	RM11307	AAAGCTCTGCAATCTTCTCTCC	GAATACGACATCAGAACAGTGC
18	RM6738	GCAAGTCGATAGGCTAATCAATCC	CATGTGTAGTGGGAAGATGTGTGG
19	RM128	TGATTTCTTGAAGCGAAGAGTGAGG	CCTCCTTGTGCTCAGCCATGC
20	RM1095	CCCATTCAGTTGATCCTGTCTGC	AGCTGGGATGCAGAAGAGTATGG
21	RM11739	TCCTCAGGGAGAAAGATGAGG	TTCACAAACGAACTCCAAGG
22	RM3285	AAGGAACGCGAGAGAGAGAAACC	ATTCTGAGCAGGAGAGGGAAGG
23	RM3738	GTGATGGGAGAGCAGGAGAAAGG	CGCAAGTGGACCTCTTATTGTGC
24	RM5794	AATCCGTCAACCCACCATGAACG	TTGCAGACTCATGGACACATGG
25	RM5310	GGGACCAAGACCTTCCCAATGC	GCGGAAGCAGGAGAAATCGTAGC
26	RM3362	ATTGCGTGGGATATGGACTATGC	CCTCTTCAATTCCCTTCTCTGC
27	RM12276	GTCGACGGCTTCTCAAGATTGG	TGAGACCTCTGTGAAGGCACCTCG
28	RM6840	CGACTGGAAGAAAGGATCATGG	CACACTACCAAGACTCCGCTATGG
29	RM6842	CCGTGCATCTCGTACCTAACCC	TGCACACACAATTAGAGGAAGAAGG
30	RM6367	GCAACCACGACATCAAGAAACC	GGAGGTTAGTGTCTCGGAGTGG
31	RM12368	GAGATAAGTGCCACGATTGATTC	GGAGCCGTACGAGTAATCTCTGC
32	RM12478	CTAGGGTTTTCGGGGCGGATGG	CCGCGGTTGACGTAGATGATGG

33	RM6616	TCAAGATCAACGCACTCCTCTCC	TCGTACGAGCAACAGGTGGTAGC
34	RM4355	GGGATGAGAGTAGAAGGCACAAGG	GCTTAATGCCCTTTGATCGTTGC
35	RM555	TTGACATGCGAAATGGAGATGG	TTGGATCAGCCAAAGGAGACCC
36	RM12923	AAATGCACAGGCATTCGTAGACC	GAAAGAAGTGGATGGAGGACATGG
37	RM6375	CGAATGGAACGACAAAGAGATGC	ATAGATCGACAACGTAGATCCACAGG
38	RM452	GTGGACTTGGCGAGATGCTACG	GTTAAGGGCAGCCACCAGATCG
39	RM550	GCTCTGTCGCGAAGTCTTCTTCC	GGCTAAGCCACATGAGACAGG
40	RM13069	GGAGGGTTATTCTCGCGTAAAGG	GACCCCTTCACTTTCCGTCAGC
41	RM13170	GCCTCTTACCCCTCATCCTTCTCG	CGCCTGCCACCTTTATCTTGAACC
42	RM6509	GTAAGACGGACGCCATGGAAGC	GACAGCACCTGCTCGAACTGC
43	RM13541	CTCCTCGCTTCGTCCACTTCC	CCATGTGTCAACCGACTCAACG
44	RM13542	CTTGCTTCAAGCGGTGGTGACG	CACGTGCTCCACTCCAGATCC
45	RM6617	CTCCTCCTCCACCTCTACTCC	TAATAGATGGCGATGGACGAAGG
46	RM13611	CTCTTGAACGGCTGCACGAAAGG	CGCGAGAATGGTAGGTGGATCG
47	RM573	TCATGTTGACGACACATACACG	CTCTTCTCCCTGGACCACACC
48	RM425	ACCACAGCAGGTGGAACAGG	GCTAGCTAAGCCCAACACCAACG
49	RM14001	TGTGGCTGGCTCCGATACC	ACCCTGCAGGATCATCAGAACG
50	RM6307	GCCTCACCTCATCATCACAACG	TGGTCTTCTCTACTGTTGTTCC
51	RM1063	TTGATCCACTATCGTGGCCTACC	TGGCAAGTGCACCTGCAAGG
52	RM208	AGTACCACCACTTCTCTGCAAGC	TCGATTGGCCATGAGTTCTCG
53	RM3850	GCCTCGAAGAAAGCATGTCC	AAGCGACCTGTACCTCAACTTCG
54	RM7485	TCATGGAAATGCCAGTTTCTCC	TTCTTCGATTAACTAGCCTCGACAGC
55	RM5807	CGCCATGCCCTAACCTATTATTGTC	ATCGTCATCCATCCAGCAAGC
56	RM3654	AGCGAGAGAGATGGAGATGG	GCGGCGGATATAAAGAGAATGG
57	RM3265	CCGTCTCTCTGTTGTTGTTCTGC	TGGTTCGCCCAACAATTAAGACG
58	RM60	CAAGTTCACCCGCCCTTCTCG	TTTCCATCATTAGCAGGCAGTAGC
59	RM14303	GTCTGTGCGCTCCTTGTCTAGC	CCTGGACCAATTTGATGTTGG
60	RM14327	GATGCAGTAGGAACACCAACACAGC	ATCGAGTACCAAGTGCCTGTGC
61	RM5474	GTGGGTTTGTGTTTGAGAGACG	GTGTTGGTGAGCATAGCAGTTGG
62	RM3392	AGCAACCAACCCAGTAGTTAGCC	GCTCATTTGCTGCTGTGTTAGC
63	RM175	GACGGAGGAGTTCGAGAGGAAGC	GTGAAGCGGACTAGCGGGAGAAGG
64	RM14593	GCACACCCGCTCACACTTGC	GCTCCGGTTTCTCGAGGAGTTTACC
65	RM1256	TTCTTCTTCTGCTTCTGTTCC	CAAAGCCATATAAACCCCTAGCC
66	RM5748	TATCCAAGCCTCCATACATAGTGC	ACAAGAGGTGAAGAACTGGATGAGC
67	RM3204	CTTACACACATGGCCCCACATGC	TCCCTCTCTCTCACTCTCCCAACC

68	RM15064	AACGTAACACAGCTGAGCAGCTACC	ATCTCCTGCAGCCTCCACACC
69	RM15065	ATAGAGTCTGTCGGGAGGAAAG	ACTGAGTTCCAATGGCTCAACG
70	RM7642	TTAGATCACGATACCTAGGGATGC	CGAAGAAAGAGAGCAGCAAAACG
71	RM15281	CGGGCTTATATCTTTGGCAATGG	GCCTCCTCCTCCTCTTTCTCG
72	RM5626	GGACGCCACCTTCCTCTTCTGCG	CGGTCAATAAACGCCATTAGACCAAGC
73	RM15567	TGCATCGATCCTTTCTTTGTCC	CCGGGACGCTATATGTTCTTCC
74	RM426	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTTCATTGTAGAGTGC
75	RM15721	CAGCCAAAGCTCTCCAATTAACCC	TGTGCCCTTCTGATCTGATGTTGC
76	RM15741	AGCTGTTAGCCGACTTGTCAACC	TCTCCAACAGTTCTTCGATTGTCC
77	RM3525	CGCAAAACGACACAAAGTCAATACC	CTCAGCTCATCAAGACCTCAAGC
78	RM1373	TGCTATACCCAAATGTCCAAGC	ATCTTCTGAGTGTCTGCCAAGC
79	RM16140	CGAGATGGCGTCAGCGAAGG	AAATGGCGAAACAAGGCTGTCAACC
80	RM3684	CGACGCAACCTATACCTTGACAAAGG	GGTGTCCAGCAAGTATTTCAACC
81	RM16147	GAGATCATCCTGAAACAACCACTGC	TGTCCACCCCAACCCCTCTTTACC
82	RM7389	ACGGATGCATGATCGAGATGG	TTTGAGCCGGAGGTAGTCTTGG
83	RM7585	TGCAATCGCGTAGTTGGTAGAATAGG	CTCGACTACCTCGCCATCATCC
84	RM16368	TGTCAGAGAATGACAAAGTACGC	GGATGTATATCTGCCACCAATGC
85	RM16382	ACTCTGGTATATCCAAAGCGATCC	TCACACTCCGTTTCTGTTACAG
86	RM16493	TCGGCAGCAACAACCTCTTAAACC	TCAACAAGATGACTCCCTGTAGCC
87	RM6659	TGTGGAGGCTTAGGAAATTTCTGG	TGTGAAACATGCCACGATACTGC
88	RM16559	CCTGGAACCTGGAGGTGTTCTCG	GTCGTGGACGATTTCTTCGTACGC
89	RM401	GCATGAGCTGCTCTCATTATTGTCC	GAAACGAACCAACGTTTCATCG
90	RM16717	ACAGCACTCATCATTCATGTCC	GATAACTGATCGACGCGACACC
91	RM6314	CATGCTGATATTGCGGTTTCAGG	TCAAGCCCTGCCCAACTACG
92	RM16825	AACTAAACACACCCCTGAACAGC	ATGTAGTACCCAAAGCACAAACG
93	RM5979	GTTTGAACCTCTGGTCTCAATGC	ACAGTATACGTGGCTCAATCAGG
94	RM3708	GCGTAAGACGACGAGACCTTACC	TGATGACGTGGCTTTCATTGG
95	RM3839	ATGCATGTGATGCCAAGAGTGG	GAAAGCACACTGCACACATACCC
96	RM17303	TAGCGATTGGATGGAGGCTGAGG	GGTCGCCTCGCCATTAGTTACG
97	RM17388	CGAGCCACGCACTCAACTCTATCC	GCTATGTTGACGGGCTTGTGG
98	RM349	GCTCGTCTTCTGCTCTGTGTGC	AAGTACGCGCTGTCCATCATCC
99	RM8217	TTGCCAGATGGAGGTTCTAATGC	TTATCAACATGAGACTGCGGAGTAGG
100	RM17604	TCCCTCTCTCTCCGATCCTTAGC	CACATCAACAACCAACCATGC
101	RM17616	TCGTCGTCTCTCTCTTTCATCG	GCTAACACCCGAAAGAGGCAAGC
102	RM507	TGCCCCATGTATGTGAGGTACTCC	GCCTAATCCAGGACAAAGCTACGG

103	RM1024	AACTGCCATCTCTGAAACTCTGC	CATCTCACTTCAGAAAGGATCATAGCC
104	RM17804	GTCACACCACCAATCTCTCTCC	GTCAATCTCTCTCAAGAACTGACG
105	RM5374	TTAGGGTTAGGCTGTTGTGTACTGG	CGCTGGATCACAACACTGTAGC
106	RM17863	GGACGTCATGATTGTGCTTGG	GTCATTGGCTTGCAGGATACACC
107	RM17886	AAGAGATGTGTGGTTCAACTCC	CCTAGGTATTCCCAAGAGAGTCC
108	RM17962	CAGCAATCTCTATCACTGCAACC	GCTAGATGACCACCTGCTGTACG
109	RM7588	GCAATTTCCGAAAGCCCATGACG	GCCCATGGGTACGTGCTATGATCC
110	RM3381	ACGAAACGCGAGCTGACAGAGG	AATAGCTGCCAGCAACTGCAACG
111	RM5140	GGCACTCGTATTTCTCAACTTCTCC	GGGTGTATCAGGAGTACAGGTTGC
112	RM6229	TTACACTGCTCCACAGCCATCC	ATTCGATCGACGCCGGTAGGG
113	RM18451	ATATACAGCGCGGACATTGTGG	CATGTCATCTTCACGCGAATCC
114	RM18614	TGGCGCAATATCTCTCTCAATTCC	TGCCACTTGTGTGTTGTTCTGC
115	RM6054	AGGCTCTTCGGCTTCATCTCC	GGTCTCTGATCAGTTTGTCTTGG
116	RM3486	GGAGTCGGCACGTAGTAGAGG	GTCGGTACTATTCTGCTCCATCG
117	RM538	CAGTTTGACACAAAGCAGCAAGC	CTCCACAGCTTGAGGGAATGC
118	RM3790	CGATCAACCACCTCAACTACTGC	TGTGCATGTGGACACGTAATGC
119	RM3170	GCAGTGTCAATCTCATGAAACCTACC	CAGACTCCAAGCACCCATAACC
120	RM19218	CGGAGGGAGTAGGTACGTAGGG	CCCATTCATCTACACTGACG
121	RM6775	AATTGATGCAGGTTACGCAAGC	GGAAATGTGTTGAGAGTTGAGAGC
122	RM7158	CATGGACTTGTAGCAGCGTACC	ATTCATCGATGGCCTCTACACC
123	RM6467	TGTTGGATTGGAATCGGAAAGC	CTCTGCTGTGCTGTGCTGTAGG
124	RM1369	CATCGATTAGCTTACATGGCAACG	ACTAGTGCAGCCGCTTCAATGG
125	RM3805	ACACCACCATCAACGTACCAACC	AAGTCGAGAGGAAGAACCAAGG
126	RM19623	CATTCTGCAGTGTCCATTGTGACC	GTGGTTGGTCTGCTCCAGTTCC
127	RM3431	AAGGGAACATTCTGGAAGACACG	ACACATTGCGTGTAGTGTGAAGC
128	RM5963	TCAAAGTTACGGGAAATGTGTGG	CTGCCATAGCTCCGTTTCTCC
129	RM3183	GTGGTGTCTAGTATGGACGAGAGG	CGGTTGGTAGACTGTAAACAAAGTGC
130	RM7583	ACTGAGCCAGCCACTGACAAAGC	CCGTAAAGCAGTCAGTCATTACAGC
131	RM19976	AATGGAGTCAATCGCACCAAGG	TTTAACTGTCCCTACGATCCTATCC
132	RM20069	GCGAGCGAGAGGAGATAGACG	CGAATTCGGCACGAGTAATAGGG
133	RM20071	GGAGGCGCCTTGTGGAACC	ATGTCATCGGAATCGGATCAAGC
134	RM20152	GGGTGGCGGTATTATAGATTCAAGG	TTAGGCCCAATAGTGGAGGAAGG
135	RM20155	GTCTCGTCTGCTGCTCTCTCC	CCTCTAACCTCGTCGGAATCG
136	RM7434	AGGCTTCTTGGAAATGGAACCTGC	GGGAATATACGTGGATGTGAGAGG
137	RM1370	GGAGGGAGGAATGGGTACACG	TTGAGAGTGAAACGAGAACCAACC

138	RM3765	ACACCATGACACCAAGGAAGG	GGATGCTTCCAATCCTCTCACC
139	RM5753	GCACCATCCGTCAGAAACAGC	TCCGAGAAAGAAACCGCTAGG
140	RM5463	AGTGCCTGTTGTTTCTCTTCG	CATGGTCAGAGCAAGTTAGTGTGG
141	RM6652	AAGCTCGTCGAGAGTAAGAAAGACC	ACCAGTCCAATCTTCGTCATCG
142	RM3394	GAGAGGGAAGGAGTTTCTTAGC	TAGTTTACACGTACCCATGTGC
143	RM20891	GTGGATGTACGTATAAGCATGTGTGC	GTACGGCTGACGGCTTTATTCC
144	RM20973	CGTGATGGTACAGGGAAGAAAGG	CGCTACACAACACACACAATCG
145	RM3224	CGCGAGGAAGAAGAGAGATCC	ATTATCGAGTGACGGAGAGAGG
146	RM3484	TCCGGTCGTCCTCATCGTATCC	GCCCTCTTGTCTCCCATCG
147	RM21238	GAGCTTCTCTCACCCATCACC	CTTCTGCAGAGGGTGTTCACCG
148	RM21242	GAGAGGAATGGAATGGAATGAGG	GAACAGGCATGTTGAAGAGTGC
149	RM21323	ATTGCGATGTTGGATGTACCG	AGCAACTCGTCAATGAACATGC
150	RM3456	TTCTCCTTGCAGACACAGATGAAAGG	GCCTCCTATATAAACCCGGCGAACC
151	RM214	GAACATGCTTCAACCATCAGG	GATCCTCTCAGTTCAGTGCAAGC
152	RM5543	GGTGCTAGGTACATGGCTGACG	AGAGCAAATCTGGGCTATCTGC
153	RM500	AGAAGTGCAGGTTGGCTCTGC	CACGAATCTCGGAGTGTCTAGGG
154	RM5875	AATAAGCGAGATGGACGAACC	TTTCCCACCAAGGGAAGATGG
155	RM2752	GATCCGCTTGACATGGATAGG	TACACGTGTGAGCGAGCTTAGCC
156	RM22105	AAGTGGAGGCGTACCTTGTTCC	CGTATCGCGTTTCGTAATGTTGC
157	RM6389	ACGAGGAGTTCGTCGCTACTGC	AGTCTCGAAACACGTCGACTTCC
158	RM420	CCTCTCACTCTGCCCTGCTCTACC	TCTCTAACTCTTGAGTGCAGCAACC
159	RM7034	GTGACCAATCCTTTGACAACTCG	TGATACATGAAACGTCCTGCTAGC
160	RM3702	CCAGCACCATCTGAAATAGAAGC	CCATTGATTAGGACGGTCAAAGG
161	RM3309	GCCTACTCAGCTTCTCTCTCTTCG	CGCCATTTACGGCAGCAACC
162	RM22418	GCAGTATCACGAGTAGCACACC	CCATCCTCTTCCCTCATCACACG
163	RM547	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC
164	RM8243	TCTACCTTAGCTGTCTGAAATTGG	CACATACCTGTTCCGTTTGATCC
165	RM22694	TTAGCTGTATTAGCCCCACATAGCC	CGCCGGTCTTCTCCTCTTAGG
166	RM22696	AGGTGAGACTGTGAGAGTCATGC	AGAGTACGAAGCCTCTATCTGACC
167	RM22837	ACCTGGGTCAGATGTCTGTTTGG	GGTAGAGCTCCATCCATCTTAGTGC
168	RM22883	TTGCCCTCCCTCAACTCATGTCC	ACAACGTGGACAAAGATGACACG
169	RM6990	ACTGGGTGTGATCCTTTCTGATGC	GTGATCCCAGATACACGATGTAGGG
170	RM6193	ACAGCTTCACGATGTTCTTGTGC	GTCAAGAAGCTCTGGGCTAACG
171	RM22997	AGTCATGGTGTGGACTGTTGG	CAAGATGGATGTGTGAACATGG
172	RM23001	CAGTTCCTCTCCTCCACCACTTCG	TGGTGGACTGGAGGGCTACTGC

173	RM23175	CGTGAAACACAAAGAACCCAGATGC	AGCAATGTCTGCCTGCTACTGC
174	RM23251	TCCGATACTCCCATAGTTAGACC	ATGTGGTTGGCTATAGTCTAGG
175	RM3845	TCGGATGAGTCCCTTCGGTTTCC	TCTCAGCTCGATCTCCTCTAGACC
176	RM3452	TGGACTTGGTCTCTCCAACTCC	CAGTATGTGTGGTGGTCAAGC
177	RM502	CATCTCTGTTCACCTTGCTTTGC	CTACCAACAACCCAAAGAAGG
178	RM23502	GGTAAAGCCTCAGAGCCCAACC	ATTTGTTCTGTTCTGTTCTCG
179	RM7631	TGAGACAGGCAACATATGCTGAGACC	GCATGTTTCATCTTTGGTCACTCATGG
180	RM23654	CTCCGATGCCCTTCTTCTCTTGG	AAAGGGAGTAGCAAGCCGAGTGG
181	RM23662	GAGAGACGATGGCACTATTGG	CGAGGAACCTTGATTGCGCATGG
182	RM23736	GGCGATACCTGCCATAGTTTCC	CCGAAAGCAATCTATGAGACACC
183	RM23742	GTGGCATGGAGAAAGGAGGAAGC	GGCCCAATATGTCACCTCCCTACCC
184	RM23743	AGGTTTGCCAGAAAGCCAGAAAGAGC	GGCCGATGGTATGATGTGACC
185	RM5799	CTTGACAAAGAGGCAACACTCC	GTTTGGTAGGTCGCAATTGTTGG
186	RM23916	GATGGTTGGTGGTTAGG	GTCCAGTGATAAGCCATGCTTGC
187	RM24085	CGACGAACCTCCTCTACCGTTTACC	CTGCGTGATCCAATCCCAAGG
188	RM3912	CACTCAGATTTGGCCGATCC	GCTGATCCAGATCTACCTGACACC
189	RM6845	CGGCAAACTCTACGAGGTAATGC	CACACGTTCTTCTCCTTCATCTCG
190	RM24271	TCTTGCAATTGACACCTCTTGAGC	AGTCCCAACAACCTGGAAGAGAGG
191	RM257	CCGTGCAACTTAAATCCAAACAGG	GGAAATCCTATATGAGCCAGTGATGG
192	RM6491	GGTGTTCCTGCGTGGCTTCC	GTTCCCGCGCTTGTCTGTCC
193	RM24718	TGACGTGGCAAGTTGACTGTGG	TAGCCGATGGAGCCACTAGAAGG
194	RM24740	AGTCAACACATGAGCGTGAGAGG	TGCTTTGGATGTACGACTTGTCC
195	RM1026	TCTGAGCTTACAGCTGCTTGACC	TGCTGCCCTAGGCTAGAAAGTCC
196	RM6797	CTCCTCCATCAGGATCATCTCG	GTGAAGCCAGCTAGGTTGAATGC
197	RM7492	CAAGGATTTGAGGGAACATGG	TTTAGAGATGGTTGCCAAGAGC
198	RM24952	AGTGGAGGAATATGACTTCAGAGG	AGAAGATGGTAGCTGGCTTGC
199	RM6179	GCGCCGCCGAGATGATAAGC	AGCGGCATCTCGTCCATCTCC
200	RM3882	AGTATTGCCACCCAGATGGCTACC	CCTTCTCAAACCTGGTGATTTC
201	RM216	GATGGTAAAGGAAGAACGTGTGC	CACATCAGACGCATCACATAGCC
202	RM6646	GGCAGTTTGATGTTGGAGAACTGC	ATTACGCGACGACATGCACACC
203	RM25149	CATGCCATTGCCCTCTGATGACC	TTGCCCGTAGAACTATTGGATCG
204	RM1126	TATTCTACTCGCAAGTCGCAAGC	GTGGATCACAAATCGGAACCTGG
205	RM25366	TCGGTCTCTGTGCCGTGATTAGG	CACCAGCGCAGCAACTAACATCC
206	RM5689	TAAAGTCCTGTAGTAGGTACACACC	CGATGAGTCAGATTGAAGTAGC
207	RM1375	GCTTGTGGCAGTTTGTATTGTGG	GGTACACTAAGTGGGCAATCAGG

208	RM25663	GCTTGAGCTCGTGGGAGG	CTCCTGCGTTCTCCATCGAAGC
209	RM3510	ATAGAAATGCCATGGAGACCAACC	AGACGACGAAGGACAGATAAGTCG
210	RM3563	TGGTACGGAAGACGAGAGATGC	TGAGGCGAGTGTCTGATAACTGC
211	RM6673	CCACCCGTCTCATGTTCTACTCG	ATGGAGATGATACACTCGCATCG
212	RM4771	AACTGAGAAACATGGACAGAAAGC	CTCAACCACACCCCTCATTCACC
213	RM590	GAGATCGAGGAGGAGGTGAGG	AGTACTGCCGATCATATGGAAGC
214	RM6327	GGAGCTGATACAACAATCAGACAGC	CGTACGCTGCTCCAGATTAGG
215	RM7173	TTTCTGTGGTCTCATCTCTCTCC	CAATGTGTGATGTGGGATTCTTGG
216	RM26063	GATCCATAATGCCCTCTTCGATTGG	AACTCCAGCAGTGAGAGCGTAGC
217	RM26105	AGCATCAATTACGCTTGCTTGC	TTCTGGTTCTTTGAGAGAGTGTGC
218	RM332	GAAGCGAAGGTGAAGAAGAAGC	CCTCCCTTGATGATACCTTGG
219	RM26108	TTGTGCCTGTGTGAGAAAGAACTAGG	GCAAAGGTAGCTAACACACATACGG
220	RM26249	AGAAACAGCTGGAACCTACAATGG	CATGCTCGGCTCCTCTACCC
221	RM536	TACCAGGATCATGTTTCTCTCC	ACTGTGAGATTGACTGACAGTGG
222	RM5590	GAGGAGGAGGAGTACATATCTGATCG	AGCAACTGGATAAGCGATTGAGG
223	RM26643	CTCCCTTCTTCTGCTGAGACACC	CAAGCTACTCATTTGGGCTCATCC
224	RM26646	GTTTCATCGGATTCTCGGTTTCAGG	GCGGAGGAACAAGACACGAAAGC
225	RM26652	CAATCCATTGCTGTTGATGC	CAAGATCTCCAAGGTGCTGAGG
226	RM26796	TTCAAGACATGGTGTGGATCTGG	GCCACTGTGCCAACATTATAACC
227	RM26998	ACGCACGCACATCCTCTTCC	CGGTTCTCCATCTGAAATCCCCTAGC
228	RM27167	GCGATCTCTCGCTTCTTGTCTCG	GCTCCGCGTCGACATGATGG
229	RM27318	TGAACCTGGACATGCCCTTATCC	ATGCGCCTTACTTTTGTGTAGG
230	RM27326	ATCAACGAGTACGCAACAGTCC	TCCTGTCTCTTACATCCTAATCG
231	RM3577	GTGACGGCAGAGGAGAGAAAGC	CAAATCTTTCTGCAATGCCACACC
232	RM7443	ATTAAACCTTCATCAGGCTACGC	AAAGTGCTGCGTGTACTTTGG
233	RM5568	GATCCAAATGGAAAGACCTGTGC	TTATTGCTTGGCGCCCTTAGTCC
234	RM3323	CATCTTGGCGTTGATACGAAGG	ACGAGTCGAGCACTACATCTCTCG
235	RM5927	TGTATAGCCCGGAAGTATGATCC	TCTGGTCTCGTCTCTCATGTGC
236	RM3747	CTTGGTTTGGTTGTGTCCAAAGC	GTTCTGATGAACAGGCCGTAGC
237	RM27542	GCCATCTTCTCGTACCTCTCTTCC	CCACCTACACCAACCAAGTACTCC
238	RM27564	CTTCTTCGAGGGTTTCTCGTTGC	CTCCTCTTATGCCCAACCACTTCC
239	RM27706	ACTCCCTCCGACACCATCATCC	ATACGGGAACCCCTCACGCTACC
240	RM1036	CTTCCCTGTCCATAGATCTGCTTGG	GAATGACCCCAACCTGTCTTCTTAGC
241	RM27877	GGAAGCCATGAAGATGTGTTGC	AATTTCTCCGAGCACCTGAAACG
242	RM27962	GGGAGTCGTGATTCTGAGACG	ATCCACGCGCAGGAGATAAAGG

243	RM27970	TCCACCACTCTGACGTCTACTAACC	CTGCGGGAAGTGTAGGAGAAGC
244	RM27973	CCACACTGCCAGGATTTAAGC	CTGTTCCCATCATCCAAATGACC
245	RM28130	CAGCAGACGTTCCGGTTCTACTCG	AGGACGGTGGTGGTGAICTGG
246	RM28404	GTGGGAGTCGAGAGGCCGATAAGG	AAAGGACGGCTCATAGGTGATGG
247	RM7018	TTTCTCTCCACCCGTTCTCACG	CAAGCATGGCCAAAGTCATGTCG
248	RM28607	AGCATTACAGTGTCCAGGTAGGG	CCTCCCTTCTTATATGCCCTTCC
249	RM6265	TTCTTCTCCAGAGACCAAGAGC	TCTTCTTCTCTTCCCTCCTCTCG
250	RM6396	ATCATCGTCGTCATCCTCTCTCC	CGTCCAGTTCGTAGGCGTATAAGG
251	RM1159	TGTGAATGCCCGTATGGATGG	TCTGAAACCATATCGTCGCATACC
252	RM6411	GTGAAGGCTGTACCATCCAACG	CGGGTATTGTGCGGTGTTCAAG
253	RM5313	TCTTCTACTCCTCGTCTTCGTTGG	CATGCAGAGCAGAGACTTCTTGG
254	RM1227	CATCGACATGTGGACCACTCC	GCCTGAGACAAGTCCCATGGTAGC

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