MAPPING QTL CONTROLLING DURABLE RESISTANCE TO RICE BLAST IN THE CULTIVAR Oryzica Llanos 5

By

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B. S., Universidad del Valle, Cali, Colombia, 1993

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the

requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Plant Pathology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2006

ABSTRACT

The rice cultivar Oryzica Llanos 5 (OL5) possesses a high level of resistance to the fungus *Magnaporthe grisea*. The number and chromosomal location of quantitative trait loci (QTL) conferring resistance against eight isolates of the blast fungus were tested in two different populations of recombinant inbred lines from the cross Fanny x OL5. Twenty one QTL were detected and associated with the resistance traits, disease leaf area and lesion type, on 9 rice chromosomes. Eight of these 21 resistance loci had significant resistance effects in both experiments, while the others had effects that were only statistically significant in one experiment. Most, but not all, of the QTL occurred in the same genomic regions as either genes with major race-specific effects or other resistance QTL that had been described in previous experiments. Most of the QTL appeared to be race-specific in their effects but it is possible some of the QTL with smaller effects were nonspecific. One of the blast isolates used was FL440, which causes limited disease on OL5 and was probably virulent on most or all of the major genes from OL5. Three QTL affected resistance to FL440 in both experiments, one of which mapped to a region on chromosome 9 where no blast resistance genes have yet been mapped. An advanced backcross strategy with marker-assisted selection for OL5 alleles in QTL regions was used to generate five BC_2F_3 populations carrying five different target regions associated with partial resistance to rice blast disease. Three of five of these populations were analyzed for segregation for resistance to the *M. grisea* isolate FL440. One QTL designated *qrbr-11.3* near the bottom of rice chromosome 11 was found to be significantly associated with partial blast resistance in 120 lines of a BC_2F_3 population ($P< 0.01$). This QTL accounted for 12.4% and 8.0% of the phenotypic variation in diseased leaf area and lesion type observed under greenhouse inoculation. Examination of the genomic sequence at the *qrbr-11.3* locus showed that twenty-nine

candidate resistance genes are present at that locus (~1.8 Mb), twenty-seven of which are predicted NBS-LRR genes. Ultimately, the information from this study can be integrated into the development of improved lines with OL5-derived QTL for resistance.

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ACKNOWLEDGEMENTS

It is a pleasure to thank those who helped me to complete this work. First and foremost, I would like to thank my major professor Dr. Scot Hulbert for his thoughtful guidance, friendship and consistent support to my studies and research in the last five years. Without him, it would be much harder if not impossible for me to make progress. I would like to express my cordial appreciation to Dr. Robert Zeigler, Joe Tohme, Dr. Barbara Valent, Dr. Clare Nelson and Dr. Michel Ransom, who took their precious time to serve on my committee and provide great guidance and assistance throughout my graduate program.

I would like to send my special thanks to our collaborators at CIAT: Dr. Fernando Correa and Gustavo Prado for collaborating with me research and for providing me with all of the assistance throughout the blast inoculation and scoring. Life blessed me with the opportunity to meet Dr. Joe Tohme. I cannot overstate the importance of his involvement in my graduate career. I am thankful to him and his group, for his support and encourage during my tenure at CIAT. My thanks also go to Myriam Cristina Duque for discussions on the use of statistical methods.

Words cannot truly express my deepest gratitude and appreciation to my best friend Gloria Mosquera, who always gave me her help, support and encourage in carrying out this work. I take this opportunity to recall with gratitude the support and technical assistance to me by Dr. Valent and her research group.

Many thanks are owed to Dr. Gerardo Gallego, Dr. Maria Mateos and Suck Singh for his help and friendship. Thank also go out to the students workers, Martha Giraldo, Shara Farlee and Dr. Chulee Yaege who have provide me with valuable aid in the

laboratory and greenhouses. I certainly want to thank the graduate student in the lab and the department whom I have overlapped: Patricia Manosalva, Busisiwe Monosi, Vanessa Segovia, Amadou Seck and Medhi Kabbage.

I thank my beloved wife Yamileth Gómez, for her love and her understanding and support to me. I would not have been able to go this far and you are appreciate more than you will ever know. I wish to express my deep gratitude to my parents Stella and Leoncio and my brother Andres Felipe, for their love, support and encouragement. Thanks for believing in me. My final thanks go to my daughter Ana Sofia, and son Daniel Andres; the little angels bringing us joy and happiness.

INTRODUCTION

Blast, caused by the fungus *Magnaporthe grisea*, is one of the most severe diseases of rice worldwide. The fungus colonizes leaves (leaf blast), panicles (panicle blast) and other parts of the rice plants, and causes crop loss in rice growing areas. To control the diseases, the use of resistant cultivars is an effective measure; thus, rice breeders have been developing resistant cultivars.

Blast resistance in rice has been classified into two types, qualitative and quantitative or partial. The first type is controlled by single genes that provide high levels of resistance, but only to specific races of the blast fungus. Partial resistance allows lesions to form but they are typically fewer in number, reduced in size or slower to develop than those produced in highly susceptible lines.

The wide scale deployment of the single genes 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.

The rice cultivar Oryzica Llanos 5 (OL5) shows a stable and high level of partial resistance to rice blast in Colombia and other blast nurseries around the world. It is regarded that the partial resistance in OL5 to different blast isolates is derived from its parents and that a qualitative and might be some quantitative genes affect its level the resistance. Despite the usefulness of this partial resistance, its genetic analysis has not yet been performed.

The concept of quantitative traits is fundamental in genetics and is also encountered in many other areas of biological sciences. Quantitative trait analyses have been performed for many decades. A modern type of study is to locate genes controlling a quantitative trait, or QTL mapping. Therefore, because the genetic OL5 is thought to be complex and unlikely to segregate in a Mendelian manner, a QTL mapping approach

was used to identify genes conferring partial resistance in OL5 to several isolates of the rice blast fungus *M. grisea*. In this study, I will work on the application of methodology for mapping QTL conferring blast resistance under certain situations such as different isolates and population size (Chapter 2). Then in Chapter III, the advanced backcross strategy using marker-assisted selection is applied to develop families segregating for a single QTL to facilitate its further verification, fine mapping and cloning. Finally, the finished sequence of the rice genome is use to identify possible candidate genes such defense and NBS-LRR type genes in the QTL regions that confer blast resistance to the blast isolate FL440, which was able to overcome the major genes in OL5, and could be nonspecific genes. But before I start a technical description of my work, some related concepts will be introduced (Chapter I). Previously proposed approaches and issues in QTL mapping are also summarized.

CHAPTER 1

LITERATURE REVIEW: STRATEGIES FOR MAPPING QUANTITATIVE RESISTANCE GENES

RICE: THE MODEL MONOCOT PLANT

Rice, a member of the grass family, is one of the three cereals on which the human species largely subsists, along with wheat and corn. In the developing world as a whole, rice provides 27 percent of dietary energy supply and 20 percent of dietary protein intake. Rice began being cultured in Asia and now is cultivated in 113 countries and on all continents except Antarctica. It is grown in a large range of soil wetness regimes, from deep flood to dryland, and in diverse soil conditions (http://www.fao.org/rice2004/en/concept.htm). Two of 23 species from the genus *Oryza* are cultivated: *Oryza sativa*, which originated in the humid tropics of Asia is also the more widely used, and *Oryza glaberrima,* from West Africa. The two main strains of *O. sativa* are japonica and indica. The differences between these two evolved both geographically and culturally over several thousand years as farming groups relocated to diverse ecosystems. Over the millennia, different types of rice evolved under cultivation in different conditions. Today, there are four general ecosystems under which rice is grown: irrigated, rain-fed lowland, upland, and flood-prone (http://www.fao.org/rice2004/en/concept.htm).

There are thousands of cultivars of *japonica* and indica rice grown around the world. Some of these cultivars carry different traits such as stiff straw stems to prevent lodging, and upright leaves, which take up and use solar energy more efficiently. Some cultivars are also adapted to the elevated temperatures and shorter days of the tropics. For example, Taichung Native 1, which was released in 1956, combined short stature with high-yield potential. When adopted by Taiwanese farmers, it yielded six to eight tons per hectare. During the 1960s the scientists at the consultative group on international agriculture research (CGIAR) further improved these varieties by using 38 different crosses to eventually generate IR8, the earliest of the modern, high-yielding rice

varieties that became recognized as "miracle rice," for its high yields. IR8, which doubled rice production yields, initiated the Green Revolution in rice. Today, more than 60 percent of the world's rice fields are cultivated with varieties with origins in the work of CGIAR scientists and breeders and their partners. More recently, another variety, IR36, with the ability to withstand a broad range of pests, has been planted on more than 27 million acres, setting a world record for acreage of a single crop variety (http://www.fao.org/rice2004/en/world.htm).

Rice is one of the most economically valueable crops in Colombia when compared to other crops that can be planted several seasons in the same year. In Latin America and the Caribbean, Colombia is the second largest rice producer. Colombia is also the host country of the Centro Internacional de Agricultura Tropical (CIAT) and the Latin American Fund for Irrigated Rice (FLAR). Rice and beans provide the principal supply of calories and protein for poor people. Colombia imports rice because its local production is insufficient to meet the high demand. (http://www.fao.org/rice2004/en/p3.htm). Breeders at CIAT have been developing rice cultivars with durable resistance. An exceptional case is the indica rice cultivar Oryzica Llanos 5 (OL5). Due to its excellent resistance to the blast fungus (*M. grisea*), genetic analysis of this cultivar will be the main topic of this thesis.

The rice genome

Among the cereal crops, such as maize, wheat, millet and sorghum, rice (*Oryza sativa*) has several attributes that make it the model monocot plant. Rice has a DNA content smaller than that of any crop plant (estimated at about 430 Mb); about three times the size of the *Arabidopsis thaliana* genome. The small genome of rice includes a large percentage (ca. 75%) of single-copy DNA (McCouch *et al.* 1988). A vast reservoir of germplasm (> 200,000 accessions) of both domestic and wild rice is available for

genetic and breeding research. Rice has proven to be the most readily transformable cereal crop (Hiei *et al*. 1994).

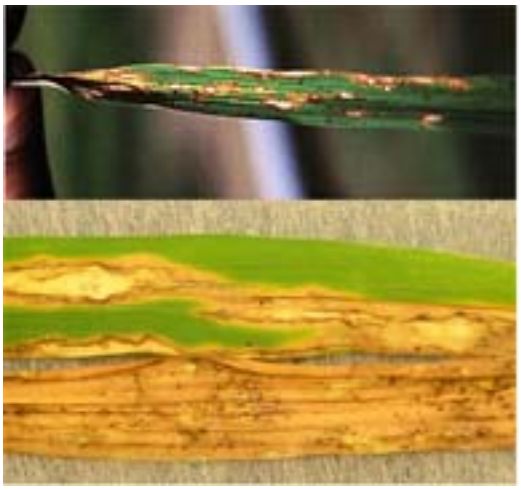
In the last ten years, two high-density molecular linkage maps of rice containing about 3000 markers have been developed in the US and Japan, making the marker density in the rice genome, on average, one marker per cM (200-300 kb) (Causse *et al*. 1994; Harushima *et al.* 1998). Over 300,000 expressed sequence tags (EST) have been deposited in the public database (Sasaki *et al.* 2005). The Rice Genome Program of Japan collaborated with the international community to sequence the rice genome with a high level of accuracy. With the completed sequence available from the International Rice Genome Sequencing Project (2005), it is expected that the genome sequence will facilitate pioneering research in functional and applied genomics. Integration of the genome sequence with the genetic map will help development of new varieties carrying agronomically important traits such as high yield potential and tolerance to both biotic and abiotic stresses. In addition to genome sequencing, assortments of other genomics projects have been initiated to produce important resources, which could serve as crucial tools in clarifying the structure and role of the rice genome. The next phase of rice genome research will focus on determining the function of approximately 35,000-40,000 predicted genes which will advance both breeding and scientific discovery.

THE PATHOGEN: *MAGNAPORTHE GRISEA*

The fungus *Magnaporthe grisea* (Hebbert) Barr (anamorph = *Pyricularia grisea*) is the causal agent of rice blast. It is a haploid filamentous *Ascomycete* with a relatively small genome of ~40 Mb divided into seven chromosomes (Dean *et al.* 2005). *M. grisea* is becoming an excellent model organism for studying fungal phytopathogenicity and host-parasite interactions. In addition to rice, this fungus can attack more than fifty other species of grasses. The fungus causes disease at seedling and adult stages on the

leaves, nodes, and panicles. In addition, Sesma and Osbourn (2004) reported a new facet of *the M. grisea* life cycle, where the fungus can undergo a different and previously uncharacterized set of programmed developmental events that are typical of root-infecting pathogens. They also show that root colonization can lead to systemic invasion and the development of typical disease symptoms on the above ground parts of the plant. On the leaves, lesions are typically spindle-shaped; wide in the center and pointed toward either end. Large lesions usually develop a diamond shape with a grayish center and brown margin (Fig. 1-1). Under favorable conditions, lesions on the leaves of susceptible lines expand rapidly and tend to coalesce, leading to complete necrosis of infected leaves.

Figure 1-1 Typical spindle-shaped leaf lesions caused by the rice blast fungus *M. grisea.*



The highly variable specific virulence of the fungus and its genetic plasticity make its control and management difficult. Thus, *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). Certain strains are able to attack other domesticated grasses, including barley, wheat, pearl millet and turf-grasses. Limited outbreaks on wheat have been reported in South America (Valent and Chumley, 1994). Widespread damage of golf courses, particularly in the Midwest (USA) where it has been attacking cool season grasses, is of particular concern (Curley *et al.* 2005). Indeed, the Centers for Disease Control and Prevention has recently recognized and listed rice blast as a potential biological weapon. Thus, no part of the world is now safe from this disease.

Unlike many phytopathogenic fungi such as the mildews and rusts, the rice blast fungus can be cultured on defined media, facilitating biochemical and molecular analyses. Early stages of the infection process, including germination, appressorium formation and penetration, can be studied *explanta*. Tools for molecular genetic manipulation have been well-developed in the last decade. Many genomic resources such as EST, BAC, genetic methodology, a physical map and the draft sequence are now publicly accessible. One of the big issues resulting from the prediction of the genes encoded in the *M. grisea* genome was that this pathogen contains more genes than its non-pathogenic cousins, *Neurospora crassa* and *Aspergillus nidulans* (Dean *et al.* 2005).

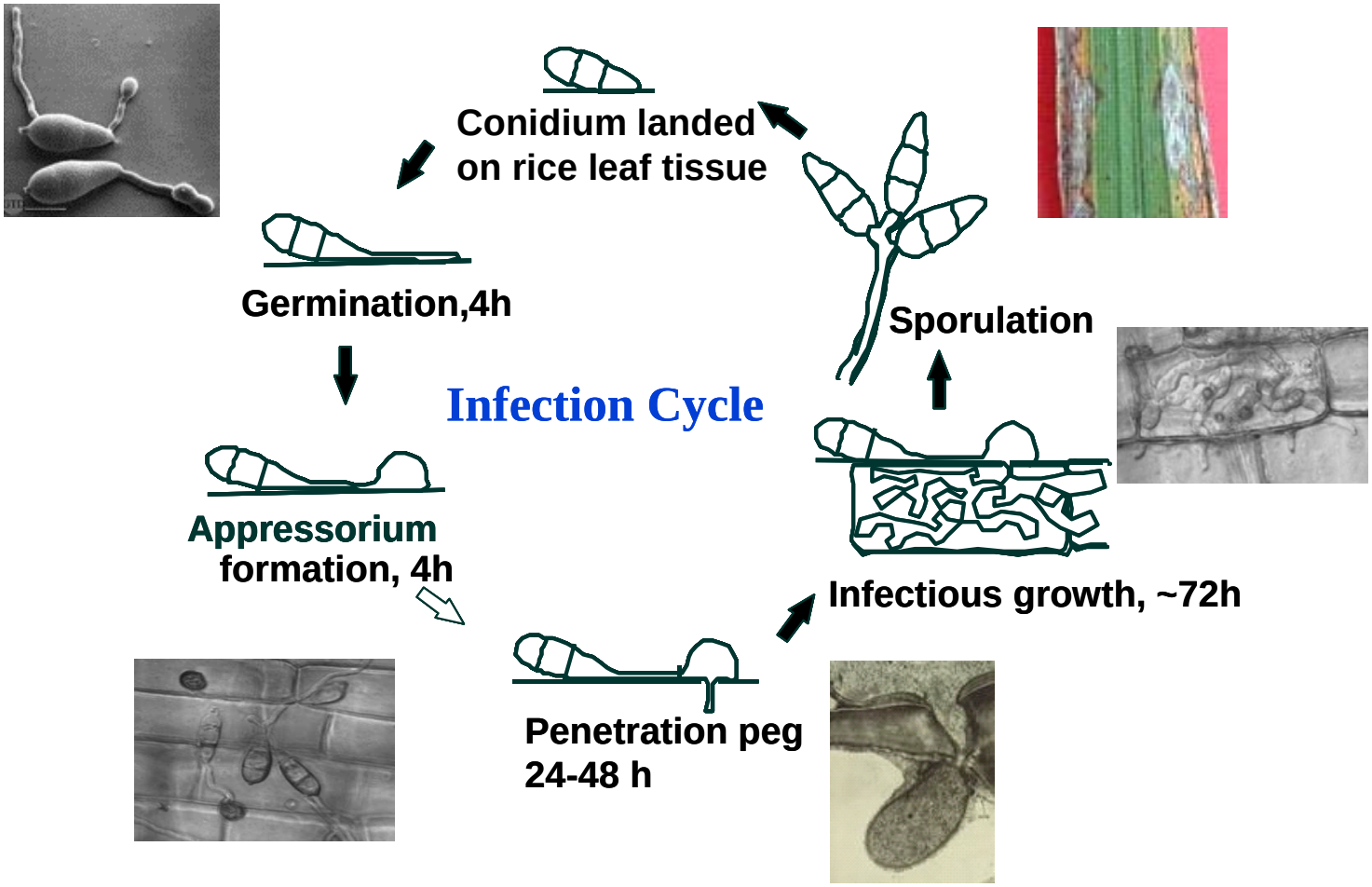
The life cycle

Infection by the rice blast fungus starts when the three-celled conidium lands on a host leaf and anchors itself to the leaf cuticle with spore-tip mucilage (Fig. 1-2). Germination proceeds with the extension of a germ tube, which undergoes hooking and

swelling at its tip and then differentiates into an infection structure called the appressorium.

During maturation, the appressorium becomes melanized, except for a well-defined pore between the appressorium and the rice leaf (Howard and Valent, 1996). The formation of this infection structure on the host surface marks the onset of the disease. A penetration peg is then driven through the host surface and the infection hypha invades and grows through the rice leaf (Talbot *et al.* 2003). At this stage, the symptoms become evident and small oval lesions begin to appear, accompanied by local chlorosis. Eventually, the growing lesions become necrotic and may coalesce. Conidia are carried by air to neighboring plants, spreading the blast disease.

Figure 1-2 The life cycle infection-related morphogenesis of the rice blast fungus *M. grisea.*



Modified from www.btny.purdue.edu/ Faculty/Xu/

PLANT DISEASE RESISTANCE

Plants are attacked by several disease-causing organisms including bacteria, fungi, viruses, and nematodes. These pathogens cause huge crop losses and the control of disease is thus a main objective of plant breeding and pathology research. Plants resist pathogen attacks both with preformed defenses such as antimicrobial compounds and by induced defense responses (Hwang *et al.* 2005). Inducible defense mechanisms can be activated upon detection of general elicitors such as bacterial flagellin and even host cell fragments released by pathogen damage (Gomez-Gomez and Boller, 2002). In addition, plants have evolved diverse recognition systems to detect proteins produced during infection by specific strains of pathogens. These 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). To understand the mechanism essential for recognition of the pathogen and induction of mutual defense reactions, more studies such as cloning and functional genomics are needed to accelerate research into the molecular basis of disease resistance.

During the last few years, many dominant *R* genes conferring complete resistance to several pathogens have been characterized from a broad range of plant species. Comparative analysis of the predicted products of these cloned *R* genes reveals that they share various conserved functional motifs allowing their separation into distinct classes (Hammond-Kosack and Jones, 1997). 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; Meyers *et al*. 2003*;* Howles *et al*. 2005*)*. These NBS-LRR genes represent a superfamily of *R* genes in both monocot

and dicot species. The NBS-LRR group can be further divided into two subclasses: those that show homology with the amino terminus of the *Drosophila* Toll protein and mammalian interleukin-1-receptor (the TIR domain); and those that do not contain that domain, but often have it substituted with a coiled-coil (CC) domain. The TIR class of genes has not been observed in monocot plants. Rather the non-TIR class appears to have amplified instead (Monosi *et al.* 2004). The NBS-LRR proteins are now widely understood as recognizing elicitors from different plant pathogens. Other identified classes encode *R*-gene proteins include the extracellular LRRs with transmembrane motifs and intracellular protein kinase domains; membrane spanning proteins with large extracellular LRRs; and those with cytoplasmic kinase domains (Ellis *et al.* 2000; Dangl and Jones 2001). By making use of the conserved motifs, it has been possible to design degenerate oligonucleotide primers that permit the amplification of conserved sequences from the genomes of diverse plant species using the polymerase chain reaction (PCR) (Aarts *et al*. 1998, Bai *et al.* 2002). This strategy provides a powerful tool that facilitates isolation of candidate *R* genes. Putative *R* gene fragments homologous to the conserved motifs can be rapidly identified by PCR and used as molecular markers to locate candidate *R* genes.

One R protein that does not fit the above classes includes *Hm1,* which codes for a toxin reductase that confers resistance to a fungal pathogen of maize (Hayashi *et al*. 2005). Barley plants carrying loss-of-function alleles (*mlo*) are defective for the MLO plasma membrane protein, which has seven transmembrane motifs. The recessive mutant alleles confer resistance against all recognized isolates of the widespread powdery mildew fungus (Peterhansel and Lahaye, 2005). Another exclusive class of *R* genes is composed of *RPW8.1* and *RPW8.2*, two similar proteins that have a putative N-terminal trans-membrane domain and a coiled-coil motif. These proteins confer broad-spectrum resistance in *Arabidopsis* to powdery mildew in a race-specific way (Xiao *et al.* 2005). Other R proteins may act in specific recognition, but have novel structures. For example, Hs1pro-1 for resistance to a

sugar beet nematode has a structure without obvious protein interaction domains and does not confer a hypersensitive response or systemic acquired resistance. The promoter region of this gene was found to control feeding-site-specific and nematode-responsive gene expression in *Arabidopsis* and sugar beet (*Beta vulgaris L*.) (Thurau *et al.* 2003). The *RTM1* and *RTM2* genes from *Arabidopsis* restrict systemic movement of tobacco etch potyvirus and do not involved in a hypersensitive response or systemic acquired resistance. These genes code for jacalin-like sequences and similarity to a small heat shock–like protein, respectively (Chisholm *et al.* 2001). The *Rpg1* gene for resistance to barley stem rust was found to encode a kinase-like protein containing two tandem protein kinase domains, a novel structure for a plant disease-resistance gene (Brueggeman, *et al*. 2002). Recently, the rice *xa5* gene for disease resistance to *Xanthomonas oryzae* pv *oryzae* (*Xoo*) was cloned and encodes the gamma subunit of transcription factor IIA (TFIIA). The TFIIA protein is commonly found in eukaryotes but has never been documented to be involved in resistance to other diseases (Iyer and McCouch, 2004).

Disease resistance genes in rice

In the last few years, several rice *R* genes acting in race-specific manners have been mapped, characterized and some of them cloned. For example, almost 50 *Pi* genes (rice blast *R* genes) have been mapped in several rice cultivars. However, only the *R* genes *Pib*, *Pi-ta* and *Pi-k*^{h} have been cloned and well-characterized.

Together with rice blast, bacterial blight, caused by *Xoo*, is one of the two most important diseases of rice. So far, nearly thirty genes for bacterial blight resistance have been phenotypically identified in rice, most of them providing race-specific resistance. Only four of them have been cloned (Gu *et al*. 2005). While most of these genes are dominant, six are recessive, including *xa5*, *xa8*, *xa9*, *xa13*, *xa19* and *xa20* (Blair *et al.* 2003). Map-based cloning approaches have been successfully applied to isolate several of the dominant genes. A number of examples include

Xa21, which encodes a leucine-rich repeat (LRR)/kinase receptor protein; (Song *et al*. 1995), *Xa1*, encoding a nucleotide binding site (NBS)-LRR protein; (Yoshimura *et al*. 1996*)*; *Xa26*, which has a structure similar to *Xa21* (Sun, *et al*. 2004). Gu *et al*. (2005) reported the cloning of the *Xa27* gene from rice and the corresponding *avr* gene *avrXa27* from *Xoo*. Both the resistant and susceptible alleles of *Xa27* encode similar proteins. However, the expression of the resistant allele is induced when challenged by bacteria carrying the corresponded *avrXa27*, whose product is a nuclear localized type-III effector.

Using bioinformatics tools and comparative-sequence approaches, the finished sequences of the rice chromosomes 11 and 12 were analyzed to classify the type and distribution of predicted *R* genes as well as downstream defense-response genes (Choisne et al. 2005). A total of 837 genes containing LRR domains were identified. From the 837 *R* genes in the rice genome, a total of 201 R-like gene models were identified on chromosome 11. Of these, 73 have homology to the NBS-LRR class of *R*-like genes and 38 show homology to the LRR-TM-like genes. They also predicted 17 downstream defense response genes including glucanases, chitinases and thaumatin-like proteins. Most of these *R*-like genes and defense response-like genes were present in large clusters of tandem arrays indicating their origin by duplication from a few ancestral genes. A large cluster of 14 defense response genes, 12 of which are chitinases, was present in tandem at 116.2 cM (between 28,056,455 and 28,122,601 bp). Several other *R*-like genes were also arranged in similar, but smaller, clusters. The full number of *R*-like genes on chromosome 12 was less than half of the number on chromosome 11. On chromosome 12, 88 predicted genes showed homology to *R*-like genes. However, 50 of these 88 predicted genes coded for an LRR motif but no NBS, CC or LZ motifs. Thus, only 18 genes showed homology to the NBS-LRR category. As with chromosome 11, the *R*-like genes and defense response genes on chromosome 12 were present in clusters. The *R*-like genes and defense response gene hot spots in

rice will be targets for future mapping and cloning of disease resistance genes. Knowing the structure of some of these gene families as well as the motifs they share will be helpful in applying reverse genetic approaches, such as virus- induced gene silencing, where a single gene or a gene family can be silenced and the function determined. A better knowledge of the effects of these individual *R* genes would also assist efforts in breeding cultivars with durable disease resistance. Thus both manual re-annotation of these *R*-like genes, as has been done for *Arabidopsis* (Meyers *et al.* 2003; Haas *et al.* 2005) and functional confirmation of the candidate resistance genes would be important for drawing practical benefits from this information.

Host specific *R* genes are typically effective against specific strains of pathogens and tend to lose their effectiveness due to shifts in the pathogen population (McDonald and Linde, 2002). Recently, Zhao *et al.* (2004) have shown that *Rxo1*, a non-host resistance gene from maize, triggers a resistant reaction in maize after challenge with *Xanthomonas oryzae* pv. *oryzicola,* which is the causal agent of bacterial leaf streak in rice. The same gene was also shown to confer a resistant reaction to some *Burkholdaria andropogonis* strains in maize and was also demonstrated to function in rice (Zhao *et al* 2005). This work presents the possibility of transferring genes from other grass species to rice to increase the arsenal of *R* genes. To achieve the goal of durable resistance in rice, more studies are required to characterize the *R* genes from rice or related species as well as resistance that is quantitatively inherited.

Known rice blast resistance genes

The genetics of blast resistance in rice has been extensively studied. The first *Pi* gene in rice was named by Kiyosawa (1966). The approach used several varieties that carried different, single resistance genes as differential cultivars (Flor, 1945) to characterize the specific virulence of different isolates of the pathogen. Using seven differential cultivars and several blast isolates, Yamasaki and Kiyosawa (1966)

described three resistance genes, *Pi-a*, *Pi-i* and *Pi-k*. Other investigators have used similar approaches, using different germplasm and blast isolates. The relationships between the different genes are sometimes difficult to determine when different blast isolates are used to characterize them. Determining their position on the rice physical map would be very helpful in this case.

To date, approximately 50 major blast resistance genes have been named and mapped on rice chromosomes (Table. 1; review in Berruyer *et al*. 2003; Sallaud *et al*. 2003; Liu *et al*. 2005, Sharma *et al*. 2005). Although rice blast is a model pathosystem, only three blast *R* genes, *Pi-b* (Wang *et al*. 1999), *Pi-ta* (Bryan *et al*. 2000*)* and *Pi-k*^{h} (Sharma *et al*. 2005) and five *Avr* genes: *PWL1* (Kang *et al*. 1995), *PWL2* (Sweigard *et al*. *1995*), *Avr1-CO39* (Farman and Leong 1998), *Avr-Pita* (Orbach *et al*. 2000), and *ACE1* (Böhnert *et al*. 2004) have been cloned and characterized. Of these, only the *Pi-ta* and *Avr–Pita* proteins have been demonstrated to interact directly (Jia *et al*. 2000). Several blast resistance genes have recently been fine-mapped, an essential starting point to map-based cloning approaches. Using random amplified polymorphic DNA (RAPD) and bacterial artificial chromosome (BAC) end markers, Liu *et al*. (2002) constructed a high-density map of the *Pi9*(t) locus, and demonstrated that *Pi2*(t) and *Pi9*(t) are physically linked in a ~100-kb interval on rice chromosome 6. Jiang and Wang (2002) identified a 118-kb DNA fragment covering the *Pi-2*(t) locus by chromosome walking using BAC clones anchored by molecular markers tightly linked to the locus. Chauhan *et al*. (2002) genetically mapped a rice blast resistance locus *Pi-CO39*(t) to a region of 1.2 cM in length on the short arm of rice chromosome 11 using simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP) and resistance gene analog (RGA) markers, and assembled three contigs of 180, 110 and 145-kb in the region by screening a genomic library of the donor cultivar (cv.) CO39 with the *Pi-CO39*(t) linked markers. Using rice genomic information and four mapping populations, Jeon *et al*. (2003) efficiently constructed a genetic and physical map of the *Pi-5(t)* locus,

locating it in a 170-kb binary bacterial artificial chromosome (BIBAC) contig on chromosome 9. In addition, they demonstrated that the *Pi-5(t)* locus is identical to the *Pi-3(t)* locus. Chen *et al*. (2005) reported the genetic and physical mapping of *Pi-37(t)*, a new gene conferring resistance to rice blast in the cultivar St. No. 1. This new *R* gene was assigned to a 374 kb interval flanked by markers RM543 and FPSM1 on chromosome 1. Using a bioinformatics approach, the location of *Pi-37(t)* was further refined to the vicinity of four candidate NBS-LRR genes on a DNA fragment of 60 kb. Using an F_2 population and SSR markers, Liu *et al.* (2005) mapped a new blast resistance gene on the short arm on chromosome 8. This novel *R* gene was designated *Pi-36(t)*. To physically map this locus, the *Pi36(t)*-linked markers were mapped on the rice genomic sequence, allowing the locus to be physically assigned to an interval of about 17.0 kb, based on the genomic sequence of Nipponbare. Sharma *et al*. (2005) reported the molecular mapping and cloning of a dominant gene *Pi-k*^{h} present in the rice cultivar Tetep. This *Pi-k*^{h} gene is the third *Pi* gene cloned so far in rice. The *Pi-k*^{h} gene was mapped between two SSR markers estimated to be 0.7 and 0.5 cM away. They identified a candidate blast-resistance gene in the region, and cloned the homologous sequence from Tetep. The *Pi-k*^{h} belongs to the NBS-LRR class of disease resistance genes. Interestingly, transcription of this gene was shown to be inducible by challenge with the blast fungus in a RT-PCR assay.

Table 1-1 Blast resistance (*Pi*) genes identified in rice.

Gene	Chr	Markers	Reference
Pi-t	1		Kiyosawa, 1972
Pi-24(t)	1	K5	Sallaud *et al.* 2003
Pi-37(t)	1	RM543	Chen *et al.* 2005
Pi-b (Pi-s)	2	RM208	Wang et al 1999; cloned
Pi-tq5	2	RG250	Tabien *et al.* 2000
Pi-14(t)	2		Pan *et al.* 1996, 1998
Pi-25(t)	2	RG250	Sallaud *et al.* 2003
Pi-16(t)	2		Pan *et al.* 1999
Pi-kur-1	4		Goto *et al.* 1970
Pi(t)	4		Causse *et al.* , 1994
Pi-(t)?	4		Tohme *et al.* 1993
Pi-5(t)	4	RG498-RG788	Wang *et al.* 1994
Pi-21	4		Fukuoka & Okuno 2001
Pi-10	5	OPF6-OPH18	Naqui and Chattou, 1996
Pi-26	5	RG313	Sallaud *et al.* 2003
Pi-i	6		Shinoda *et al.* 1971
Pi-zt (Pi-2)	6	RG64-RG456	Yokoo, 1970; Goto, 1981
Pi-8	6		Pan *et al.* 1996
Pi-9(t)	6		Pan *et al.* 1996
Pi-13(t)	6		Pan *et al.* 1996
Pi-22(t)	6		Pan *et al.* 1996
Pi-27	6	EST-2	Sallaud *et al.* 2003
Pi-3(t)	6		Mackill and Boman, 1992
Pi-tq1	6		Tabien *et al.* 2000
Pi-17(t)	7		Pan *et al.* 1996
Pi-11(t)	8	RZ617-RZ323	Causse *et al.* 1994
Pi-33	8		Berruyer *et al.* 2003
Pi-zh	8		Causse *et al.* 1994
Pi-29	8	RZ617-RZ323	Sallaud *et al.* 2003
Pi-36(t)	8	RM5647	Liu *et al.* 2005
Pi15	9		Pan *et al.* 2003
Pi5 (Pi3)	9		Jeon *et al.* 2003

Table 1-1 Cont.

Gene	Chr	Markers	Reference
Pi-28(t)	10	RZ500	Sallaud *et al.* 2003
Pi-a	11		Kiyosawa, 1967
Pi-f	11		Shinoda *et al.* 1971
Pi-k	11		Shinoda *et al.* 1971
Pi-k^{h}	11	RM 2191	Sharma et al. 2005; cloned
Pi-is-1	11		Goto *et al.* 1970
Pi-kur-2	11		Goto *et al.* 1988
Pi-1	11	RG303-G181	Causse *et al.* 1994
Pi-7(t)	11	RG103A-RG16	Wang *et al.* 1994
Pi-18	11	RZ536	Sang *et al.* 1996
Pi-44	11	AF349-AF348	Chen *et al.* 1999
Pi-30(t)	11	OPZ11-f	Sallaud *et al.* 2003
Pi-lm2	11		Tabien *et al.* 2000
Pi-sh	11		Imbe and Matsamuto, 1985
Pi-ta	12	RG869	Bryan et al. 2000 cloned
Pi-4(t)	12	RG869	Yu *et al.* 1991
Pi-6(t)	12	RG81	Causse *et al.* 1994
Pi-ta2	12	RG869	Jia *et al* . 2003
Pi-12(t)	12	RG869	Zhen *et al.* 1996
Pi-19(t)	12	RG241	Shinoda *et al.* 1971
Pi-20	12	Xnpb 88	Imbe *et al.* 1997
Pi-62(t)	12	Rz816	Imbe *et al.* 1997
Pi-157	12	RG341	Naqui and Chattou, 1996
Pi-31(t)	12		Sallaud *et al.* 2003
Pi-32(t)	12		Sallaud *et al.* 2003

Quantitative resistance

Plant disease resistance is recognized as either genetically simple (monogenic) or complex (polygenic). In most cases, monogenic resistance is race-specific and functions in a gene-for-gene manner (Flor, 1971). 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). With QTL mapping, the roles of specific loci in genetically complex traits can be described, and fundamental questions that have intrigued researchers in the field of plant pathology for decades

can be addressed. Are genes that control race-nonspecific resistance the same as "defeated" race-specific genes? Are genes that control partial resistance race-specific? What kinds of interaction exist between resistance genes, plant development, and the environment? More research is necessary to answer these questions.

Resistance to the bacterial blight pathogen, *Xoo*, has been reported to have both qualitative and quantitative components (Li *et al.* 2001). The qualitative components show strong effects typical of race-specific genes (*Xa4* and *Xa21*) against the matching avirulent *Xoo* races. The same resistance genes also appeared to provide lower levels of resistance to races that did not carry the corresponding *Avr* genes. The quantitative role of components of resistance conditioned by these *R* genes was shown as their residual effect against matching virulent races. The *xa13* gene was completely recessive without noticeable residual effects against the virulent races but showed more pronounced specificity. An important result from this study was that interaction between some of the dominant genes such as *Xa4* and *Xa21* or between them and the recessive genes, *xa5* and *xa13*, increased resistance to *Xoo*. This suggested that combinations of these genes in the same genetic pool would increase the resistance level and durability of resistance to *Xoo*.

Currently, fine mapping and cloning of QTL responsible for variation in agronomic traits is a common objective in agricultural research. 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). Several other studies have used fine mapping of the region harboring a QTL to resolve and predict the genes responsible for the variation on the trait. These studies include the physical mapping of rolled leaf QTLs (Shao *et al.* 2005) and a

grain-weight QTL, *gw3.1* in rice (Li *et al.* 2004). Isolation of these QTL holds great promise to improve world agriculture but is a challenging task.

Quantitative resistance to *M. grisea*, the rice blast fungus

One quantitative resistance system that has been especially well characterized in rice is resistance to the blast fungus (Wang *et al.* 1994; Sallaud *et al.* 2003; Talukder *et al.* 2005). In most of these studies, the association of major genes and minor QTL, environment x QTL interactions and the issue of durable resistance were all considered. The bases of these studies were recombinant inbred lines (RIL) and doubled haploid populations. In the rice blast QTL study of Wang *et al*. (1994), a durable source of resistance known as Moroberekan was analyzed for both *R* genes and quantitative (partial) resistance loci. Two dominant loci associated with qualitative resistance to five isolates of the fungus were tentatively named *Pi-5(t)* and *Pi-7(t).* These genes were mapped on chromosomes 4 and 11 of rice, and both were different from previously identified qualitative blast resistance loci. In the QTL mapping study by Sallaud *et al*. (2003), five new blast resistance loci named *Pi-24*(t) to *Pi-28*(t) were identified using a QTL mapping approach.

Another study tested the specificity of QTL for partial resistance to blast disease by using isolates for which no major *R* gene segregated in a mapping population (Talukder *et al.* 2004). Of the 18 QTL reported, eight were effective against only one isolate, seven were effective against two isolates and only three were predicted to be effective against all three isolates. Fourteen QTL mapped to previously identified QTL for blast resistance and 10 to previously identified major resistance genes. The conclusion from this study was that most of the QTL detected are race-specific and that quantitative resistance genes might be due the action of defeated *R* genes. More studies in dissecting the genes responsible for partial or quantitative resistance are necessary to distinguish the role of major genes, with

race-specific and possibly nonspecific effects, from genes that confer only small effects with unknown specificity.

QTL MAPPING

Traits

In biology, a trait refers to a (partially) genetically determined characteristic, which could be anything from human blood type to susceptibility of plants to attack by pathogens. Two kinds of traits, Mendelian and quantitative, are distinguished. A Mendelian trait is determined by a single gene (or few genes), following classical Mendelian inheritance patterns, such as 3:1 for a phenotypic ratio from a trait controlled by a single dominant gene in an F_2 family. In contrast, multiple genes could determine a quantitative trait and its value is continuous, such as plant height and human weight. Quantitative traits are very common and are important both in applied and theoretical studies. For example, increasing milk, meat or crop production or plant disease resistance all requires the manipulation of quantitative traits.

Related issues in QTL mapping

It is now common to study a quantitative trait by charactering QTL affecting it. Due to the complicated and variable features of QTL, such as magnitude of effect, genomic position, environmental effects, interactions, etc, their locations and effects are difficult to characterize. Therefore, an important task in QTL studies is 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. It can be applied to animal and plant breeding programs to perform selection of a desired trait more efficiently. In addition, knowing numbers, effects and potential interactions of

QTL could be helpful in making reasonable hypotheses concerning the inheritance of the trait in important elite cultivars for its further application on breeding.

QTL mapping has been carried out for various traits in many species. 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, monogenically-controlled trait. Modern QTL mapping is derived from this idea, with the key innovation being that defined sequences of DNA act as the linked monogenic markers. New interest was generated when studies with maize and tomatoes demonstrated that some markers explained much of the phenotypic variance of complex characters (Tanksley, 1993). As a consequence, vigorous research on QTL mapping for quantitative traits such as yield, quality, maturity, and resistance to biotic and abiotic stress was initiated in many crop species (Lee, 1996). With the development of comprehensive DNA marker maps (Tanksley, 1992; Causse *et al.* 1994), it is now possible to search for QTL throughout the genomes of most species. For example, Frary *et al.* (2000) found that the tomato *fw2.2* QTL changes fruit weight by up to 30% and Zeng *et al.* (2000) characterized the genetic architecture of the size and shape differences of the posterior lobe of the male genital arch between two species of *Drosophila* species. This has had the profound result of moving the focus in studies of polygenic traits to questions about the chromosomal locations, gene actions, and gene by genotypic interactions, also gene by environment interactions and biological roles of specific loci involved in complex phenotypes.

In every QTL mapping study, experimental design issues need to be considered. Generally, there are two types of experimental units (individuals or lines) used in QTL mapping: individuals from natural populations or from designed experiments. QTL mapping in plants usually uses two parental homozygous lines if possible, aiming for two individuals that have very different gene composition and trait values. Simple line crosses are routinely used for QTL mapping in plant and laboratory animals. They could result from crosses between two F1 parents to give F_2

families and then selfing several times resulting in recombinant inbred lines (RIL) or from a cross between an F_1 plant and one of the parental lines (backcross design). Crosses developed between inbred lines have the fewest complications. RIL have been most commonly used in QTL studies over the F_2 and backcross population. Taking an F_1 plant through multiple rounds of self-fertilization can easily generate them. The resulting lines have little within-line genetic variance and only the genetic variance between lines is considered. These inbred lines are highly homozygous and always pass the same allele to all of their offspring.

Data for QTL mapping usually have two components: marker data and trait values. Marker data includes marker genetic map position and marker genotype. Trait values can be continuous, such as disease leaf area, or they may be categorical, such as leaf size denoted by large, medium and small. Sample size needs to be considered when planning the experimental design. With a greater sample size, detection of QTL with smaller effect is more likely (Zeng 1994; Vales *et al.* 2005).

Markers and maps

As mentioned above, one component of observed data in QTL mapping experiments is the markers. Various properties of different types of markers are important to consider in QTL mapping experiments.

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. The distinction can be at different levels such as phenotype, protein or DNA. Phenotypic traits can be markers if the variation observed in the population of interest is entirely explained by a single Mendelian factor. At the protein level, allozymes can be used as markers. These are soluble proteins with different mobility on an electrophoresis gel. The

mobility difference is a result of unequally charged protein due to amino acid substitutions. 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. Variation in DNA sequence is detected by hybridization and polymerase chain reaction (PCR) based methods. Commonly used DNA markers include restriction fragment length polymorphism (RFLP). The RFLP may result either from mutation in restriction endonuclease sites or from deletions or insertions of DNA between the sites. Polymorphisms detected by PCR result from insertion and deletions between, and mutation in primer binding sites. PCR based markers include sequence tagged sites (STS), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequenced repeats (SSR or microsatellites), variable number of tandem repeats (VNTR or minisatellites) and single nucleotide polymorphisms (SNP). Among these 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 (Röder *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.

Maps and map construction

A genetic map describes orders and positions of identifiable landmarks on DNA. Theses 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. Estimating recombination frequency between two positions generates a genetic map. In contrast, having 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 by Lander *et al*. (1987). Assembling sequences or DNA fragments into contigs allows construction of a physical map. Two strategies are commonly used for genome sequencing: hierarchical sequencing and shotgun sequencing. Hierarchical sequencing works as a top-down approach: it starts with cutting and cloning the genome into large ordered DNA fragments. These are then sequenced, typically by sub-cloning many smaller overlapping fragments of each large clone, sequencing these and assembling the sequences into a large sequence contig representing the whole original clone. In contrast, shotgun sequence is a bottoms-up approach: small fragments of genomic DNA from the whole genome are sequenced and these are assembled into a genomic sequence using computer algorithms (Tammi *et al.* 2002).

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. Since the traits of interest are, by nature, genetically complex, environmental factors and genetic background potentially have an enormous impact on results. This is one of the most powerful applications of QTL mapping (i.e. analyzing gene x gene and gene x environment interactions), but it also means that many large, time-consuming experiments need to be carried out to analyze a system thoroughly.

Finally, QTL mapping, like any genetic study, is only as good as its phenotypic scoring method. In studies of disease resistance, factors all the way from a suitable inoculum to difficulties in quantitative estimation of resistance make QTL mapping more challenging. Fortunately, powerful computer software programs are now available to analyze QTL mapping results (Nelson, 1997; Manly *et al.* 2001; Broman *et al.* 2003; Wang *et al.* 2005) and better DNA marker systems have been developed to simplify the technique and increase marker density.

QTL mapping methods

Various statistical methods have been developed for QTL mapping. The most commonly used methods for QTL mapping are based on the maximum-likelihood method. From simple to more complicated, four approaches are commonly used: single marker analysis (SMA), interval mapping (IM); composite interval mapping (CIM) and multiple interval mapping (MIM).

Single marker analysis (SMA)

SMA tests the association between marker genotypes and trait values using t-tests, ANOVA models or regression. In other words, it tests trait value differences

among markers groups. SMA is the least informative of the analyses, because recombination (*r*), as well as the additive (*a*) and the dominant (*d*) effects of a QTL may be confounded. SMA often fails to give reliable estimates of numbers and positions of QTL and the magnitude of their effects (McMillan and Robertson, 1974, Lander and Botstein, 1989).

Interval mapping (IM)

Thoday, in 1961 introduced interval mapping and a mathematical treatment of this method was presented by Lander and Botstein (1989). IM uses two observable flanking markers to construct an interval within which to search for QTL along the chromosomes. A map function, either Haldane or Kosambi, is used to translate from recombination frequency to distance or vice visa. Then, a LOD score is calculated at each increment in the interval. Finally, the LOD score profile is calculated for the whole genome. When a peak has exceeded a threshold value, there is evidence that a QTL has been found at that location (Zeng, 1994).

Composite interval mapping (CIM)

Jansen and Stam (1994) and Zeng (1994) developed CIM. This method is an extension of IM that places certain markers into the model as cofactors. CIM fits parameters for a target QTL in one interval while simultaneously fitting partial regression coefficients for background markers to account for variance caused by non target QTL. In theory, CIM gives more power and accuracy than simple IM because the effects of other QTL are not present as residual variance.

Multiple interval mapping (MIM)

MIM 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. MIM 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.

ADVANCED BACKCROSS QTL STRATEGY

The advanced backcross (AB-QTL) mapping strategy integrates the processes of QTL detection and introgression from wild germplasm into elite material (Tanksley and Nelson 1996). Instead of an F_2, F_2-derived RIL or double haploid population, this approach uses BC_2 or BC_3 populations for the discovery and mapping of trait loci. Thus, molecular-marker and phenotypic analyses are performed at a more advanced generation when the recurrent parental line alleles are at higher frequency. Once favorable alleles for various loci or QTL are identified, only a few more backcrosses and/or selfs are necessary to develop near-isogenic lines that can be tested and possibly used for variety development. The AB-QTL method was first applied in tomato (Tanksley *et al*. 1996) and has since been adapted for use in rice (Xiao *et al.* 1995, 1998; wheat (Huang *et al*. 2003), maize (Ho *et al*. 2002) and pepper (Rao, *et al*. 2003). Simulations suggest that AB-QTL would be effective in detecting QTL that are additive, dominant, and partially dominant and over-dominant in effect. However, recessive QTL from the donor parent would go undetected in an AB-QTL analysis (Tanksley and Nelson, 1996).

Advanced backcross QTL has several advantages over conventional QTL analysis. First, major negative QTL can be eliminated at early stages during population development by selection with specific markers. The probability of finding QTL with epistatic effects among alleles from the wild parent is reduced since lines are skewed towards alleles from the recurrent parent. Once putatively beneficial QTL are identified from such analysis, whole-genome marker selection can be used to identify the backcross lines from which quantitative trait loci-near isogenic lines (QTL-

NIL) could be isolated. By using QTL-NIL and marker information, map based cloning of QTL is more feasible than using families derived from F_2.

Frary *et al*. (2004) used an AB-QTL mapping strategy to identify loci for yield, processing and fruit quality traits in tomato in BC_2 and BC_2F_1 populations derived from the interspecific cross *Lycopersicon esculentum* E6203 × *L. pennellii*. They found a total of 84 different QTL. For 23 traits analyzed for which allelic effects could be deemed favorable or unfavorable, 26% of the identified loci had *L. pennellii* alleles that enhanced the performance of the elite parent.

Advanced backcross QTL analysis was used by Li *et al*. (2004) to identify favorable loci affecting yield and yield components from *Oryza sativa*, cv. Jefferson x *O. rufipogon.* They used an isogenic population developed by advancing NIL lines through five generations of backcrossing to cv. Jefferson and seven generations of selfing. The NIL approach was used for fine mapping of a grain-weight QTL (gw3.1). The locus was associated with transgressive variation for grain size and grain weight in this population. Analysis of a syntenic region in maize showed a QTL affecting kernel size, suggesting that favorable alleles of this locus may have been selected in the early process of domestication in both cereals.

Ho *et al.* (2002) applied an AB-QTL strategy to identify QTL of agronomic importance in a cross between two elite inbreds of maize. In wheat, AB-QTL analysis was used to identify QTL for yield and yield components in a BC_2F_2 derived population from a cross between a German winter wheat and spring-type synthetic hexaploid wheat (Huang *et al.* 2003).

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CHAPTER 2

MAPPING QTL CONTROLLING DURABLE RESISTANCE TO RICE BLAST

ABSTRACT

The rice cultivar Oryzica Llanos 5 (OL5) shows a high level of resistance to the blast fungus. The number and chromosomal location of quantitative trait loci (QTL) conferring resistance against eight isolates of the blast fungus, were tested in different experiments using two different populations of recombinant inbred lines from the cross Fanny/OL5. Twenty one QTL were detected and associated with the resistance traits, disease leaf area and lesion type in 9 rice chromosomes. Most, but not all, of the QTL mapped to genomic regions previously recognized to carry major blast R genes or QTL. Eight of these resistance loci were statistically significant in both populations while the others were significant in only one of the two experiments. Most of the QTL showed race-specificity to the different blast isolates since, they affected several but not all of the eight isolates. Resistance to the blast isolate FL440, which was partially virulent on the resistant parent, was controlled by QTL with small effects, some of which could be nonspecific.

INTRODUCTION

Rice blast, caused by *Magnaporthe grisea,* is a devastating disease because of its wide distribution and its destructiveness under conductive conditions. The disease has been identified in 85 rice-producing countries in both tropical and temperate zones, destroying crops in Latin America, Africa and Asia (Roca *et al.* 1996). Genetic resistance has been, and will continue to be, the major method of disease control of blast. However, varieties carrying genes that confer high levels of resistance (*R* genes) typically lose their resistance after a few years (Chen *et al.* 2003). These *R* genes function in a gene-for-gene fashion (Jia *et al.* 2000) so the pathogen can adapt by mutating or deleting the corresponding avirulence gene. Approximately 50 Pi genes (blast *R* genes) have been described and mapped in the rice genome (reviewed in Liu *et al.* 2005 and Sallaud *et al*. 2003) and at least three have been molecularly characterized; the *Pi-ta* (Bryan *et al.* 2000), *Pi-b* (Wang *et al.* 1999) and *Pi-k*^{h} (Sharma *et al.* 2005) genes on chromosomes twelve, two and eleven, respectively. Some genes contributing to blast resistance in rice have smaller quantitative effects (Wang *et al.* 1994, Fukuoka and Okuno 2001) and it is not clear whether they are functioning in a race-specific manner. One thing that is clear is that some genes with incomplete resistance effects can be race-specific; examples include *Pif*, *Pb1* and *Pi21* (review in Chen *et al.* 2005). Many rice geneticists believe that at least some genes affect quantitative resistance function in a nonspecific fashion (Chen *et al.* 2005, Fukuoka and Okuno 2001). Such a gene might include a transcription factor that controls the expression of defense genes or possibly a component of a defense-signaling pathway. Genetic analyses of resistance have identified resistance QTL in various germplasm and environments (Sallaud *et al.* 2003, Chen *et al.* 2003, Talukder *et al.* 2004). The detection of QTL represents the first step toward dissecting their molecular basis and their individual phenotypic effects in different environments, and also the first step in their manipulation in breeding material by selection of linked markers.

Rice blast researchers have concentrated considerable effort on the classification of isolates of the pathogen into apparently asexually derived lineages and transfer of genes conferring resistance to those isolates into commercial rice cultivars. With the exception of the two commercial rice cultivars, Oryzica Llanos 5 (OL5) released in 1989, and Fedearroz 50, released in 1998, the blast resistances in Colombian commercial rice varieties have typically broken down one to three years after release (Table. 2-1; Correa-Victoria and Martinez, 1995). The resistance in OL5 is still effective today, not only in Colombian production fields where the disease is still very prevalent, but also in rice-blast nurseries in Colombia, Brazil, The Philippines, Thailand, Indonesia, China and Korea (Correa-Victoria, 2005 personal communication). In Colombia, its resistance has been evaluated to several hundred isolates of the fungus, which grouped into six lineages based on molecular marker analysis. OL5 was inoculated with 202 isolates from six lineages at the International Rice Research Institute in The Philippines. In both experiments it showed resistance to all the isolates (Correa-Victoria and Zeigler, 1993). The resistance genes in OL5 were derived from five different progenitor cultivars through traditional breeding methods. Analysis of these and other cultivars with well-characterized blast isolates has indicated that all of them exhibit susceptibility to the different lineages of the pathogen and resistance to others (Fig. 2-1; Correa-Victoria *et al.,* 2004). Based on its pedigree, OL5 could potentially carry the *Pi-2* and *Pi-z R* genes on chromosome 6, *Pi-33* on chromosome 8, *Pi-ta*^{2} on chromosome 12, *Pi-b* on chromosome 2 and *Pi-k* and *Pi-sh* on chromosome 11 (Table. 2-2). The durable resistance of the cultivar OL5 could therefore be the result of the complement of race specific *R* genes, which in combination may have conferred resistance to all Colombian lineages for more than 15 years. Alternatively, the resistance could in part be due to the accumulation of genes with smaller but potentially nonspecific effects. Because of its complex origin and the interaction of many genes, the genetic basis of its resistance durability is not clear.

In the present study, we mapped QTL for two components of partial resistance to blast disease based on phenotypic data from 120 F_6 recombinant inbred lines (RIL) and 231 independent F_5 RIL from a Fanny x OL5 cross. Fanny is a highly susceptible japonica rice cultivar with no known genes conferring resistance to any rice blast isolate. Our objectives were to: (1) estimate the number, genomic position and genetic effects of the OL5 genes controlling resistance to eight different isolates of *M. grisea*, belonging to five different genetic lineages. (2) Determine the repeatability of resistance QTL by examining them in two different RIL populations, derived from the same parents, but inoculated in separate experiments and (3) compare our predicted loci with previous QTL and resistance loci reported for blast disease. We found that blast resistance in OL5 is due to the combined effects of multiple loci with major and minor effects. Some of these mapped to regions of previously identified *Pi* genes but two mapped to regions with no reported *Pi* genes.

Table. 2-1 Colombian rice cultivars, year of release, source of resistance and year in which virulent *M. grisea* isolates were observed. Modified from Correa-Victoria, *et al.* (2004)

Cultivar	Source of Resistance	Year of Release	Resistance breakdown	Years of Resistance
Cica 4	Peta	1971	1972	1
Cica 6	IR-822-432	1974	1975	1
Cica 7	Colombia 1	1976	1978	2
Cica 9	C 46-15	1976	1977	1
Cica 8	Tetep	1978	1980	2
Metica 1	Colombia 1	1981	1982	1
Oryzica 1	C 46-15, Colombia 1	1982	1985	3
Oryzica 3	Colombia 1, Tetep	1984	1985	1
O. Llanos 5	IR36, Colombia 1, 5685, Cica9. Cica7	1989	Not Yet	> 15
O. Caribe-8	Tetep, IR665, Colombia 1, Cica9	1993	1995	2
Fedearroz 50	IR665, Colombia 1, 5685, Cica9	1998	Not Yet	> 6

Figure 2-1 Genealogy of OL5 and the sources of its resistance to the blast fungus *M. grisea.* Progenitor cultivars and the complementary blast reaction of them to the Colombian blast lineages (SRL) are indicated. Modified from Correa-Victoria *et al.* (2004)

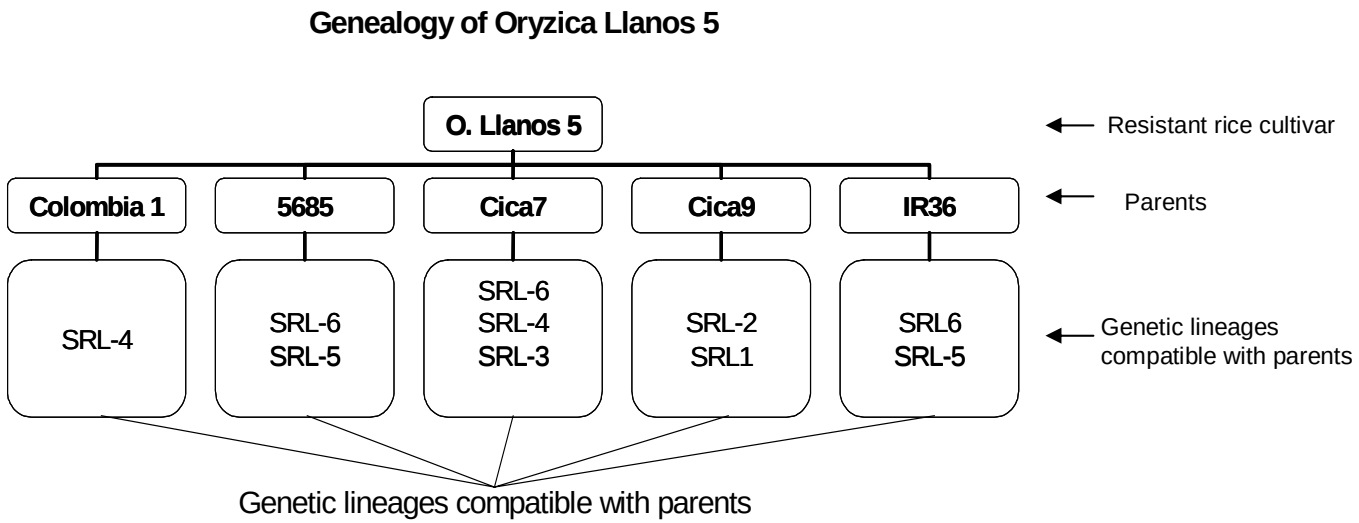


Table 2-2 Possible resistance genes present in commercial rice cultivars from Colombia inferred from inoculation with *M. grisea* isolates carrying the corresponding avirulence genes. Modified from Correa-Victoria *et al.* (2004)

	Resistance Genes								
Rice Cultivar	***Pi-b (2)***	***Pi-z (6)***	***Pi-2 (6)***	***Pi-33 (8)***	***Pi-sh (11)***	***Pi-k (11)***	***Pi-kh (11)***	***Pi-1 (11)***	***Pi-ta2 (12)***
Oryzica 2	X		X		X	X	X	X	X
Oryzica 3						X			X
Cica-8	X					X		X	X
Cica-9			X						X
IR 22					X	X			X
Oryzica Llanos 4			X			X	X		
Oryzica Caribe-8			X		X	X			
Oryzica Llanos 5	**X**	**X**	**X**	**X**	**X**	**X**			**X**
Fedearroz-50	X	X	X	X	X	X			X

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

Two recombinant inbred line (RIL) populations were used. The RIL populations were developed at the Centro International de Agricultura Tropical (CIAT) in Cali, Colombia and completed in 1997 and 1999. The first population consisted of 120 F_6 RIL and the second one of 231 F_5 RIL. The first population was examined for resistance in experiment 1 and the second in experiment 2. Both populations were derived from a cross between Fanny, a highly susceptible japonica rice cultivar and OL5, an Indica rice cultivar that has excellent blast resistance properties.

PATHOGEN INOCULATION AND DISEASE SCORING

Experiment 1 was carried out under greenhouse conditions at CIAT between June and July 2001. Experiment 2 was conducted over the same months in 2002. Eight *M. grisea* isolates from five different genetic lineages (Levy *et al.* 1993) were used in the study. All of them were highly virulent on Fanny, while only isolate FL440 produced lesions on OL5. Ten rice seedlings of each line along with the parent were grown in individual pots (4 inches) for 21 days before inoculation. Groups of fifteen pots were then placed in 55 cm long by 35 cm wide by 40 cm tall aluminum-framed transparent plastic-covered mist chambers. The entire inoculation experiment was replicated twice for both populations for each of the eight *M. grisea* isolates. The blast inoculation was carried out as described by Correa-Victoria and Zeigler (1993). In brief, a conidial suspension of 1x105 spores ml^{-1} and 0.5% gelatin in sterile water was sprayed onto the rice seedlings (25 ml per chamber). The inoculated seedlings were placed in a greenhouse maintained at 25 °C and sprayed with water twice each day. The plants were scored for disease infection 7 days after inoculation. Two components of partial resistance, percentage disease leaf area (DLA) and lesion type

(LT) were estimated and were considered separate traits. The most seriously diseased leaves from each plant of each line were used for visually estimating LT and DLA. Disease severity of 27 day old plants was scored using a rating from 0 to 100% for DLA and from 0 (highly resistant: no symptoms), 1-2 (lesions 1-2 mm, no sporulation), 3 (round lesions 2-3 mm with little sporulation), and 4 (spindle shaped lesions of more than 3 mm with heavy sporulation) for LT (Fig. 2-2). The scoring of both traits, DLA and LT, was based on methods of Correa-Victoria and Zeigler, (1993).

Figure 2-2 Disease leaf area (DLA) and lesion type (LT) scale used to evaluate the blast infection. The picture was taken at 7 days after inoculation. (Courtesy of G. Prado, CIAT)

MOLECULAR MARKERS AND SSR DETECTION

Plant DNAs were isolated from leaf tissue using a modified CTAB method (Hulbert and Bennetzen, 1991). The F_6 RIL were examined using restriction fragment length polymorphisms (RFLP) and simple sequence repeat (SSR) markers, while the F_5 RIL were examined only with SSR markers. RFLP probes were selected from the interspecific rice map (Causse *et al*. 1994). Five restriction enzymes were used for the RFLP probes: *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I. Southern transfer and hybridization were performed as described by Gallego *et al*. (1995). The Megaprime DNA labeling system (Amersham Life Science, NJ) was used to label DNA probes. For both experiments marker surveys were first conducted to identify polymorphic markers from the available rice SSR (Fig. 2-3). Polymerase chain reaction (PCR) conditions for the SSR markers were as described in Panaud *et al*. (1996), Temnykh *et al*. 2000 and McCouch *et al*. 2002, with the following modifications: the total reaction was scaled down to 15 µl and the following thermal cycle profile was used: 94°C for 4 min, followed by 11 cycles of 94°C for 45 s, 65°C for 45 s and 72°C for 45 s. The annealing temperature was decreased 1°C per cycle for these initial 11 cycles. The initial cycles were followed by 24 cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 45 s. The 72°C step was extended to 5 min for the last cycle. The PCR reaction was performed in PTC200 96U thermocycler (MJ Research, Watertown, Mass). The PCR products were run on 5% polyacrylamide gels containing 7 M urea, using a Bio-Rad Sequi-Gen GT sequencing cell. When parental polymorphic fragments differed in migration by less than 2 cm, up to sixteen polymorphic markers were multiplexed on a single polyacrylamide gel. Each marker was loaded on the gel separately, starting with the one with the smallest amplified fragments, and run 10 min at 100 watt before loading the next marker (Fig. 2-4). Amplified fragments were detected using a silver staining procedure (Promega, Madison, Wis.).

Figure 2-3 Screening for polymorphisms between Fanny and OL5 parents using rice SSR markers. Twenty-four SSR primer pairs were used to PCR amplify DNA from the two parents and the products separated on acrylamide gels as described in the text. The gel was stained with silver nitrate. The 20 bp ladder is included in the far left lane and between markers 238B and 541. F and O are the Fanny and OL5 parents.

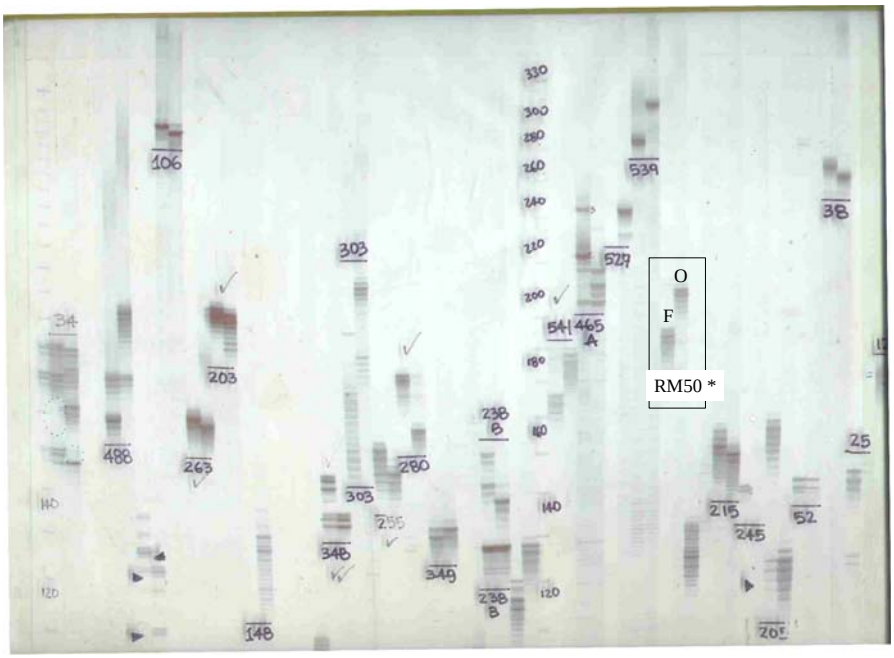
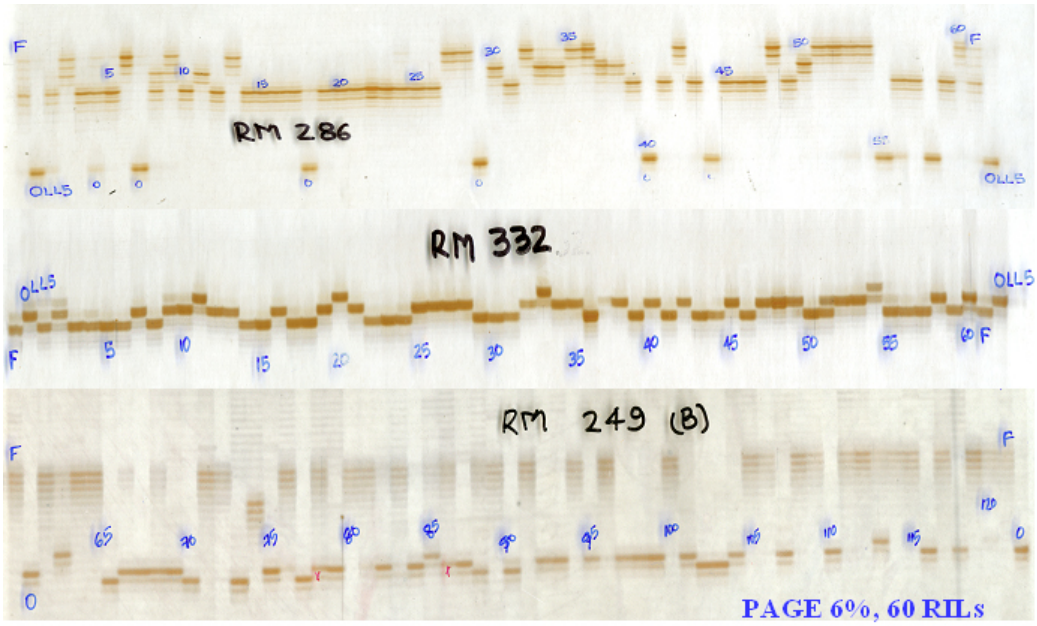


Figure 2-4 PCR products for three different rice microsatellite (RM) markers using DNA from the parents (F = Fanny and OLL5 = OL5) and experiment 1 progeny running in 6% of acrylamide gel and stained with silver nitrate. Only 60 samples plus the two parents for each SSR are shown.



LINKAGE MAPPING

Two genetic maps, one consisting of 350 molecular markers (RFLP and SSR) for experiment 1 and 201 SSR for experiment 2, were constructed using the multipoint functions performed by Mapmaker/EXP v. 3.0 (Lander *et al*. 1987). The two-point LOD score threshold was set to 6, and r_{max} to 0.3. Ordering of markers was achieved using the 'order', 'try' and 'ripple' commands, which calculate likelihood ratios for the different possible multipoint orders. Graphical representations of the linkage groups were produced with Mapmaker (Macintosh ver. 2.0, Proctor *et al*. 1993). Conversion of recombination fractions into centimorgans (cM) was performed using the Kosambi mapping function (Kosambi, 1944). Correspondence of linkage groups and the order of the markers on chromosomes were inferred based on the genetic linkage map of rice (Causse *et al*. 1994, Temnykh *et al*. 2000) and also from the rice physical map (www.gramene.org). Finally, an integrated genetic map with data from all the RFLP and SSR markers used was constructed to facilitate presentation of the locations of previous *Pi* genes, as well as QTL identified in this analysis.

MAPPING QUANTITATIVE RESISTANCE LOCI

QTL were identified using the composite interval mapping (CIM) and multiple interval mapping (MIM) approaches of Windows QTL Cartographer version 2.5 (Wang *et al.* 2005). Both QTL mapping methods were used to localize loci with major or minor effects on resistance and to search for epistatic interactions between QTL. For CIM, automatic forward-backward stepwise regression was used for the selection of cofactors (forward $P < 0.01$, backward $P < 0.01$). Model 6 was used to scan the genome at 1-cM intervals, using a window size of 10 cM. The likelihood value of the presence of a QTL was expressed as the log10 likelihood ratio (LOD) score. We defined an experiment wise error threshold of $P < 0.01$. For each trait analyzed

among two experiments with each of the eight isolates, a significance threshold was evaluated by performing 1,000 permutations (Doerge and Rebai, 1996) to determine LOD significance levels at $P < 0.01$ (Table 2-3). The approximate proportion of the phenotypic variation explained by the QTL (R^2) was estimated in both the CIM and MIM models. The QTL position was estimated as the point where the maximum LOD score was found by CIM. QTL detected in a single screen that were offset but overlapped by at least one marker were joined. When the same QTL interval was detected in both populations, either using CIM or MIM, we report the values with the higher R^2 and LOD scores. MIM was performed in order to resolve inaccurate QTL and confirm positions of QTL identified by CIM as well as to identify epistatic interactions between QTL. The MIM analysis was performed as described in Wu *et al.* 2005, with the following modifications: for the initial model to be used in MIM analysis, we first scanned through composite interval mapping results as a starting point. The Akaike information criterion (AIC) was used and set to c (n) = 2 when searching for epistatic interactions as recommended for Windows Cartographer.

Table 2-3 Level of significance threshold (LOD) obtained by permutation test at P < 0.01for DLA and LT across the two experiments

Exp. 1			Exp. 2		
	Permutation			Permutation	
ISOLATE	DLA	LT	ISOLATE	DLA	LT
FL440	2.5	2.7	FL440	4.5	3.5
CARIBE-8	4.0	9.5	CARIBE-8	3.0	9.0
CEYSVONI	3.5	2.9	CEYSVONI	5.0	2.7
CICA9	4.0	3.5	CICA9	2.4	2.8
F47	20.4	5.5	F47	4.8	2.4
SELECTA	13.4	3.7	SELECTA	7.8	18.0
F-54	3.0	3.5	F-54	8.0	5.8
METICA	4.5	3.0	METICA	2.7	3.1
	LOD			LOD	

NOMENCLATURE

QTL were designated as describe in Tabien *et al.* 2002 with the following modifications. A "*qrbr*" prefix indicates a QTL for rice blast resistance. This is followed after a hyphen by the number of the chromosome where the QTL was mapped. When multiple QTL are mapped in the same chromosome, an additional dot and numbers are added in ascending order from the top to the bottom of the chromosome name to distinguish between them. For example, a locus name *qrbr-9.1* means a QTL for blast resistance located towards the top of chromosome 9 (Fig. 2-8)

RESULTS

DISTRIBUTION OF RESISTANCE IN THE RI POPULATIONS

The frequency distributions of DLA and LT scores obtained in the two experiments with the eight isolates were examined to determine if they approximated normality (Fig.2-5). Chi-square analyses (data not shown) indicated that no normal distributions were followed for any of the eight isolates for either trait in either experiment. In general, both experiments showed a high percentage of individuals with DLA below 20% among the eight isolates (Fig. 2-5a). In contrast, the frequency distributions for LT for both experiments were very dependent on the blast isolate used. A higher percentage of individuals in the two experiments had LT scores of either 4 or 0 depending on the blast isolate used (Fig. 2-5b). For example, when challenged with the FL440 and F-54 isolates, both experiments had a high proportion of individuals with LT scores of 3 and 4. However, when challenged with the Metica and F-47 isolates, most of the lines had LT scores of 0 and 1 (Fig. 2-5b). Other isolates such as Cica-9 and Selecta showed dissimilar frequency distributions in the two experiments (Fig. 2-5).

The resistance segregation in both experiments varied dramatically depending on the blast isolate used. All of the isolates were virulent on Fanny with LT scores of 3 and 4 and DLA between 60 and 80%. In contrast, the LT scores were 0 for OL5 in both experiments except for the FL440 isolate, which was able to produce some LT scores of 1, 2 and 3 but with a DLA that was typically less than 10% (Fig. 2-6). Interestingly, only three lines in experiment 1 and five in experiment 2 were as resistant to the FL440 isolate as OL5, indicating that a combination of several genes was required to achieve this level of resistance.

Figure 2-5 Frequency distributions of disease leaf area DLA (a) and lesion type LT (b) across the two experiments.

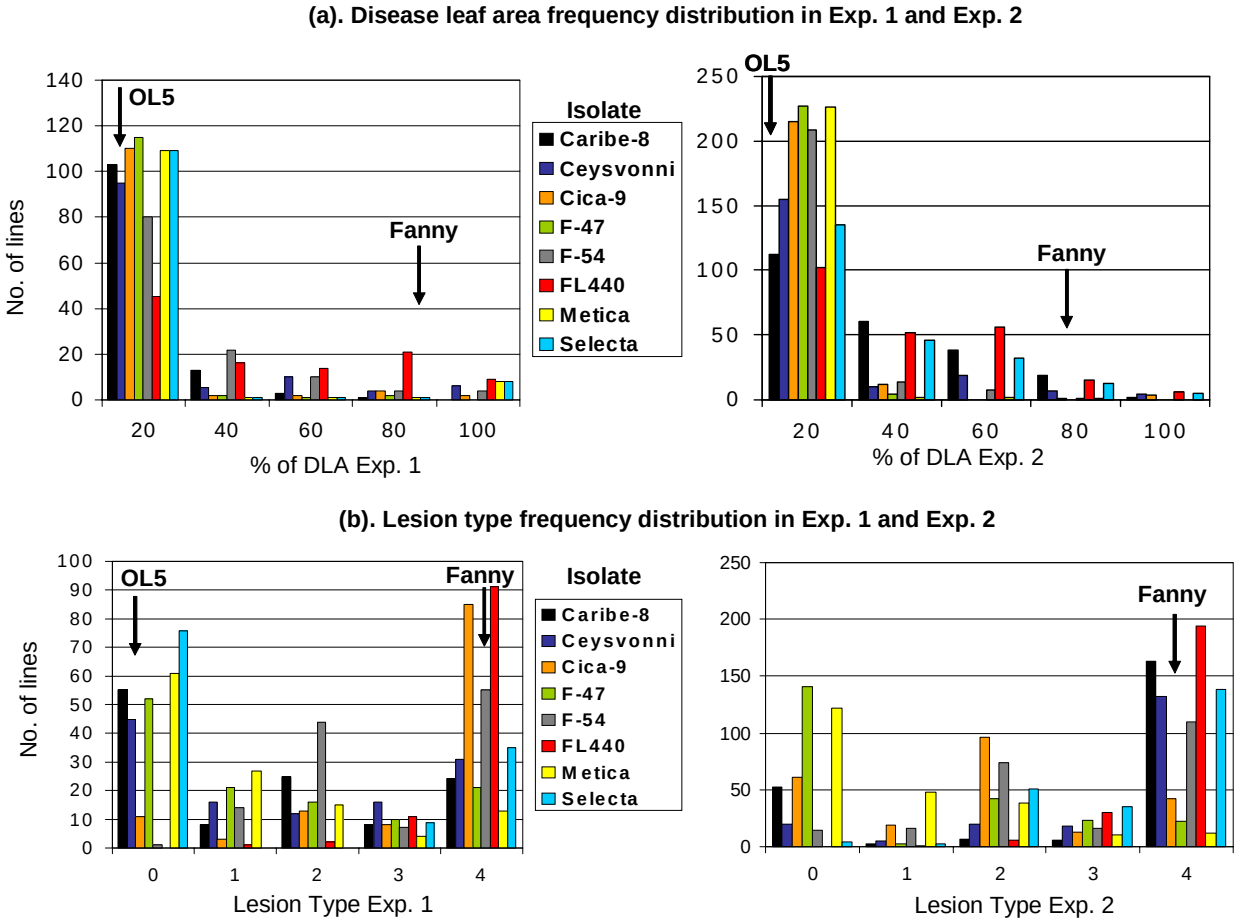
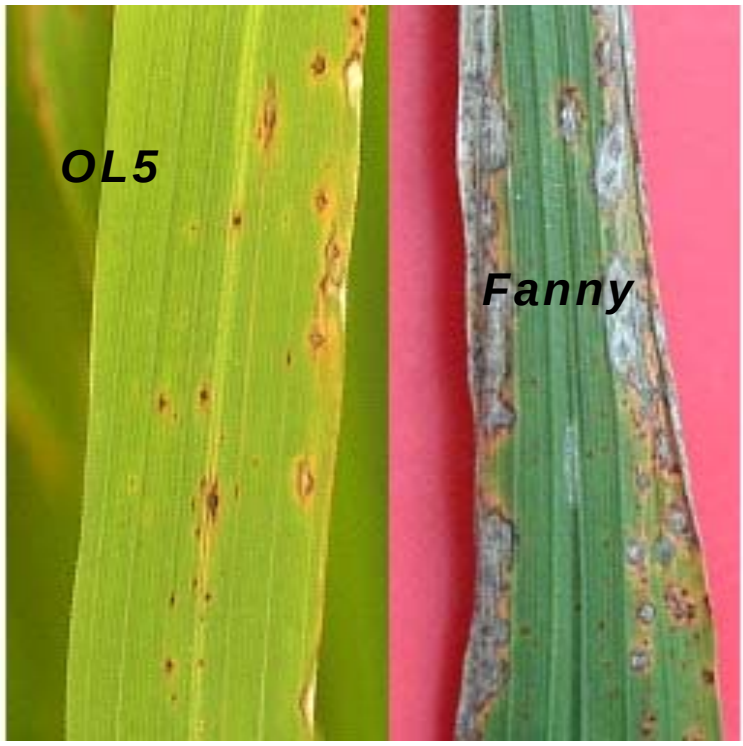


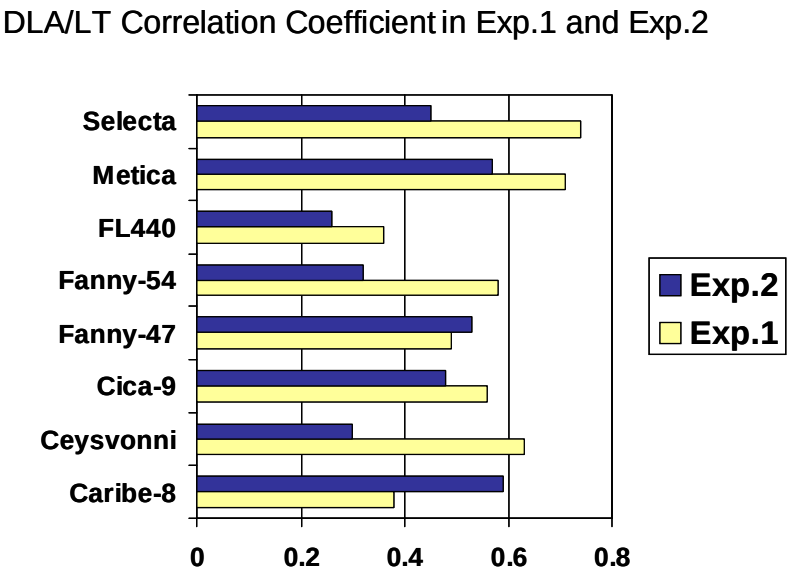
Figure 2-6 Rice cultivars OL5 (left) and Fanny (right) inoculated with isolate FL440 of *M. grisea*. Severe blast lesions are observed in Fanny and few lesions in OL5. The picture was taken at 7 days after inoculation.



TRAIT CORRELATIONS

Correlations between the two components of partial resistance (DLA and LT) among the individuals in each experiment were calculated for each of the eight isolates (Fig. 2-7). There was a positive correlation for the two traits in both experiments with each isolate. However, the strongest correlations for DLA and LT were found with the Selecta and Metica isolates (0.74 and 0.71 respectively) in experiment 1. In general, the correlations were higher in experiment 1 than experiment 2. The weakest correlations were found for the FL440 isolate with values of 0.26 and 0.35 in experiment 1 and 2, respectively. The poor correlations were partly due to the presence of lines with few, but relatively large (LT of 3 and 4), lesions resulting in a DLA of less than 10%. The resistance exhibited by the different lines was quite different phenotypically and also depended on the blast isolate used.

Figure 2-7 Correlation coefficients between disease lesion area (DLA) and lesion type (LT) for each of the eight isolates in both experiments 1 and 2.



QTL MAPPING

A total of 21 different loci were mapped, each associated with one or more of 58 statistically significant reductions in DLA or LT to one or more of the eight isolates used in this study. These loci were associated with LOD scores above the threshold value (equivalent to $P < 0.01$) determined by the permutation test for one of the traits in at least one of the two experiments. The resistance loci mapped to nine of the 12 rice chromosomes, with none mapping to chromosomes 5, 7 or 10. Of the 58 significant resistance traits identified, 36 (62%) were identified in experiment 1 and 22 (38%) in experiment 2. Twenty (34%), corresponding to eight loci, occurred in both experiments (Table 2-4, Fig 2-8). Thirty eight were detected only in one of the two experiments; 23 from experiment 1 and 15 from experiment 2. QTL that are significant in only one of the two experiments are referred to as experiment-specific QTL.

None of the QTL identified had statistically detectable effects on all eight isolates. However, some traits required high LOD scores to be statistically significant because of their abnormal distributions. For example, the lesion type trait in experiment 2 challenged with the Caribe-8 isolate required a LOD of 9.0. While at least 2 resistance loci were identified for each blast isolate in each experiment, these were often experiment-specific. For four of the 21 loci identified, the putative resistance allele was contributed by Fanny. These mapped on four chromosomes but all four were experiment-specific (Fig 2-8).

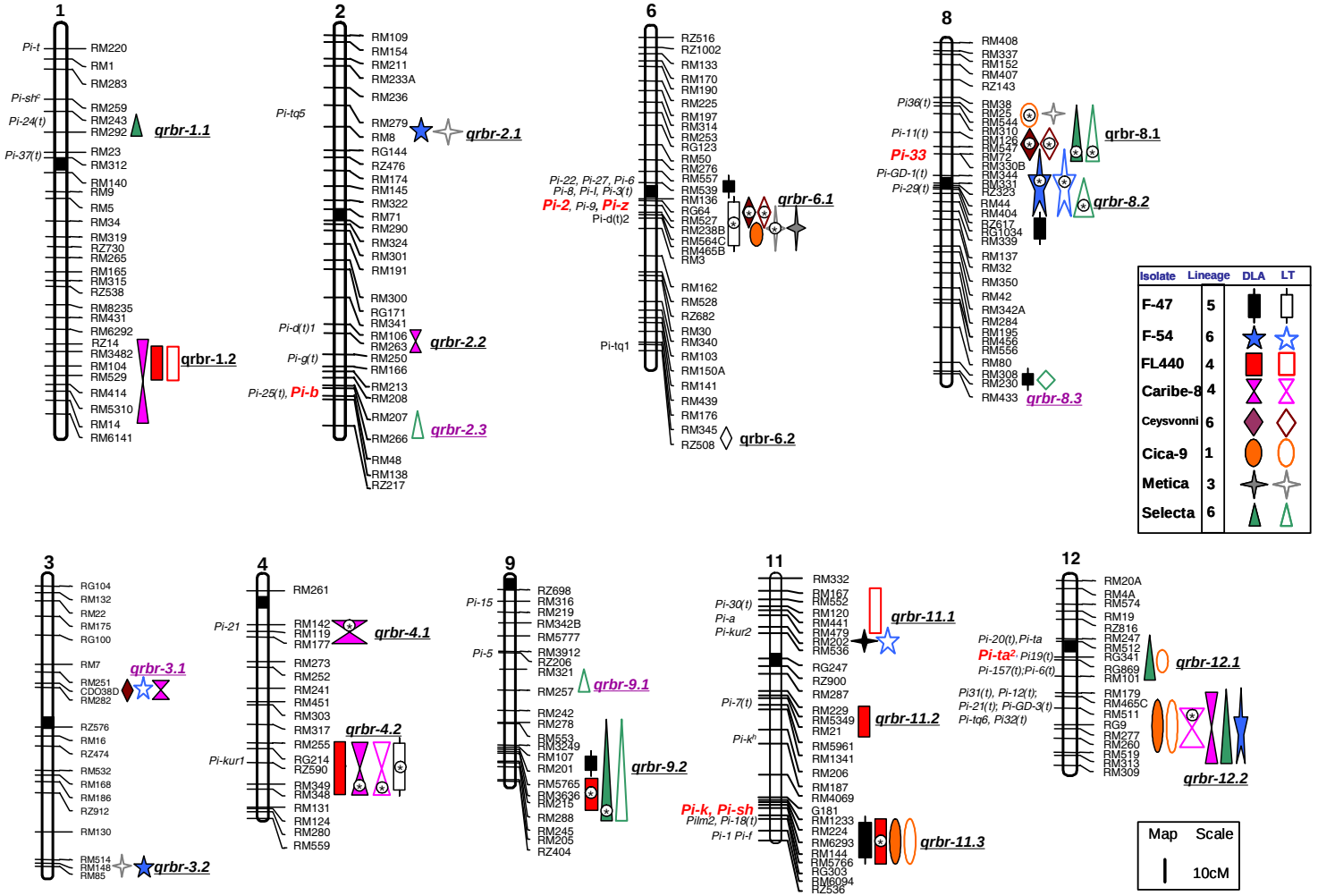
LOCI ASSOCIATED WITH QUANTITATIVE RESISTANCE

Three QTL, *qrbr-8.1*, *qrbr-8.2* and *qrbr-8.3*, were identified on chromosome 8 (Fig. 2-8). QTL *qrbr-8.1* and *qrbr-8.2* were clustered in the general region of the centromere or the short arm of chromosome 8 (Fig. 2-9). QTL *qrbr-8.1,* in the interval between RM38 and RM547, affected resistance to four isolates with five DLA or LT traits significant in both experiments. This locus controlled 57.3% of the phenotypic

variance in resistance to the Selecta isolate in experiment 1 (Table 2-4; Fig 2-8). One trait, (LT to isolate Metica) that mapped to this locus was significant in experiment 2. QTL *qrbr-8.2,* with resistance effects for three isolates, was identified in the interval of markers RM72 and RM339, near the centromere. Resistance to isolate Caribe-8 DLA and LT as well as resistance to isolate Selecta were significant in both experiments. DLA after inoculation with isolate F-47 was significant only in experiment 1. At least five race-specific *R* genes have been mapped in this region in previous studies including *Pi-11*, *Pi-33*, *Pi-29*, *Pi-GD-1(t)* and *Pi-36*. *Pi-33* was previously mapped close to the SSR marker RM72 (Berruyer *et al.* 2003). The *Pi-33* gene has been shown to confer resistance to isolates from lineage 1, 3, 4 and 6 but not 5 (Table 2-5, Correa-Victoria *et al.* 2004). Since isolates Caribe-8 (lineage 4), Selecta (lineage 6) and F-47 (lineage 5) were affected, it seems likely there are at least two *R* genes in this region in OL5. Our results support the idea that OL5 carries *Pi-3*3 as proposed by Correa-Victoria et al. (2004), and this gene could account for the *qrbr-8.1* locus.

QTL *qrbr-8.3* on chromosome 8 was mapped in the region between SSR markers RM308 and RM230 (Fig. 2-8). No *Pi* genes have yet been reported in this region; however this QTL was experiment-specific and was inherited from the susceptible parent Fanny.

Figure 2-8 Genetic map of rice linkage groups constructed using the two Fanny x OL5 RIL populations used in experiment 1 and 2. The map contains 265 markers. Approximate positions of *Pi* genes mapped in previous studies are indicated at the left of the chromosomes (see text for references). QTL were identified for LT and DLA against 8 different isolates as shown. For QTL designations in purple, the predicted resistant alleles were inherited from Fanny. *Pi* genes thought to be present in the pedigree of OL5 are shown in red. Resistance traits that were statistically significant in both experiments are marked with an asterisk.



⊗ QTL mapped at the same locus in both experiments

Figure 2-9 Location of the chromosome 8 QTL for DLA and LT blast resistance traits for six blast isolates with LOD scores above the threshold (2.5) using CIM in experiment 1. The bars represent the 1-LOD confidence interval. Most of the resistance traits mapped to two loci flanking the centromeric region (arrow).

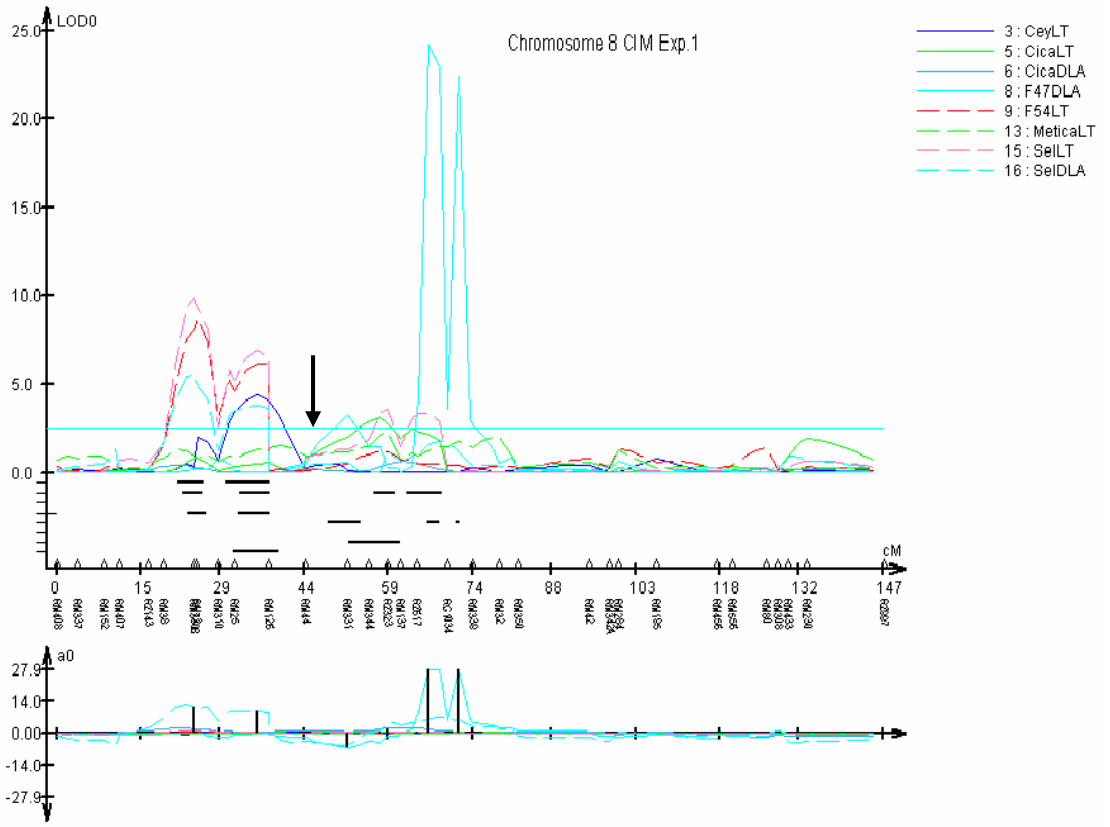


Table 2-4. Genomic intervals associated with quantitative blast resistance traits (DLA and LT) in experiment 1 and/or experiment 2. Loci with statistically significant effects in both experiments are shown in bold. Only QTL with LOD values above the threshold obtained from the permutation test (P< 0.01) are shown.

Isolate	QTL	Chromosome	Trait [a]	Interval Markers	LOD [e]	R^2 [b]	Effect [c]
Caribe-8	qrbr-1.2	1	DLA	RZ14-RM14	11.6	22.2	8.2
	qrbr-2.2	2	DLA	RM106-RM263	5.3	14.7	2.0
	qrbr-3.1	3	DLA	RM251-RM282	5.2	4.4	-2.4
	qrbr-4.1	**4**	**DLA**	**RM142-RM119**	**4.7**	**5.3**	**2.8**
	qrbr-4.2	**4**	**DLA**	**RM255-RM348**	**9.1**	**12.7**	**4.2**
	qrbr-12.2	12	DLA	RM179-RM313	11	31.7	19.5
	qrbr-4.2	**4**	**LT**	**RM255-RM348**	**10.5**	**22.8**	**1.4**
	qrbr-12.2	**12**	**LT**	**RM511-RM260**	**12.1**	**62.7**	**1.9**
Ceysvonni	qrbr-3.1	3	DLA	RM251-RM282	3.9	8.7	-8.2
	qrbr-6.1	**6**	**DLA**	**RM136-RM527**	**6.3**	**25.1**	**12.3**
	qrbr-8.1	**8**	**DLA**	**RM310-RM72**	**7.5**	**25.5**	**20.5**
			Epistatic[d]	qrbr-6.1 x qrbr-8.1	11.1	12.8	8.8
	qrbr-6.1	**6**	**LT**	**RM136-RM527**	**7.7**	**32.6**	**1.3**
	qrbr-6.2	6	LT	RM345-RZ508	2.9	4.8	0.4
	qrbr-8.1	**8**	**LT**	**RM310-RM72**	**5.1**	**8.9**	**0.5**
	qrbr-8.3	8	LT	RM308-RM230	2.7	8.0	-0.7
Cica-9	qrbr-6.1	6	DLA	RM238B-RM564C	2.7	5.0	3.2
	qrbr-11.3	11	DLA	RM1233-RM5766	4.7	9.5	5.8
	qrbr-12.2	12	DLA	RM465C-RM519	3.5	7.9	3.8
	qrbr-8.1	**8**	**LT**	**RM25-RM544**	**2.9**	**5.8**	**0.3**
	qrbr-11.3	11	LT	RM1233-RM5766	7.5	15.8	0.7
	qrbr-12.1	12	LT	RG341-RG869	4.1	11.2	0.6
	qrbr-12.2	12	LT	RM465C-RM519	4.3	10.2	0.5
F-47	qrbr-6.1	6	DLA	RM557-RM539	6.5	15.9	3.5
	qrbr-8.2	8	DLA	RZ617-RM339	22.6	27.5	0.7
	qrbr-8.3	8	DLA	RM308-RM320	6.1	9.1	-2.9
	qrbr-9.2	9	DLA	RM107-RM201	5.8	11.5	3.1
	qrbr-11.3	11	DLA	RM1233-RM5766	6.3	16.1	3.4
	qrbr-4.2	**4**	**LT**	**RM255-RM348**	**7.6**	**11.9**	**0.6**
	qrbr-6.1	**6**	**LT**	**RM136-RM465B**	**7.6**	**13.3**	**0.8**
F-54	qrbr-2.1	2	DLA	RM279-RM8	3.7	13.1	6.2
	qrbr-3.2	3	DLA	RM514-RM148	3.0	7.1	6.2
	qrbr-8.2	**8**	**DLA**	**RM547-RM404**	**10.1**	**36.2**	**10.6**
	qrbr-12.2	12	DLA	RM179-RM313	20.8	31.7	9.6
			Epistatic[d]	qrbr-8.2 x qrbr-12.2	10.1	9.9	8.9
	qrbr-3.1	3	LT	RM251-RM282	3.0	8.2	-0.3
	qrbr-8.2	**8**	**LT**	**RM547-RM404**	**30.8**	**62.9**	**1.0**
	qrbr-11.1	11	LT	RM479-RM536	28.1	19.2	1.0

Table 2-4 cont.

Isolate	QTL	Chromosome	Trait [a]	Interval Markers	LOD [e]	R^2 [b]	Effect [c]
FL440	qrbr-1.2	1	DLA	RM3482-RM529	2.9	17.6	12.7
	qrbr-4.2	**4**	**DLA**	**RM255-RM348**	**2.5**	**7.8**	**0.4**
	qrbr-9.2	**9**	**DLA**	**RM5765-RM215**	**3.0**	**9.3**	**9.2**
	qrbr-11.2	11	DLA	RM229-RM21	3.1	4.6	4.2
	qrbr-11.3	**11**	**DLA**	**RM1233-RM5766**	**2.9**	**15.1**	**12.1**
	qrbr-1.2	1	LT A	RM3482-RM529	3.9	12.9	0.2
	qrbr-11.1	11	LT	RM167-RM479	2.5	10.0	0.3
			Epistatic[d]	qrbr-1.2 x qrbr-11.1	4.7	13.7	-0.5
Metica	qrbr-6.1	6	DLA	RM123-RM465B	5.8	13.1	9.1
	qrbr-11.1	11	DLA	RM479-RM536	2.6	3.8	1.5
	qrbr-2.1	2	LT	RM279-RM8	3.2	25.2	0.6
	qrbr-3.2	3	LT	RM514-RM148	3.9	8.7	0.4
	qrbr-6.1	**6**	**LT**	**RM136-RM465B**	**2.6**	**11.5**	**0.5**
	qrbr-8.1	8	LT	RM25-RM544	2.8	15.2	0.5
			Epistatic[d]	qrbr-6.1 x qrbr-8.1	2.0	6.0	0.4
Selecta	qrbr-1.1	1	DLA	RM243-RM292	8.3	8.1	0.4
	qrbr-8.1	**8**	**DLA**	**RM38-RM72**	**10.1**	**27.4**	**14.7**
	qrbr-9.1	9	DLA	RM321-RM257	7.7	15.5	-16.3
	qrbr-9.2	**9**	**DLA**	**RM278-RM288**	**7.5**	**4.9**	**8.8**
	qrbr-12.1	12	DLA	RM247-RM101	3.7	7.8	8.1
	qrbr-12.2	12	DLA	RM179-RM313	7.0	23.4	17.4
			Epistatic[d]	qrbr-8.1 x qrbr-9.2	7.3	4.6	7.8
	qrbr-2.3	2	LT	RM207-RM266	3.1	5.2	-0.4
	qrbr-8.1	**8**	**LT**	**RM38-RM72**	**10.0**	**57.3**	**1.9**
	qrbr-8.2	**8**	**LT**	**RM344-RM404**	**5.7**	**10.1**	**0.7**
	qrbr-9.1	9	LT	RM278-RM288	4.7	7.3	0.6

[a] DLA = percentage diseased leaf area; LT = Lesion type. [b] R^2 = percentage of variance explained in the experiment in which the locus had the largest effect [c] Additive effect: a > 0, resistant allele from OL5; a < 0, resistant allele from Fanny. [d] Epistatic = digenic interaction between QTLs for the same trait as predicted by multiple interval mapping (MIM). [e] LOD = $\log_{10}$ of the odds ratio

Table 2-5 Resistance effects of different *Pi* genes to isolates of *M. grisea* from Colombia. The lineages of the different isolates (L1-L6) are listed below the isolate designations. Genes that are considered to be possibly present in OL5 as predicted by its pedigree are highlighted in gray. R = Resistant reaction.

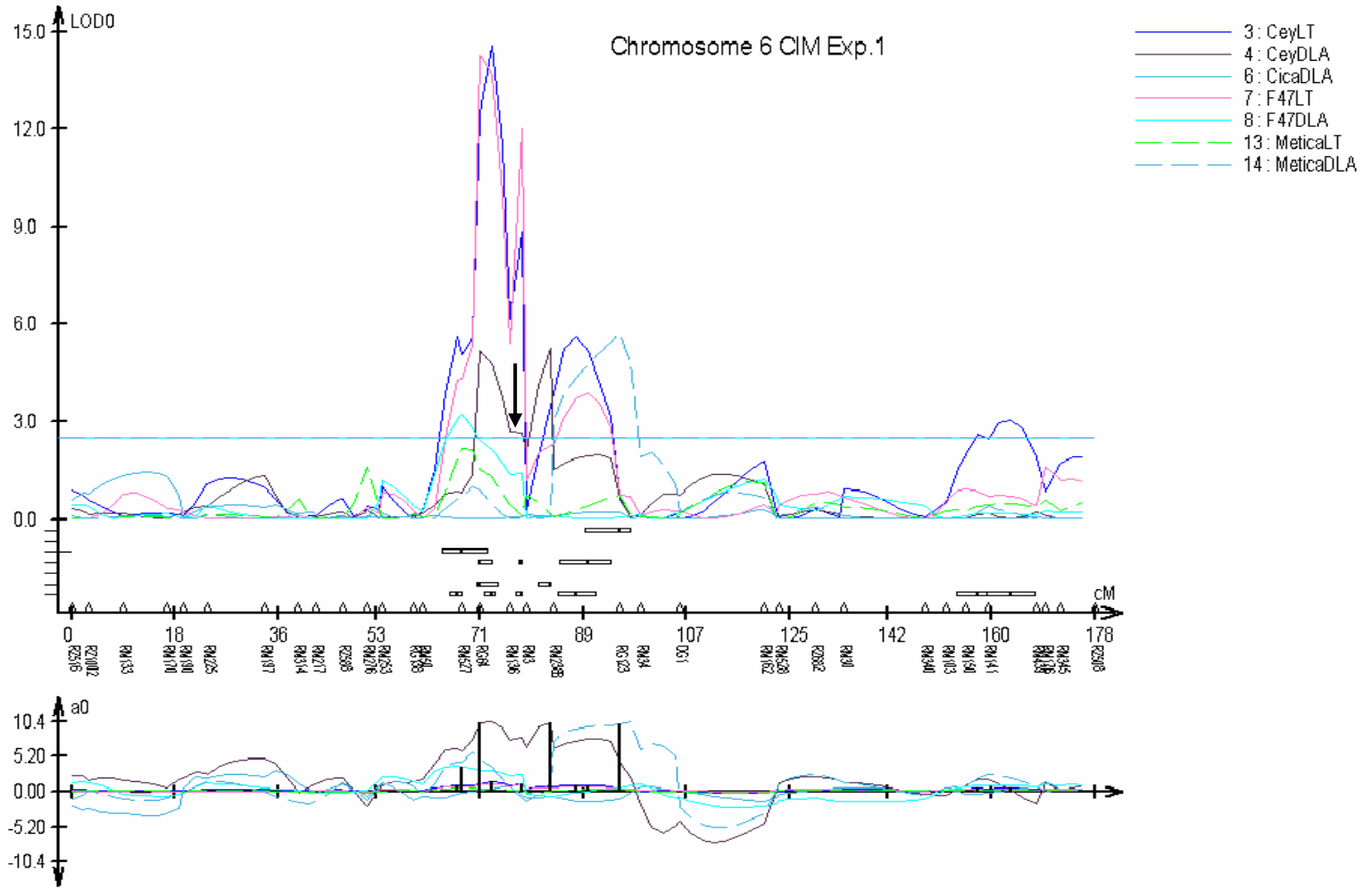
			Cica-9	Metica	FL440	Caribe-1			F-47	F-54	Ceysvonni	Selecta
			Isolate/Genetic Lineage									
Rice cultivar	Chr.	R gene	L1	L3	L4	L4	L4	L4	L5	L6B	L6B	L6
F 145-2	2	*Pi-b*	R			R						R
CT 13432-54	6	*Pi-2*							R			
OU 244	6	*Pi-z*							R	R	R	R
Toride	6	*Pi-z^t*							R		R	R
CT 13432-55	8	*Pi-33*	R	R		R				R	R	R
Norin 2	11	*Pi-sh*		R					R			R
CT 13432-68	11	*Pi-1*	R		R	R	R	R				
K 3	11	*Pi-k^h*			R	R	R	R				R
F 80-1	11	*Pi-k*						R				R
F 128-1	12	*Pi-ta^2*					R	R				

Modified from Correa-Victoria, 2004

Two loci (*qrbr-6.1* and *qrbr-6.2*) were identified affecting resistance to isolates Ceysvonni, F-47, Cica-9 and Metica on chromosome 6. The *qrbr-6.1* locus was located near the centromere between markers RM557 and RM465B (Fig 2-8). Resistance to isolate Ceysvonni was significant for both DLA and LT in both experiments and the phenotypic variance explained by the locus was 25.1% and 19.4% of the DLA in experiments 2 and 1, and 32.6% and 23.8% of LT in the same experiments, respectively. The resistance trait lesion type with isolate F-47 was also affected by *qrbr-6.1* in both experiments and explained 13.3% of the variation in experiment 1 (Table 2-4). At least 10 *R* genes conferring high levels of blast resistance have been mapped in the centromere region of chromosome 6. Two of those, *Pi-2* and *Pi-z,* were reported to possibly be present in OL5 based on its

pedigree (Correa-Victoria, 2003). *Pi-2* has been reported to be effective against isolates from lineage 5 like F-47, and *Pi-z* provided resistance to these as well as lineage 6 isolates like Ceysvonni (Table 2-2 and Table 2-5) but not lineage 1 (Cica-9) or 3 (Metica). The *qrbr-6.2* locus was located in the interval between markers RM345 and RZ508. A LT QTL to isolate Ceysvonni mapped to this interval but was significant in experiment 1 (Table. 2-3, Fig. 2-10).

Figure 2-10 QTL mapped for the DLA and LT blast resistance traits in Fanny x OL5 above the threshold (LOD 3.0) identified on rice chromosome 6 using CIM in experiment 1 to four blast fungal isolates. The bars represent 1-LOD confidence interval. Most of the resistance traits mapped to one locus in the centromere region (arrow). The lower contour shows the additive effect where (+) values = allele from OL5 and (-) values = alleles from Fanny



Three loci, *qrbr-11.1, qrbr-11.2* and *qrbr-11.3,* on chromosome 11 contributed to resistance to isolates FL440, Metica, F-54, F-47 and Cica-9. The *qrbr-11.3* locus was located between markers RM1233 and RM5766 and affected resistance to three isolates, but only the DLA trait with isolate FL440 was significant in both experiments (Table 2-4; Fig. 2-8). At least seven *R* genes have been mapped around this locus and one of them, *Pi-k*^{h}, was recently cloned (Table 2-2) (Sharma *et al*. 2005). Two of these *R* genes, *Pi-k* and *Pi-s*h have been predicted to possibly be present in OL5 (Table 2.2) based on the pedigree of the cultivar. Both *Pi-k* and *Pi-sh* confer resistance to isolates from genetic lineage 6. *Pi-k* also confers resistance to some lineage 4 isolates and *Pi-sh* confers resistance to Colombian lineage 5 isolates. Neither gene confers resistance to lineage 1 isolates like Cica-9 (Table 2-4). Thus, *Pi-k* or *Pi-sh* alone could explain the QTL and would also be necessary to postulate a second *R* gene in this region if the resistance effect on Cica-9 is real. A second locus (*qrbr-11.2*) conferring resistance to isolate FL440 was identified between the markers RM229 and RM21 (Fig. 2-8) but was significant in experiment 2. A third locus (*qrbr-11.1*) was located between the markers RM167 and RM536 in the short arm of chromosome 11. This locus affected isolates Metica, FL440 and F-54, but all of them occurred in only one of the two experiments (Fig 2-8).

Two QTL on chromosome 12 affected resistance to isolates Selecta, Cica-9, Caribe-8 and F-54 (Fig. 2-8). The *qrbr-12.2* locus was identified between markers RM179 and RM 313 and affected resistance to four of the isolates, but only LT with isolate Caribe-8 was significant in both experiments. This QTL accounted for 62.7% of the variation in lesion type in experiment 2 and 21.1% in experiment 1 (Table 2-4). This was the largest effect estimated for any of the QTL for resistance traits for any of the eight isolates. The QTL *qrbr-12.1* mapped near the centromere between markers RM247 and RM101. Effects on one Selecta DLA trait (experiment 2) and one Cica-9 LT

trait (experiment 1) were mapped in this locus but both were significant in only one experiment. Several race-specific blast resistance genes have been mapped in the centromeric region of chromosome 12. *Pi-ta*^{2} is one of the genes predicted to be potentially present in this region in OL5 (Bryan *et al.* 2000; Table 2-2). The large effect this QTL has on resistance to isolate Caribe-8 (lineage 4) is consistent with the possibility that *Pi-ta*^{2} is involved in the quantitative resistance. However, the physical position of *Pi-ta*^{2} has not been yet accurately determined so it would be premature to conclude that this gene controls the resistance observed fin this region.

Two QTL affecting resistance to isolates Selecta, FL440 and F-47 were identified on chromosome 9 (Fig. 2-8). The QTL *qrbr-9.2* mapped between the markers RM278 and RM201 and had a significant effect on DLA for FL440 and Selecta in both experiments. They had relatively small effects, explaining 9.3% and 4.9% of the phenotypic variance in experiment 1 for isolates FL440 and Selecta, respectively (Table 2-4). No *Pi* genes have yet been reported at this locus. QTL *qrbr-9.1* was mapped between markers RM321 and RM257, and the resistant allele was inherited from the susceptible parent Fanny. The locus had a significant effect on Selecta LT in experiment 1, explaining 15.5% of the variance but had no significant effect in experiment 2.

Two QTL were identified on chromosome 4. QTL *qrbr-4.1* mapped to the region between RM142 and RM177 and affected DLA after inoculation with Caribe-8 in both experiments (Fig. 2-8, Table. 2-3). The QTL *qrbr-4.2* mapped to the region between RM255 and RM348 and affected resistance to FL440, Caribe-8 and F-47 isolates. Four resistance traits were affected by this QTL, DLA with FL440, DLA and LT with Caribe-8, and LT with F-47, and they were significant in both experiments (Fig. 2-8), explaining between 7.8% and 11.9% of the phenotypic variance.

Partial resistance to isolates Caribe-8, F-54, Ceysvonni, and Metica was controlled by two QTL on chromosome 3. One of these QTL, *qrbr-3.1* (between RM251

and RM282), affected resistance to three isolates and in all three cases the predicted resistant allele came from the Fanny parent but was significant only in experiment 1. The other QTL on chromosome 3 (*qrbr-3.2*), was mapped between the markers RM514 and RM148 and had an experiment-specific effect on isolate F-54, but the resistance allele was inherited from OL5. The locus also had a small effect on both traits for this isolate in experiment 2 but the effect was not statistically significant (LOD ~0.5; Fig. 2-11). No *Pi* genes have previously reported on chromosome 3. Thus, our results suggest that there are two loci conferring partial resistance to rice blast located on rice chromosome 3, but because their effects were experiment-specific, they require further analysis for verification.

Figure 2-11a. Identification of QTL on rice chromosome 3 for DLA and LT traits to four blast isolates in two experiments. (a) QTL mapped in experiment 1. The QTL at the first locus (left) was inherited from Fanny. The bars indicate the 1-LOD confidence interval of the two loci. The small graphic (bottom) show the additive effect where (+) values = allele from OL5 and (-) values = alleles from Fanny.

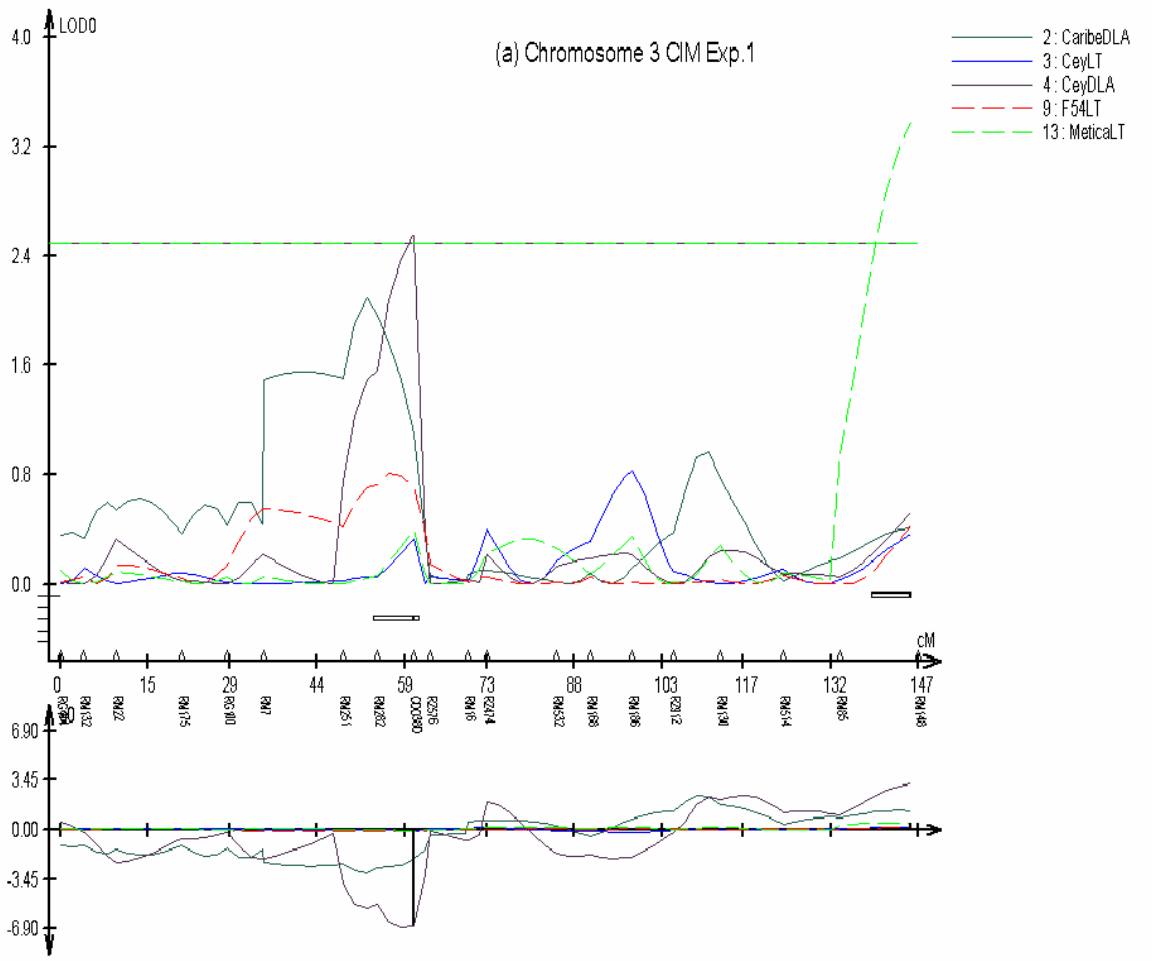
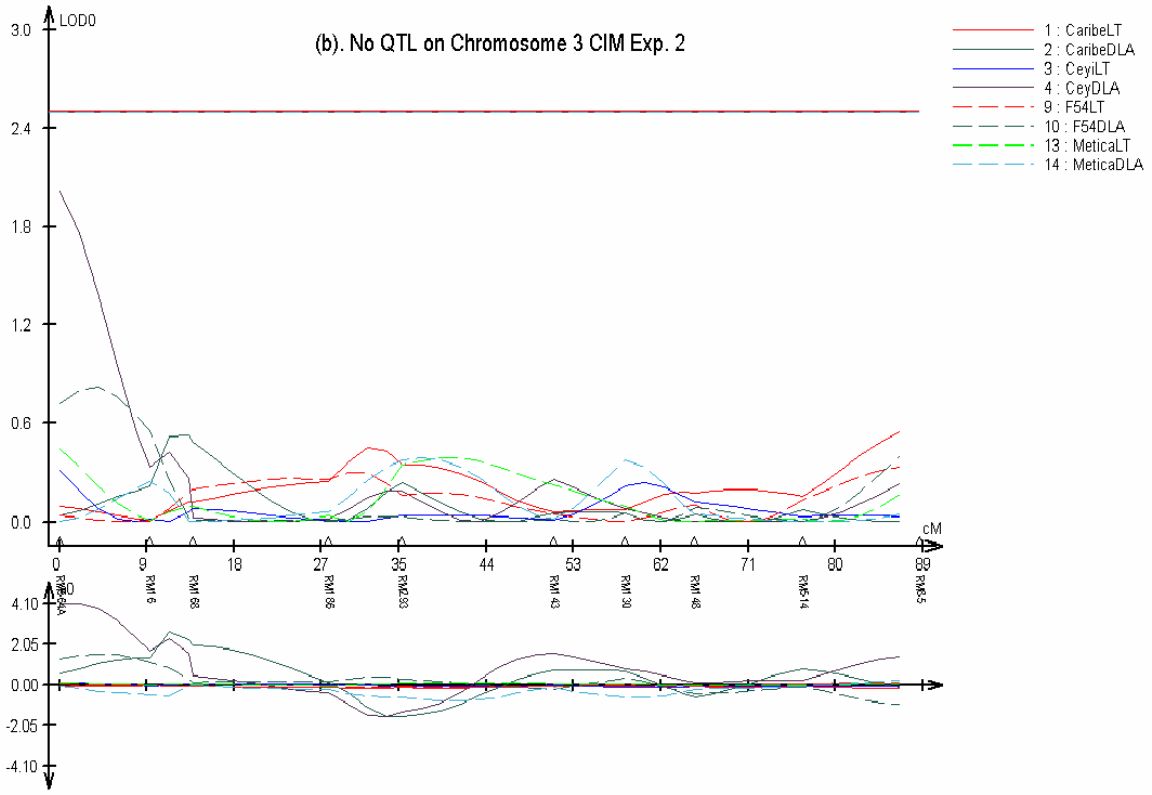


Figure 2-11 b QTL mapped in experiment 2 with low LOD values for isolates Metica and F-54 (right side in the figure) were found in the same region as QTL found in experiment 1.



Two QTL affecting resistance to isolates Selecta, Caribe-8 and FL440 were identified on chromosome 1 (Fig 2-8). The QTL *qrbr-1.2* mapped between markers RM3482 and RM5310 and affected resistance to the Caribe-8 and FL440 isolates. The locus explained 17.6% of the variation in DLA and 12.9% of the variation in LT for the FL440 isolate in experiment 1 but had no significant effect in experiment 2 (Table. 2-3, Fig. 2-12). Similarly, resistance to Caribe-8 was also identified in this locus in experiment 1 and explained 22.2% of phenotypic variance but was not statistically significant in the experiment 2. There is no previous report of *Pi* genes in this region. QTL *qrbr-1.1* was mapped between the markers RM259 and RM292 and confers resistance to the Selecta isolate, but its effect was also experiment-specific.

Figure 2-12a Likelihood plots of QTL associated with DLA and LT on rice chromosome 1. QTL based on mapping populations in exp. 1 (a) and exp. 2 (b) using composite interval mapping. The bars indicate the most likely positions of the QTLs. The horizontal dashed lines represent the minimum LOD required for significance.

a.

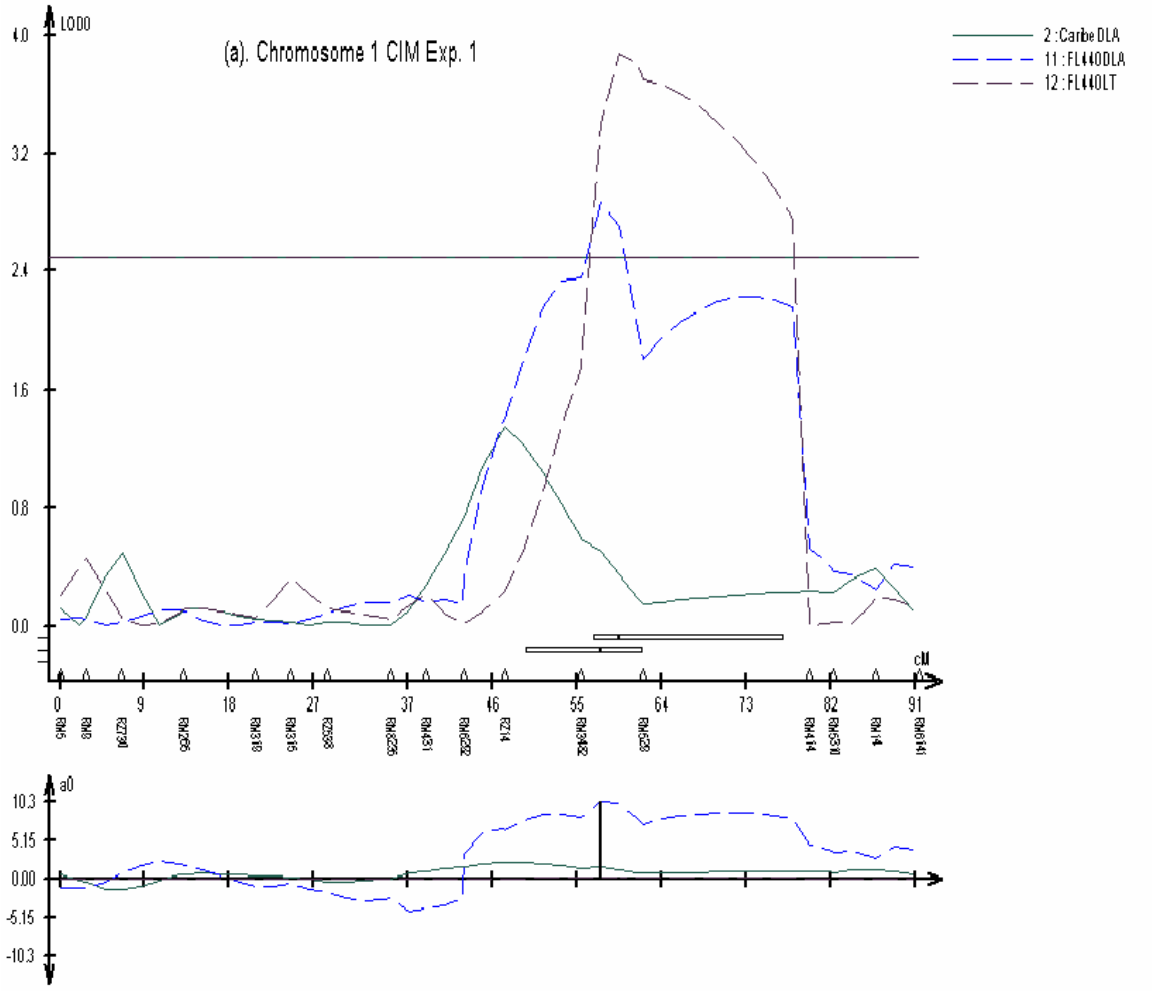
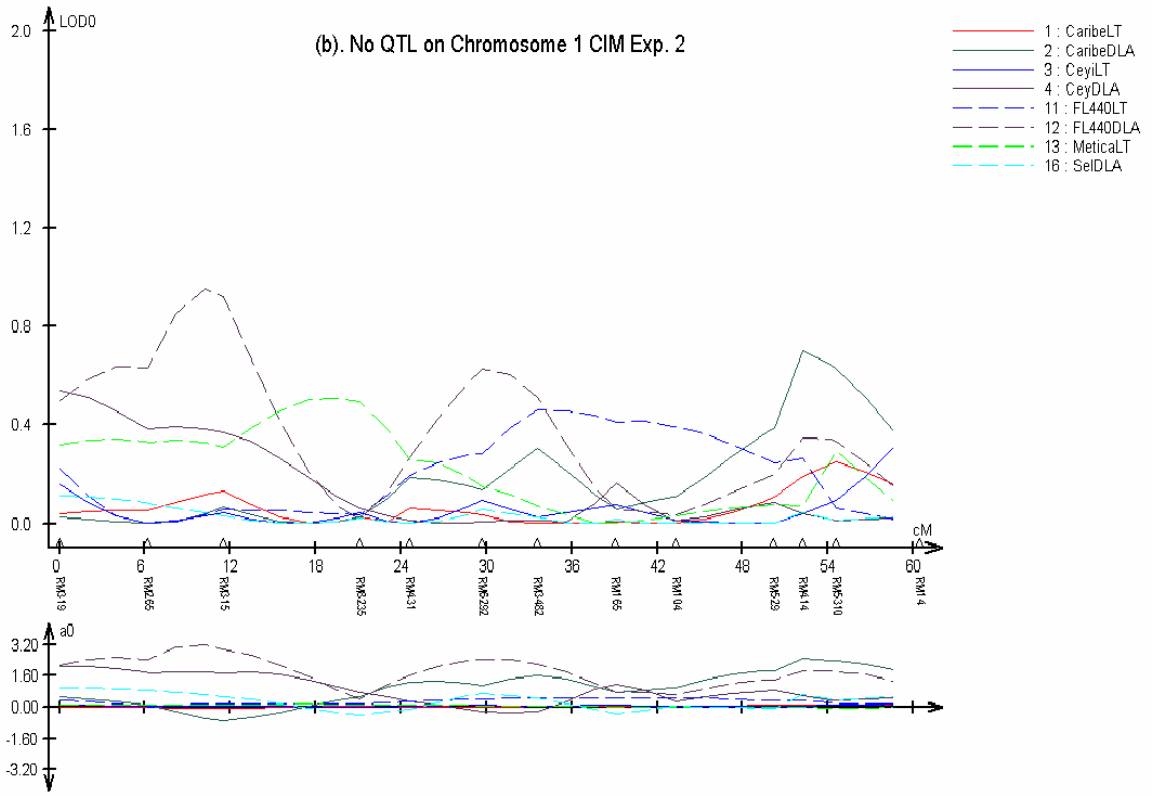


Figure 2-12 b

b.



POSSIBLE CONTRIBUTIONS OF PREVIOUSLY DESCRIBED, AND NOVEL, *PI* GENES

Eight of the twenty-one loci had statistically significant effects in both experiments, two on chromosomes 8 and 4 and one on chromosomes 6, 9, 11 and 12. Most of these loci correspond to map positions to which Pi genes have already been mapped. QTL *qrbr-8.1* on chromosome 8 affected resistance to isolates Cica-9, Ceysvonni, and Selecta from lineages 1, 3 and 6 respectively (Fig. 2-8). A closely linked QTL, *qrbr-8.2*, conferred partial resistance to isolates F-54 and Selecta, both from lineage 6. These results suggest that at least one gene is conferring partial resistance to different isolates from lineage 6, and a different gene (or genes) is affecting isolates from

lineages 1 and 3. A possible candidate for the *qrbr-8.1* QTL is the *Pi-33* gene since it is thought to be present in OL5 pedigree. The *Pi-36 (t)* and *Pi11* genes are other possibilities, but they are not known to be present in Colombian germplasm. *Pi-GD-1(t)* and/or *Pi-29* could possibly account for the resistance contributed by *qrbr-8.2*, since the genes have been mapped 5.4 cM and 8.9 cM from the RFLP marker RZ617, respectively, but neither gene was present in OL5 based on its pedigree. The QTL *qrbr-4.1* on chromosome 4 was unique in that it conferred quantitative resistance to only one isolate, Caribe-8 (Fig. 2-8). The other QTL (*qrbr-4.2*) on chromosome 4 conferred resistance to isolates FL440 and Caribe-8, both from lineage 4 and F-47 from lineage 5. No *R* genes have been described in Colombian germplasm on chromosome 4 that are effective against isolates from lineages 4 and 5 (Table 2-2, Table 2-4) but two Pi genes, *Pi-kur1* and the recessive *pi-21* gene, both from japonica cultivars, have previously been mapped near both loci (Goto *et al.*1988; Fukuoka and Okuno, 2001) (Fig.2-8, Table 2-5). Since the OL5 cultivar is an indica rice cultivar, it seems unlikely that these genes are in its pedigree.

The QTL *qrbr-6.1* on chromosome 6, conferred partial resistance to isolates F-47 and Ceysvonni, from lineages 5 and 6, respectively (Fig. 2-8, Table 2-5), and also had experiment-specific effects on isolates from two other lineages. Based on the genes thought to be present in Colombian germplasm, (Table 2.4), this gene is likely to be *Pi-z* because it affects isolates from these two lineages (Correa-Victoria, *et al.* 2004).

Several *Pi* genes have been mapped to the genetic region on chromosome 11 where the resistance QTL *qrbr-11.3* mapped in both experiments. The best candidate for this gene is probably *Pi-k*, which is thought to be present in the pedigree of OL5 and confers resistance to lineage 4 isolates like FL440 (Table 2-4, Table 2-6). The recently cloned *Pi-k*^{h} gene also maps to this region but does not confer resistance to lineage 4

isolates. Its physical position on rice chromosome 11 is also approximately 2 Mb above the interval predicted for the QTL in OL5 (Sharma *et al*. 2005).

The QTL *qrbr-12.2* on chromosome 12 that conferred resistance to Caribe-8 in both experiments and had experiment-specific effects on resistance to several other isolates mapped near a region where many *Pi* genes have been mapped (Fig. 2-8). Of those *Pi* genes, *Pi-ta*^{2} is the best candidate to contribute to the resistance in OL5 because it is present in its pedigree and confers resistance to isolates from lineage 4 such as Caribe-8 as well as lineage 6 isolates such as F-54 and Selecta (Table 2-4, Table 2-6). QTL *qrbr-9.2* on chromosome 9 was statistically significant in both experiments and mapped to a region where no *Pi* genes had been previously mapped (Fig. 2-8). The locus had a modest effect however, and did not have R^2 values above 15% for any of the isolate/experiments combinations (Table 2-3). Given the relatively small resistance effect of this locus, it is possible it affects resistance to the other isolates, but the effects were not detectable in the current experiment.

Table 2-6. Putative association between QTL with significant effects (R^2 > 13%) and *Pi* genes predicted to be present in the OL5 parent. [a] Number in parentheses indicates the % of variation accounted for the locus in the experiment in which it had the largest effect. *Pi* gene names followed by question marks indicate that the gene has not been predicted to be in the OL5 pedigree or that no *Pi* genes have yet been mapped to that region. (*) Means that this QTL was experiment-specific but has a R^2 > 13%.

Chromosome	Interval	Isolate (%R^2)[a]	Pi gene	QTL
1	RM3482-RM529	FL440 (17.6)	*Pi-?*	*qrbr-1.2*
		Caribe-8 (22.2)	*Pi?*	
2	RM279-RM8	Metica (25.2)	*Pi-tq5?*	*qrbr-2.1*
		F-54 (13.1)		
	RM106-RM263	Caribe-8 (14.7)	*Pi-b*	*qrbr-2.3*
4	RM255-RM348	Caribe-8 (22.8)	*Pi-kur1?*	*qrbr-4.2*
6	RM136-RM527	Ceysvonni (32.6)	*Pi-z*	*qrbr-6.1*
		F-47 (13.3)	*Pi-2*	
		Metica (13.1)	*Pi-2; Pi-z*	
		Ceysvonni (25.1)	*Pi-z*	
8	RM38-RM72	Selecta (53.7)	*Pi-33; Pi36(t)?; Pi11?*	*qrbr-8.1*
		Ceysvonni (62.9)	*Pi-33; Pi36(t)?; Pi11?*	
		F-54 (25.5)	*Pi-33; Pi36(t)?; Pi11?*	
		Metica (15.2)	*Pi-33; Pi36(t)?; Pi11?*	
	RZ617-RM339	F-47 (27.5)	*Pi-GD-1(t)? Pi29?*	*qrbr-8.2*
9	RM321-RM257	Selecta (15.5)	*Pi-5?*	*qrbr-9.1*
11	RM1233-RM5766	FL440(15.1)	*Pi-k?*	*qrbr-11.3*
		Cica-9 (15.8)	*Pi-sh*	
		F-47 (16.1)	*Pi-sh*	
12	RM179-RM313	F-54 (31.7)	*Pi-ta*^{2}	*qrbr-12.2*
		Selecta (23.4)	*Pi-ta*^{2}	
		Caribe-8 (62.7)	*Pi-ta*^{2}	

As mentioned above, most of the QTL detected in this study map to locations previously identified as containing *Pi* genes with large, race-specific resistance effects or QTL with smaller effects on blast disease (Fig. 2-8). This is consistent with the idea that the OL5 cultivar carries multiple *Pi* genes with major and minor effects, and that those account for at least part of the high level of resistance it has shown over time. Several other QTL mapped to positions where no *Pi* genes have yet been mapped, but this QTL was experiment specific (except for the locus on chromosome 9) so should be regarded

with caution. These include QTL on chromosomes 1 and 8 and two QTL on chromosome 3. QTL *qrbr-1.2* affected three experiment-specific resistance traits and was mapped in experiment 1 in the RM3482-RM529 marker interval on chromosome 1, and two of them had R^2 above 15% (Table 2-4). Isolates FL440 and Caribe-8, both from lineage 4, were affected by this QTL (Fig. 2-8; Table 2-6). Although this locus was significant in experiment 1, there was a low LOD value (< 0.7) observed with these isolates in the same region (Fig. 2-10). The predicted resistance allele from QTL *qrbr-8.3*, on chromosome 8, was inherited from the susceptible Fanny parent and conferred resistance to isolates F-47 and Ceysvonni. Another experiment-specific locus (*qrbr-3.1*) on chromosome 3 was also inherited from Fanny and conferred resistance to isolates Ceysvonni, Caribe-8 and F-54 but the R^2 values were modest. Another QTL *qrbr-3.2* at the bottom of chromosome 3, also with low R^2 values, was inherited from OL5, and conferred partial resistance to isolates F-54 and Metica.

CANDIDATE GENES FOR THE QTL CONFERRING RESISTANCE TO THE FL440 ISOLATE

Little is known about the molecular basis of genes that contribute quantitatively to resistance. Genes that act in a gene-for-gene fashion can have small effects or can have small resistance effects on isolates that do not carry the corresponding *R* genes (Hu *et al.* 1997; Li *et al.* 2001). Other defense response genes or defense signaling genes might also be good candidates. The DNA sequences corresponding to loci on chromosomes 4, 9 and 11 that confer partial resistance against isolate FL440 in both experiments and *qrbr-1.2* on chromosome 1 were examined for possible candidate genes. QTL on chromosomes 6, 8 and 12 that had larger effects (R^2 > 20%) and affected both DLA and LT in both experiments were not considered because they mapped to loci with *R* genes that have been cloned, such as *Pi-ta* on chromosome 12 (Bryan *et al.* 2000) and *Pi-k*^{h} on chromosome 11 (Sharma *et al.* 2005) or physically

mapped like *Pi-2* and *Pi-9* on chromosome 6 (Liu *et al.* 2002) and *Pi-36* (Liu *et al.* 2005), *Pi-GD-1(t)* (Liu *et al.* 2004) and *Pi-33* (Berruyer*, et al.* 2003) on chromosome 8; or they were located in regions with many predicted candidates, such as clusters of NBS-LRR genes (Monosi *et al.* 2004, Table 2-7).

The microsatellite markers spanning four putative resistance loci were integrated with the Nipponbare DNA sequence *in silico* using the GRAMENE and TIGR databases. This was possible because the genetic map is linked to the rice physical map wherever sequence information is available for the markers. Predicted genes in these two databases, such as NBS-LRR genes, receptor kinases and other protein kinases were considered good candidates for race-specific *R* genes (Bai *et al*. 2002; Howles *et al*. 2005). Other genes, such as those coding for proteins commonly induced in defense reactions, were also considered as possible QTL (Ramalingam, *et al* 2003; Wisser *et al.* 2005). The putative resistance QTL *qrbr-1.2*, located in the interval between RM6292 and RM529 (~1.44 Mb) on chromosome 1, included one NBS-LRR gene and three possible defense genes (Table 2-7). The second QTL *qrbr-9.2* mapped in the interval between RM278 and RM288 (~1.87 Mb) on chromosome 9 which included two NBS-LRR and three possible defense genes (Table 2-7). The defense genes corresponded to one ascorbate peroxidase and two thaumatin-like proteins, typical of those induced by a variety of phytopathogens in many plants. The QTL *qrbr-4.2* interval on chromosome 4 contained 5 NBS-LRR proteins and four defense genes (Table2-6). Finally, the *qrbr-11.3* locus on chromosome 11 harbors 27 predicted NBS-LRR proteins, one defense gene and one JAMyb-family transcription factor (Table 2-7).

Table 2-7 Candidate NBS-LRR and defense genes in the QTL regions conferring resistance to isolate FL440 of *M. grisea* on chromosomes 1, 4, 9 and 11. The SSR markers used in this study are also shown.

Marker	*Chr.*	*bp*	*Gene*	*Protein*	*QTL*
RM6292	1	39202220			
10449	1	39481144	LOC_Os01g67980	putative thiol protease	
RM3482	1	39699704			
10451	1	39856209	LOC_Os01g68660	oryzacystatin	
10452	1	39858624	LOC_Os01g68670	oryzacystatin	
RM104	1	40147548			
10030	1	40541026	LOC_Os01g70080	NBS-LRR	
RM529	1	40651364			
RM255	4	30518352			
10609	4	30579431	LOC_Os04g52210	diterpene cyclase	
10610	4	30587813	LOC_Os04g52230	diterpene cyclase	
10611	4	30595893	LOC_Os04g52240	diterpene cyclase	
10612	4	30955956	LOC_Os04g52720	oxalate-oxidase	
10098	4	31217547	LOC_Os04g53160	NBS-LRR	
10096	4	31160979	LOC_Os04g53050	NBS-LRR	
10094	4	31138740	LOC_Os04g53030	NBS-LRR	
10093	4	31113927	LOC_Os04g52970	NBS-LRR	
10099	4	31415285	LOC_Os04g53500	NBS-LRR	
RZ590	4	32193711			
RM349	4	32243656			
RM348	4	32394720			
RM278	9	19033543			
RM107	9	19721560			
10232	9	19814069	LOC_Os09g34150	NBS-LRR	
10233	9	19819867	LOC_Os09g34160	NBS-LRR	
RM201	9	19826860			
RM5765	9	20442217			
10778	9	20736410	LOC_Os09g36560	thaumatin-like protein	
10779	9	20745831	LOC_Os09g36580	thaumatin-like protein	
RM215	9	20837155			
10780	9	20852557	LOC_Os09g36750	ascorbate peroxidase	
RM288	9	20912111			

Table 2-7 cont**.**

Marker	*Chr.*	*bp*	*Gene*	*Protein*	*QTL*
10329	11	25831137	LOC_Os11g43700	NBS-LRR	
G181	11	26132682			
10330	11	26406046	LOC_Os11g44580	NBS-LRR	
RM1233	11	26456128			
10331	11	26672305	LOC_Os11g44960	NBS-LRR	
10332	11	26684649	LOC_Os11g44970	NBS-LRR	
10333	11	26711543	LOC_Os11g45050	NBS-LRR	
RM224	11	27123770			
10334	11	26720036	LOC_Os11g45060	NBS-LRR	
10335	11	26732682	LOC_Os11g45090	NBS-LRR	
10336	11	26769392	LOC_Os11g45130	NBS-LRR	
10337	11	26785664	LOC_Os11g45160	NBS-LRR	
10338	11	26792478	LOC_Os11g45180	NBS-LRR	
10339	11	26800570	LOC_Os11g45190	NBS-LRR	
10340	11	26878226	LOC_Os11g45330	NBS-LRR	
10341	11	27056210	LOC_Os11g45620	NBS-LRR	
10863	11	27123771	LOC_Os11g45740	JAMyb Oryza sativa	
10342	11	27140738	LOC_Os11g45750	NBS-LRR	
10343	11	27141649	LOC_Os11g45760	NBS-LRR	
10344	11	27157662	LOC_Os11g45790	NBS-LRR	
10345	11	27185021	LOC_Os11g45840	NBS-LRR	
10346	11	27243159	LOC_Os11g45920	NBS-LRR	
10347	11	27244227	LOC_Os11g45930	NBS-LRR	
10348	11	27268880	LOC_Os11g45970	NBS-LRR	
10349	11	27270758	LOC_Os11g45980	NBS-LRR	
10350	11	27335825	LOC_Os11g46070	NBS-LRR	
10351	11	27370513	LOC_Os11g46130	NBS-LRR	
10352	11	27379180	LOC_Os11g46140	NBS-LRR	
10353	11	27434046	LOC_Os11g46200	NBS-LRR	
10354	11	27435146	LOC_Os11g46210	NBS-LRR	
RM5766	11	28225397			
10355	11	28169486	LOC_Os11g47780	NBS-LRR	
RM144	11	28158704			
10864	11	28179212	LOC_Os11g47810	metallothionein-like protein	
RM6094	11	28284056	117.9		

DISCUSSION

Blast resistance in the cultivar OL5 was found to have very complex inheritance. Eight resistance QTL were identified that were statistically significant in both of the two mapping experiments conducted. The two experiments were conducted with separate, independently derived lines from the same cross allowing the verification of some loci with relatively small effects (e.g. R^2 < 15%). Evidence for several other experiment-specific QTL was generated but the results were significant in only one of the two experiments. Some of these latter loci may represent genes with small effects, which are affected by the environment or near the threshold of significance in QTL mapping experiments. Differences in the frequency distributions of resistance to some of the isolates were observed between the two experiments indicating environmental differences between the two experiments. This presumably reflects the segregation of different resistance genes which may function at different stage in the infection process. This difference indicates that the environment where the disease assay was performed made an appreciable difference in resistance to some of the isolates. Similar experiment- or environment-specific effects have been observed in other QTL mapping analyses (Talukder, *et al.* 2005; Xu, *et al.* 2004).

Many of the QTL identified in the present study mapped to regions where blast resistance traits had previously been mapped. Seven of eight QTL that were detected in both experiments mapped to regions of known *Pi* genes. Resistance traits mapped as QTL for blast resistance have also been mapped to several of these loci (Sallaud, *et al.* 2003; Tabien *et al.* 2002; Wen *et al*. 2003, Chen *et al.* 2003). Several of the *Pi* genes that mapped to these areas are thought to be present in rice lines in the pedigree of OL5 and are therefore good candidates for the genes controlling the resistance (Table 2-2). One QTL, *qrbr-9.2*, on chromosome 9 was in a region where no *Pi* genes had been designated, but a QTL had been reported for blast resistance (Chen *et al*. 2003). QTL

had been previously mapped to most of the other QTL identified in OL5; including putative OL5 QTL that only had significant effects in one experiment. These regions include: QTL *qrbr-1.2*, *qrbr-2.2*, *qrbr-3.1*, *qrbr-8.1* and *qrbr-9.2* are located in the same five regions as QTL for resistance to rice blast identified in a cross between cultivars Zhenshan 97 and Minghui 63 (Chen *et al.* 2003). Nine QTL (*qrbr-1.2, qrbr-3.1, qrbr-6.1, qrbr-6.2, qrbr-8.1, qrbr-9.2, qrbr-11.2, qrbr-11.3 and qrbr-12.1*) coincided with nine QTL identified for blast in lines derived from a cross between the indica IR64 and the japonica cultivar Azucena (Ramalingam, *et al.* 2003). Four QTL (*qrbr-6.1, qrbr-8.2, qrbr-11.1 and qrbr-12.1*) were also identified in a double haploid (DH) population derived from an IR64 by Azucena cross (Sallaud *et al.* 2003). The *QTL qrbr-1.2, qrbr-4.2*, *qrbr-6.1* and *qrbr-12.1* occurred at the same positions as QTL for blast resistance in a RIL derived from a Bala x Azucena cross (Talukder, *et al.* 2004). Four QTL (*qrbr-4.1*, *qrbr-4.2, qrbr-9.1* and *qrbr-12.1*) occurred at the same locations as the four blast resistance QTL mapped in a Nipponbare x Owarihatamochi cross (Fukuoka and Okuno, 2001). One of those four QTL (*qrbr-4.1*) occurred at the same interval as a recessive *R* gene (*pi-21*). The *qrbr-4.1* QTL was also identified in a Zhong 156 x Gumei 2 cross (Wu *et al.* 2005). Four QTL, *qrbr-1.1*, *qrbr-11.1*, *qrbr-2.2* and *qrbr-3.1* coincided with four QTL mapped in a cross between Zhong 156 and Gumei 2 (Wu *et al.* 2005).

The genes controlling these resistances could be identical or allelic to those controlling the QTL in these other crosses, thus supporting the idea that at least some of them represent a real resistance QTL from the OL5 and Fanny lines. Most of the experiment- specific QTL in the present study were not significant in both experiments even when the statistical criterion was relaxed (e.g. $P < 0.01$ to $P< 0.05$). For example, QTL *qrbr-1.2* had highly significant effects on resistance to FL440 and Caribe-8 in experiment 1 but an LOD score of less than 1.0 in experiment 2. When the combined effects of other QTL with larger effects account for the large proportion of the genotypic

variance, it can be difficult to detect additional QTL with small effects. Map coverage with genetic markers was thorough in both experiments, so this should not have had much of an effect on QTL detection. Another possible reason for inconsistent QTL detection across experiments could also be the different environmental conditions under which experiments were conducted. Different resistant mechanisms could be affected by different environmental conditions.

Since blast resistance in many rice cultivars is short-lived, recent genetic analyses have focused on resistances that appear to have relatively durable or broad-spectrum effects (Wang *et al.* 1994; Tabien, *et al* 2000; Jeon *et al.* 2003; Correa-Victoria *et al.* 2004; Wu *et al.* 2005). The molecular basis of this resistance is not well understood but genetic evidence indicates it may be controlled by successful combinations of genes with large race-specific effects or combinations of many genes with minor effects (Johnson 1981; Wang *et al*. 1994; Jeon *et al*. 2003). Race specific genes like *Pi-1*, *Pi-2* and *Pi-9*, *Pi-z* have been recognized to confer resistance to many isolates of the blast fungus from different geographical regions (Chen *et al.* 1996; Liu *et al.* 2002). These genes have been identified in cultivars whose resistance remained effective in the regions they were grown, like the African cultivar Moroberekan (Wang *et al*. 1994; Inukai *et al*. 1996), the Korean cultivar Suweon 365 (Ahn *et al*. 2000) and the Chinese cultivar Sanhuangzhan 2 (Liu *et al.* 2004). The molecular bases of Colombian rice cultivars conferring resistance to blast have not been documented. The OL5 cultivar was developed by combining resistances from several cultivars with different sources of resistance and selecting for high levels of resistance to different genetic lineages of the pathogen. The presence of multiple genes with large effects made thorough characterization of all the QTL difficult, and the use of multiple blast isolates was a critical component of the analysis. It is difficult to determine whether some of the QTL represent *Pi*-type genes with race- specific effects or genes with potential nonspecific

effects. Because OL5 carries so many resistance genes, there are typically several genes conferring resistance to each of the isolates. The presence of genes conferring large resistance effects to a given isolate obscures the effects of genes with smaller effects and may even make them impossible to detect.

A nonspecific QTL with moderate affects may not show a statistically significant contribution to resistance in a population challenged with a blast isolate to which multiple genes with major effects were functioning. The blast isolate FL440 was selected for partial virulence on OL5 in an attempt to identify an isolate to which the genes with major race specific effects would not function. The QTL identified with this isolate had modest effects with none of them accounting for more than 17% of the genetic variation when averaged over the two experiments. Two of the three QTL that were significant in both experiments to isolate FL440 isolate mapped to regions where *Pi* genes have previously been mapped while no *Pi* genes have been mapped to the chromosome 9 locus. The resistance effects of these genes seems relatively small for major race-specific *Pi* genes, but if their resistance is controlled by typical *R*-gene like sequences they may simply have small race-specific effects or possibly residual effects that are nonspecific. The effects of these genes should be tested with multiple isolates in lines where they are isolated from the other QTL. Identification of genes with nonspecific effects would provide useful breeding tools even if the effect of each individual locus is small.

The genes in each of the QTL regions that have been predicted from analyses of the rice genome sequence provide possible candidates for the genes underlying these traits. A preliminary examination of these genes might shed light on whether the genes underlying specific QTL are race specific or potentially nonspecific. When the three genomic regions conferring resistance to isolate FL440 in both experiments were examined, NBS-LRR genes were found in all three regions, leading the idea that *R* genes would be involve in the QTL observed. In fact the *qrbr-11.3* region contains 23

NBS-LRR in a genomic region of ~ 1.8 Mb and several race specific *Pi* and *Xa* (bacterial blight resistance) genes also map to this region. The NBS-LRR genes are known for their race specific effects on resistance, although some NBS-LRR genes or gene clusters may also have nonspecific effects. On the other hand, if any of these loci actually do have nonspecific effects, there are also other predicted genes that could account for the effects. These include an oxalate-oxidase gene and a family of diterpene cyclase encoding genes at the chromosome 4 QTL, Thaumatine like protein encoding genes at the chromosome 9 QTL and a JAMyb transcription factor at the chromosome 11 QTL. Transcription of the latter gene was even shown to be up-regulated after blast infection in microarrays experiments (Gloria Mosquera, personal communication). Further genetic studies in those regions are needed to determine if any of these QTL is nonspecific and correspond to the genes that control these resistance effects.

CONCLUSIONS

The large numbers of QTL identified conferring blast resistance demonstrate the durability of OL5 is due to many genes with variable effects. Eight of the QTL were identified in both experiments while others were experiment specific. Many of the experiment specific QTL, however, corresponded to positions of previously mapped QTL, lending credibility to their existence in OL5. The QTL with minor resistance effects to isolate FL440 could be nonspecific, since they had small effects and some mapped to regions with no *Pi* genes.

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CHAPTER 3

ADVANCED BACKCROSS QTL ANALYSIS TO MAP GENES INVOLVED IN RESISTANCE TO RICE BLAST

ABSTRACT

An advanced backcross strategy with marker-assisted selection for donor alleles in QTL regions was used to generate five BC_2F_3 populations carrying five different target regions associated with partial resistance to rice blast disease. Three of five of these populations were analyzed for segregation for resistance to the *M. grisea* isolate FL440. One QTL designated *qrbr-11.3* near the bottom of rice chromosome 11 was found to be significantly associated with partial blast resistance in 120 lines of a BC_2F_3 population ($P< 0.01$). This QTL accounted for 12.4% and 8.0% of the phenotypic variation in disease leaf area (DLA) and lesion type (LT) observed under greenhouse inoculation with blast isolate FL440, indicating that both traits are controlled by the same gene. Analysis of the *qrbr-11.3* locus based on genomic sequence of the corresponding region of the reference *japonica* cv. Nipponbare, show that 29 candidate genes are present at that locus (~1.8 Mb), 27 of which are predicted NBS-LRR genes. Two other QTL, *qrbr-9.2* and *qrbr-1.2*, were not found to be significantly associated with resistance in their corresponding advance backcross mapping populations, suggesting that those QTL effects are difficult to reproduce because their resistance effects are small or because the environment plays an important role in their expression.

INTRODUCTION

Disease resistant crops have been produced through traditional breeding methods for many years, but many varieties have failed due to their rapid loss of resistance. The use of molecular approaches has allowed genes conferring disease resistance to be analyzed at a more detailed level. Molecular approaches have been applied to examine the complexity of plant defense mechanisms and host–pathogen recognition. This knowledge has led to better use of marker-assisted selection (MAS) to refine breeding strategies.

In the last few years, significant advances have been made in understanding the molecular basis of disease resistance in agronomically important species. However, there are still many aspects that need to be explored. In most studies of disease resistance, the focus has been on single, dominant genes with “Mendelian” inheritance because they are easy to genetically manipulate and map in a suitable segregating population. Flor’s "gene-for-gene" hypothesis states that a resistance gene in the plant has a corresponding avirulence gene in the pathogen (Flor, 1971). In some cases, plant resistance proteins interact directly with avirulence proteins from the pathogen (Tang *et al*. 1996; Jia *et al.* 2000), leading to recognition and a hypersensitive resistance response. Studies on crop plant-pathogen systems have revealed that when breeders introduce varieties carrying such resistance genes, pathogen isolates often arise that lose the protein that is recognized. Once the pathogen population shifts to this new form, the resistance ‘breaks down’ because the resistance gene is no longer effective (Ohtsuki and Sasaki, 2005).

Some resistances to plant pathogens are believed to be polygenic and quantitative. This type of resistance is complicated and poorly understood. Quantitative resistance is believed to be controlled by many genes that individually contribute a small

effect on the phenotype and resistance effectiveness is expressed on a continuous scale in a segregating population. Multiple efforts to understand the molecular bases of these complex mechanisms are underway using QTL analysis and map-based cloning approaches (Ayliffe and Lagudah, 2004).

The use of molecular markers now provides the ability to understand the genetic basis of quantitative traits. Molecular markers and genetic maps allow one to detect quantitative trait loci (QTL) controlling a wide variety of traits, including, yield and yield components, aluminum tolerance, and also resistance to pathogens. They also offer a complementary approach to breeding, since selection can be based on genotype. Combining breeding methods and QTL mapping approaches allows partial resistance loci to be manipulated like major genes. Marker-assisted selection can be used to manipulate each target QTL in various breeding strategies, like pedigree or backcross breeding (Steele *et al.* 2005). Thus, the genetic markers can provide a way to successfully target *R* genes present in highly resistant lines, to be used in breeding programs for isolating those beneficial genes that are often lost when traditional methods of selection are used. Marker assisted selection in plant breeding programs is usually a two-stage process. In the first stage a QTL analysis is performed. The parental lines which differ for at least one quantitative trait are crossed to develop a segregating population such as an F_2, recombinant inbred lines (RIL) or double haploid (DH) population in which molecular markers are used to identified linked QTL. The second stage is to take advantage of these QTL and use them in a breeding program to develop a superior variety. However, QTL discovery and variety development utilizing those QTL are both time consuming processes.

Advance backcross QTL analysis (AB-QTL analysis), was proposed as a method for the simultaneous discovery and transfer of valuable wild germplasm into elite

cultivars (Tanskley and Nelson, 1996). The process is similar to marker-assisted back-crossing (MABC; Steele et al. 2005). This approach integrates the process of marker-based QTL detection with variety improvement while exploiting the potential of the natural genetic variation existing in adapted germplasm. In this method, BC_2 or BC_3 populations are derived from an intercross allowing the identification and mapping of valuable donor QTL alleles. Thus, QTL are at once transferred to lines with the genetic background of the adapted parent, which are then tested.

By using the AB-QTL method, the breeder can accelerate crop improvement by allowing rapid development of near isogenic lines (NILs) containing the QTLs of interest, derived directly from the advanced backcross population where the positive QTL were identified (Goodstal *et al.* 2005). Superior lines can be developed from those NILs, where most of the genome is the same as the elite recurrent parent. AB-QTL has been employed in QTL studies of tomato (Frary *et al*. 2004; Foolad *et al*. 2002), and rice (Li *et al.* 2004; Steele *et al.* 2005).

The highly resistant indica rice cultivar OL5 was released by CIAT breeders in Colombia in 1989. Its resistance is still effective, not only in Colombia but also around the world, where it has been tested with hundreds of isolates of the blast fungus (Correa-Victoria, personal communication). The previous chapter described a QTL mapping approach that was used to identify several QTL that each affected resistance to one or more of eight isolates of the blast fungus (*M. grisea*). Most of the loci mapped to regions of known *Pi* genes, particularly on chromosomes 6, 8, 11 and 12. They were effective against several isolates and for some resistant traits the loci accounted for more than half of the genetic variation in the population. For isolates like FL440, which was the only one of the eight used that caused lesions on the resistant OL5 parent, six putative QTL were identified that affected resistance (Fig. 3-1). Only three of the QTL, on

chromosomes 4, 9 and 11, were statistically significant in two different experiments. The effects of these QTL were modest, with no R^2 values above 20% in either experiment. The other three loci were experiment-specific, but one of them, *qrbr-1.2* on chromosome 1 explained 17.6% of DLA and 12.9% of LT in experiment 1 and occurred in the same interval as another experiment specific QTL for the Caribe-8 isolate. Two of the putative QTL, *qrbr-9.2* and *qrbr-1.2,* affecting resistance to the FL440 isolate, mapped to regions on chromosomes 9 and 1 where no major race-specific genes had yet been reported; but QTL for blast resistance have been reported (Tabien *et al.* 2002, Chen *et al.* 2003). The QTL *qrbr-11.3* mapped near the bottom of chromosome 11 and could therefore correspond to *Pi-sh* or *Pi-k* if these genes have a relatively small effect on resistance to FL440, or could be another gene in this region. It is therefore possible that the genes conferring resistance to FL440 are defeated *R* genes with residual effects. Alternatively, they may be other types of genes with small effects on resistance and these effects could, in theory, be nonspecific. To characterize the specificity of their effects they need to be isolated individually into lines with homogeneous backgrounds and tested with different blast isolates. An AB-QTL approach would be useful for this purpose.

In the present study, an AB-QTL approach combined with marker-assisted selection was used to target five segments of four chromosomes for introgression and further fine mapping. All five segments were predicted to carry QTL for partial resistance to blast disease (disease leaf area and lesion type). BC_2F_3 populations were developed from an inter-specific cross between OL5, a worldwide recognized, highly resistant indica rice cultivar and Fanny, a highly susceptible *japonica* accession. I describe the selection in two backcross (BC) generations and three rounds of self-fertilization using MABC after challenge with the FL440 blast isolate. Ultimately, the information from this study can be integrated into the development of improved lines with OL5-derived QTL for resistance.

The objectives for this study were:

1. To generate advanced lines using an AB-QTL strategy with marker-assisted selection that can be used for dissecting QTL that confer resistance to the FL440 blast isolate.
2. To validate the *qrbr-1.2*, *qrbr-9.2*, *qrbr-11.3* QTL in BC_2F_3 families.
3. To identify candidate genes in the five targets QTL regions using the rice genome sequence database.

MATERIALS AND METHODS

BACKCROSSING AND SELECTION

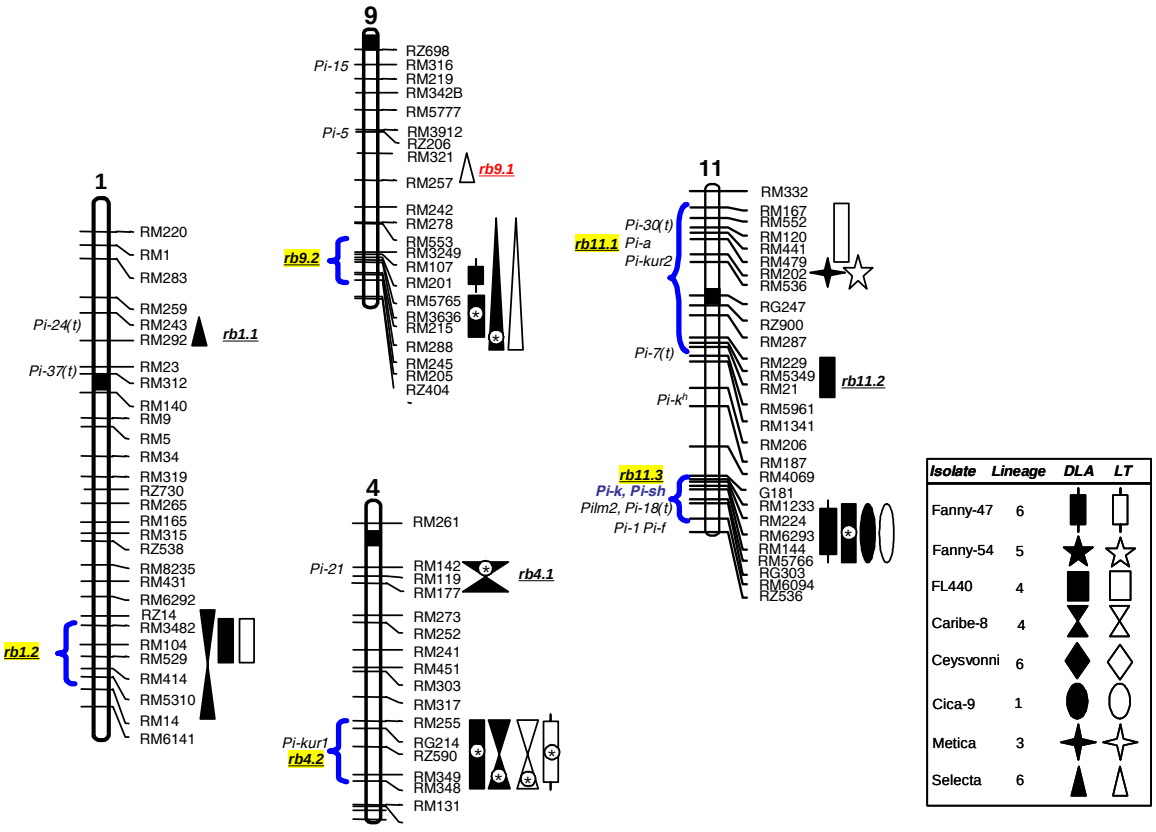
Five chromosomal regions with potential effects on resistance to isolate FL440 were selected for introgression and further analysis using an AB-QTL strategy. The five target chromosome segments with QTL from OL5 are designated *qrbr-1.2*, *qrbr-4.2*, *qrbr-9.2*, *qrbr-11.1* and *qrbr-11.3* (Fig. 3-1) and were estimated to carry genes for resistance to the blast fungus as indicated by QTL analysis (Chapter 2).

Population development was carried out at Kansas State University (KSU), Manhattan, Kansas, USA and the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia, beginning with a cross between the cultivar Fanny as the female parent and OL5 as the male parent (Fig. 3-2). The recurrent parent in this study was Fanny, a highly susceptible rice cultivar. Fanny was chosen for this study because it is highly susceptible and lacks any known major resistance genes for blast. The donor parent was the indica rice cultivar OL5. It has a high level of disease resistance against hundreds of isolates from *M. grisea* around the world and its resistance is still effective today and therefore provides a starting point for further improvement. All the inoculation assays with the FL440 isolate of *M. grisea* and the second BC were carried out at CIAT. The first BC and all of the genetic characterization was carried out at KSU.

A MAS approach was carried out using simple sequence repeat (SSR or microsatellite) markers for introgression of OL5 alleles in the five target segments and to lose these alleles in several non-target regions. In the first backcross, selection was made with SSR that had previously been mapped in the Fanny /OL5 population and laid within the region containing the target QTL (Fig. 3-1). When RFLP markers were used in the previous mapping study, closely linked SSR markers were selected to replace them by comparing their positions on the genetic or physical rice maps

(http://www.gramene.org). SSR analysis was performed as described in Chapter 2. Individuals that were heterozygous at alleles in the target segment were selected.

Figure 3-1 Rice chromosomes 1, 4, 9 and 11 showing the five QTL regions (blue) that conferred partial blast resistance to isolate FL440 of *M. grisea*. Other isolates affected by the same locus are also shown. The *Pi* genes mapped in previous studies are indicated at the left of the chromosome.

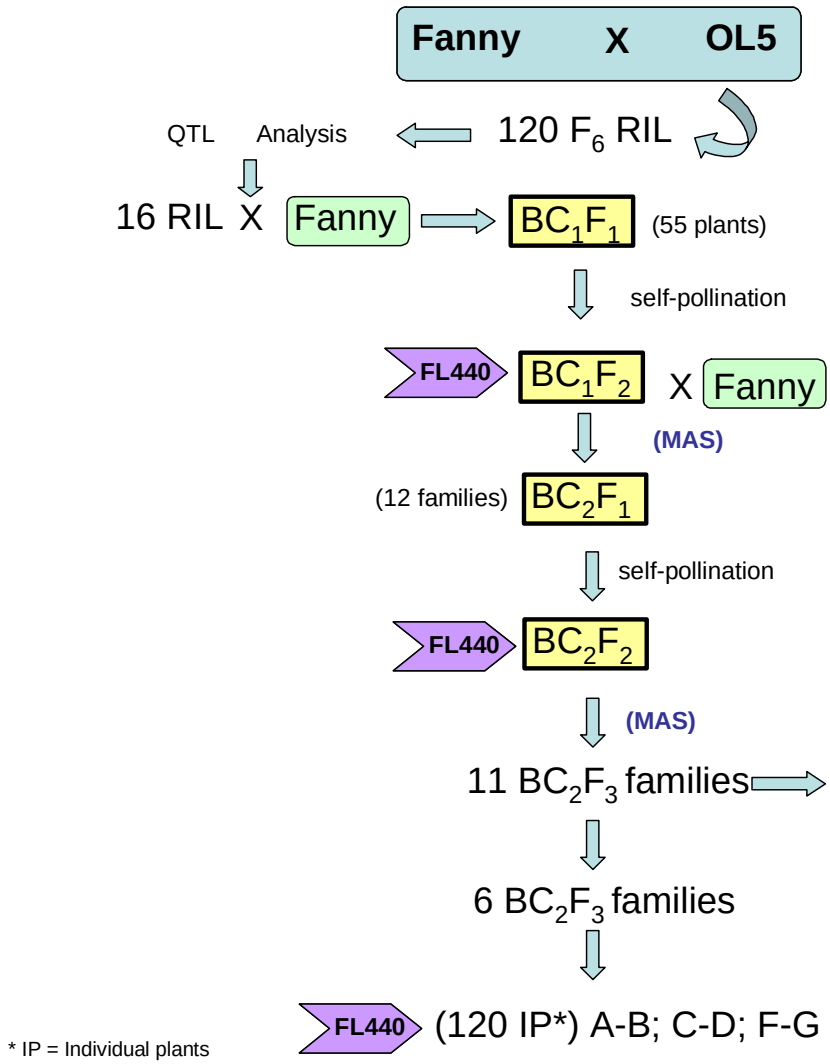


The AB-QTL strategy began with selection of sixteen of 120 F_6 RIL progeny chosen as carrying OL5 alleles in each of the five QTL regions and then backcrossed to Fanny (Table 3-1). About 10 seeds from each of these 16 initial RIL as well as seeds from Fanny (used as recurrent parent) were planted into 25 cm pots containing a greenhouse soil mixture in the summer of 2003 at KSU. When flowering coincided, emasculation of the Fanny plants was done by removing caryopses with scissors to expose the anthers and the stigma. An aspirator attached to a suction pump was used to suck the anthers from the flowers and also passed over the stigmas to suck away any pollen grains present on their surface. Pollen from each of the sixteen individual plants was shaken into different Fanny panicles to facilitate pollination by dispersing the pollen from the male (RIL) to the female parent (Fanny). Panicles containing emasculated flowers were covered with paper bags to prevent contaminating cross-pollination. Plants were maintained in a greenhouse with a day/night temperature set at 29/21°C.

Plants resulting from this first BC (BC_1) were self fertilized at KSU to produce BC_1F_2 families which were sent to CIAT. A total of 55 BC_1F_2 families were planted in the greenhouse at CIAT and inoculated with isolate FL440 21 days after planting (Fig. 3-2). The families were scored for resistance seven days after inoculation and any plants that were noticeably more resistant than the Fanny control plants were considered resistant. Plants that appeared more resistant than the control Fanny from 12 BC_1F_2 families, that carried OL5 alleles at the target locus, were backcrossed a second time to Fanny to generate BC_2 plants in the fall of 2003 at CIAT. Plants resulting from this second BC (BC_2) were planted in the greenhouse at CIAT, grown to maturity, and then self-fertilized to generate BC_2F_2 families, which were inoculated with FL440. From these, a subset of 11 BC_2F_2 families, designated families A to K (Table 3-2), were selected based on the same criteria as the BC_1 population. A total of 3900 individual seed derived from these eleven BC_2F_2 families were sown into 4-inch pots containing a greenhouse soil mixture

at KSU in the summer of 2005. Some of these 11 BC_2F_2 families derived from the same BC_1F_2 plant were combined for use in QTL mapping. For example, population AB was derived from pooling the A and B families, both of which segregated for locus *qrbr-1.2*; populations C, D and E, all segregated for *qrbr-11.3*; families F and G segregated for *qrbr-9.2*; families H, I and J all segregated for *qrbr-11.1* and family K segregated for *qrbr-4.2*. Finally, a subset of 120 random plants derived from the AB, CD and FG BC_2F_2 families was used for SSR marker analysis and BC_2F_3 families derived from those BC_2F_2 plants were sent to CIAT for inoculation and evaluation in fall of 2005.

Figure 3-2 Schematic representation of the backcrossing strategy used to transfer the OL5 alleles at the five putative QTL carrying regions for fine mapping of the QTL. MAS was employed in both $BC_{(n)}$ F2 generations to select the most desirable genotypes.



	BC2F2	# Seed
A	126 A-1-13	450
B	126 A-1-7	231
C	107 A-24-13	387
D	107 A-24-21	345
E	107 A-24-18	72
F	76 C-2-9	420
G	76 C-21-8	510
H	76 C-22-13	420
I	76 C-22-17	270
J	76 C-2-2	366
K	107 C-2-14	330

Table 3-1 Selection of sixteen lines from the experiment 1 RIL family as carrying OL5 alleles based on SSR markers across important regions of the rice chromosomes. The chromosome number and the segment with OL5 alleles are highlighted.

		RIL															
RR	**Chr**	**5**	**11**	**25**	**30**	**41**	**60**	**62**	**76**	**87**	**91**	**92**	**105**	**107**	**110**	**124**	**126**
RM246	1	S	S	R	S	S	R	R	S	R	R	R	R	S	S	S	S
RM34	1	S	S	S	S	S	S	R	S	S	R	R	R	R	R	S	R
RM106	2	S	S	R	R	S	S	S	R	R	S	S	S	R	R	R	R
RM263	2	S	R	R	R	S	R	S	S	R	S	S	S	R	R	R	R
RM130	3	S	S	S	R	S	S	R	S	R	S	R	S	R	S	S	S
RM148	3	S	R	S	S	S	R	R	R	R	S	R	R	R	S	S	R
RM203	3	R	S	S	S	R	R	S	R	R	R	R	S	S	R	S	-
RM85	3	S	S	S	S	S	R	R	R	R	S	R	S	R	S	S	-
RM124	4	S	S	R	S	S	R	R	S	R	S	R	-	R	R	R	S
RM131	4	S	S	R	S	S	R	R	S	S	S	R	S	R	R	R	S
RM255	4	-	S	R	S	R	R	R	R	-	R	R	S	R	S	S	S
RM280	4	S	S	R	S	S	R	R	S	R	S	R	S	R	R	R	S
RM303C	4	R	R	R	R	S	R	R	R	R	S	R	S	R	S	R	R
RM348	4	S	S	R	S	R	R	R	S	S	S	R	S	R	S	S	S
RM349	4	S	S	R	S	R	R	R	S	S	S	R	R	R	S	S	S
RM136	6	S	S	S	S	S	S	R	-	S	R	S	R	S	-	S	S
RM238B	6	S	S	S	S	S	S	R	R	S	R	S	S	R	R	R	R
RM276	6	S	S	S	R	S	R	R	R	R	R	R	R	S	R	S	R
RM3	6	S	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R
RM50	6	-	S	S	R	S	R	R	R	R	R	R	R	R	R	R	R
RM38	8	S	S	S	S	S	S	R	S	R	-	-	R	S	S	R	S
RM72	8	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S
RM126	8	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S
RM137A	8	R	-	S	S	S	R	R	R	R	S	-	S	-	-	R	S
RM25	8	R	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S
RM310	8	R	R	S	S	-	S	S	R	R	S	R	S	S	S	S	S
RM331	8	S	R	R	R	R	S	S	R	R	S	S	S	S	S	R	S
RM339	8	S	S	R	R	R	S	S	R	R	S	S	S	S	S	R	S
RM342A	8	S	R	R	R	R	S	S	R	R	S	S	S	R	S	R	R
RM342B	8	S	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R
RM344	8	S	R	R	R	R	S	S	R	R	S	S	S	S	S	R	S
RM350	8	S	S	R	R	R	S	S	R	R	S	S	S	R	S	R	R
RM42	8	S	S	R	R	R	S	S	R	R	S	S	S	R	S	R	R
RM44	8	S	S	R	R	R	S	S	R	R	S	S	S	S	S	R	S
RM205	9	R	R	S	S	S	S	S	R	S	S	S	S	R	R	R	S
RM215	9	S	R	S	R	S	S	S	R	S	R	S	S	R	R	R	S
RM245	9	S	R	S	S	S	S	S	R	S	S	S	S	R	R	R	S
RM244	10	S	S	S	S	R	R	R	R	S	R	R	S	R	R	S	R
G181	11	R	R	R	R	S	S	R	R	S	S	R	R	R	S	S	R
RG303	11	R	S	R	R	S	R	R	R	R	S	R	S	R	-	S	R
RM144	11	R	S	R	-	-	S	R	R	S	S	R	S	R	S	S	S
RM181	11	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RM224A	11	R	R	R	R	R	S	R	R	S	S	R	R	R	S	S	R
RM224B	11	R	S	R	R	S	S	R	R	S	S	R	S	R	S	S	R
RM224C	11	R	S	R	R	S	R	R	R	R	R	R	S	R	R	S	S
RZ536	11	R	S	R	R	S	S	R	R	S	S	R	S	R	S	S	R
RG9	12	S	R	R	R	R	S	R	S	R	S	S	S	R	S	S	S
RM101	12	S	S	R	R	R	R	R	R	R	R	S	R	R	S	S	S

Table 3-2 Eleven BC_2F_2 derived families (A to K) selected by SSR markers as segregating at five QTL regions. R^2 = percentage of the phenotypic variance to blast isolate FL440 explained by the QTL in the previous study (Chapter 2). Families segregating for the same locus are indicated by the same color.

	BC2F2	# Seed	Chr	QTL	R2 (%)	Trait	Experiment
A	126 A-1-13	450	1	qrbr-1.2	17.6	DLA	1
B	126 A-1-7	231					
C	107 A-24-13	387					
D	107 A-24-21	345	11-q	qrbr-11.3	15.1	DLA	1 and 2
E	107 A-24-18	72					
F	76 C-2-9	420	9	qrbr-9.2	9.3	DLA	1 and 2
G	76 C-21-8	510					
H	76 C-22-13	420					
I	76 C-22-17	270	11-p	qrbr-11.1	10.0	LT	1
J	76 C-2-2	366					
K	107 C-2-14	330	4	qrbr-4.2	7.8	DLA	1 and 2

PATHOGEN INOCULATION AND DISEASE SCORING

M. grisea isolate FL440 was used to inoculate lines derived from BC_1F_2, BC_2F_2 and BC_2F_3 families. Fanny and OL5 were also inoculate with FL440 and used as controls in the phenotypic scoring for resistance in the segregating families. Blast inoculations were carried out as described (Correa-Victoria and Zeigler, 1993) except that a concentration of $4x10^5$ spores ml^{-1} was used. The scoring of the resistance traits, DLA and LT, were evaluated in the subset of 120 random plants derived from the AB, CD and FG BC_2F_2 families based on methods of Correa-Victoria and Zeigler, (1993). Because of the large number of lines and limited space in the greenhouse at CIAT, families E, H, I, J and K were not evaluated.

MOLECULAR MARKERS AND QTL ANALYSIS

The molecular marker scoring was preformed as described in Chapter 2. QTL were identified using interval mapping (IM) and composite interval mapping (CIM). The

primary analysis using IM was performed using QGENE (Nelson, 1997). The QTL detected by IM and CIM corresponded well (data not shown). Therefore only the data from IM are presented. Parameters for CIM, from QTL Cartographer (Wang *et al*. 2005), were as used in Chapter 2. To identify the significance threshold for each trait, an empirical threshold was determined by permutation for IM and CIM using 1000 permutations for both traits in each chromosome (Doerge and Rebai 1996). For IM, the experiment-wise significance level of $P < 0.01$ corresponded to an average LOD > 2.39 across the traits, while the level of $P < 0.05$ corresponded to a LOD > 1.79. For CIM the experiment-wise level of $P < 0.01$ corresponded to an average LOD > 2.44, while the level of $P < 0.05$ corresponded to a LOD > 1.78. A putative QTL was reported if detected in at least at an experiment-wise significant threshold of $P < 0.05$.

RESULTS

SSR MARKERS AND PHENOTYPIC SELECTION

A total of 36 SSR markers that detected polymorphism between Fanny, the recurrent parent and OL5, the donor parent, were used to genotype 22 BC_1F_2 families in the five targets and several non-target QTL regions (Fig. 3-3 and Fig 3.4). The non-target regions were those regions (chromosomes 6, 8, 12) where QTL with major effects were also found for isolates other than FL440. Families that carried OL5 alleles in the five QTL regions and that also appeared to be segregating for resistance were selected. A total of 12 BC_1F_2 families were selected and resistant plants from each of these families were backcrossed again to Fanny. The resulting BC_2F_1 progeny were grown to maturity and self-fertilized to produce BC_2F_2 families. A total of 36 BC_2F_2 families that appeared to segregate for resistance to FL440 were analyzed for OL5 alleles at target QTL. Eleven of those 36 families were selected. As mentioned in Material and Methods, they were designated families A to K (Fig. 3-5; Table. 3-2).

Figure 3-3 SSR marker analysis in 22 BC_1F_2 derived families for target and non-target QTL regions. A total of 12 families carrying OL5 alleles that also were segregating for resistances to isolate FL440 were selected (pink box). Blue boxes indicate OL5 alleles were detected for that SSR.

BC_1F_2 SSR analysis for heterozygous Fanny/OL5 alleles

		Chr 9						Chr 11							
	BC2F1	Locus 1			Locus 2			Locus 1				Locus 2			
KSU	CIAT	RM219	RM3912	RM524	RM242	RM107	RM3249	RM202	RM6897	RM21	RM5961	RM1233	RM224	RM5766	RM6094
1	126A-1														
2	126A-11														
3	126A-21														
4	126B-12														
5	126B-21														
6	126B-22														
7	126C-1														
8	126C-21														
9	126E*11														
10	107-2														
11	107A-24														
12	107B-11														
13	107C-2														
14	107C-21														
15	107C-22														
16	107D-21														
17	110DX-1-21														
18	76C-1														
19	76C-2														
20	76C-3														
21	76C-21														
22	76C-22														

		Chr 4						Chr 1				Chr 5		
		Locus 1			Locus 2			Locus 1				Locus 1		
KSU	CIAT	RM3836	RM348	RM6748	RM131	RM280	RM5608	RM431	RM3482	RM14	RM5310	RM26	RM274	RM31
1	126A-1	Line 1			Line 1									
2	126A-11	Line2			Line2									
3	126A-21	Line 3			Line 3									
4	126B-12	Line 4			Line 4									
5	126B-21	Line 5			Line 5									
6	126B-22	Line 6			Line 6									
7	126C-1	Line 7			Line 7									
8	126C-21	Line 8			Line 8									
9	126E*11	Line 9			Line 9									
10	107-2							Line 10						
11	107A-24							Line 11						
12	107B-11							Line 12						
13	107C-2							Line 13						
14	107C-21							Line 14						
15	107C-22							Line 15						
16	107D-21							Line 16						
17	110DX-1-21							Line 17						
18	76C-1							Line 18						
19	76C-2							Line 19						
20	76C-3							Line 20						
21	76C-21							Line 21						
22	76C-22							Line 22						

		Chr 2			Chr 6			Chr 12		
		Locus 1			Locus 1			Loci 1		
KSU	CIAT	RM236	RM203	RM8	RM527	RM557	RM136	RM247	RM512	RM313
1	126A-1									
2	126A-11									
3	126A-21									
4	126B-12									
5	126B-21									
6	126B-22									
7	126C-1									
8	126C-21									
9	126E*11									
10	107-2	Line 10								
11	107A-24	Line 11								
12	107B-11	Line 12								
13	107C-2	Line 13								
14	107C-21	Line 14								
15	107C-22	Line 15								
16	107D-21	Line 16								
17	110DX-1-21	Line 17								
18	76C-1	Line 18								
19	76C-2	Line 19								
20	76C-3	Line 20								
21	76C-21	Line 21								
22	76C-22	Line 22								

Figure 3-4 Screening for OL5 alleles in 22 BC_1F_2 families using rice SSR markers. Thirty six primer pairs were used to PCR amplify DNA from Fanny, OL5 and the 22 families. Only six rice SSR markers are shown. Arrows indicate the Fanny and OL5 alleles (bands).

SSR analysis in 22 BC_1F_2 families

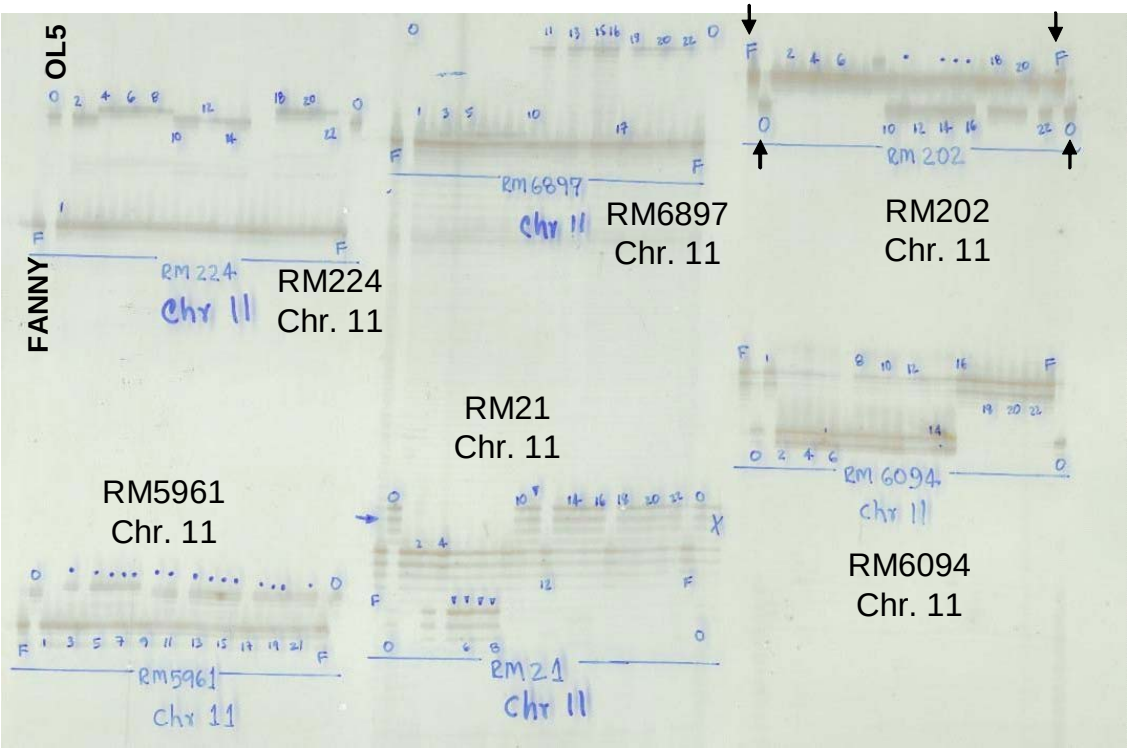


Figure 3-5. Eleven BC_2F_2 families (A to K) selected with SSR markers as carrying OL5 alleles and also segregating for resistance to isolate FL440. Colored boxes indicate the presence of OL5 alleles. Families carrying the same locus are indicated with identical letter codes and highlighted with the same color.

		Chr 1 *(rb1.2)*					Chr 9 *(rb9.2)*				
	BC2F2	RM431	3810	RM3482	RM14	RM6141	RM242	RM3249	RM3636	RM1026	RM6707
A	126 A-1-13	A	A	A							
B	126 A-1-7	B	B	B	B						
C	107 A-24-13										
D	107 A-24-21										
E	107 A-24-18										
F	76 C-2-9						F		F	F	
G	76 C-21-8						G	G	G	G	
H	76 C-22-13										
I	76 C-22-2										
J	76 C-22-17										
K	107 C-2-14										

		Chr 11									Chr 4 *(rb4.2)*				
		rb11.1					*rb11.3*								
	BC2F2	RM167	RM441	RM479	RM202	RM21	RM1233	RM224	RM5766	RM6094	RM3836	RM348	RM6748	RM131	RM5608
A	126 A-1-13														
B	126 A-1-7														
C	107 A-24-13						C	C	C	C					
D	107 A-24-21						D	D	D	D					
E	107 A-24-18						E	E	E	E					
F	76 C-2-9														
G	76 C-21-8														
H	76 C-22-13	H		H	H	H									
I	76 C-22-2	I		I	I	I									
J	76 C-22-17	J	J	J	J										
K	107 C-2-14											K	K	K	K

Partial resistance was evaluated in the A, B, C, D, F and G BC_2F_3 families by estimating percentage DLA and lesion type against the rice blast isolate FL440. The donor parent, OL5, showed good, but not complete, resistance to FL440 while Fanny, the recurrent parent, was susceptible. None of the 120 lines tested in each of the three families showed complete resistance to FL440. Significant differences in DLA and LT were observed among the families. Distribution of percentage of DLA and LT are shown in (Fig. 3-6). Among these traits, DLA showed the widest range of variation. In contrast lesion type showed a distribution that was skewed towards the LT scores of 2 to 4. In general, a high percentage of individuals with DLA between 0% and 20% among the three populations were observed. When considering lesion type, values of 0 or 1 were not observed, less that 10 families showed average LT of 2, and most of them had values of 3 and 4. The lesion type scores indicated that the families were highly susceptible to FL440. It is important to note, however, that the A and B families were the most susceptible with LT scores of 4, but their DLA values were typically in the range of 0 to 10% and 10-20%. Under the inoculation conditions used, the fungus was therefore able to produce large lesions type 4 but the DLA scores were below 20 % (Fig. 3-7).

Figure 3-6 Frequency distributions of average DLA and LT of the combined AB, CD, and FG BC_2F_3 populations inoculated with blast isolate FL440. The arrows indicated the average scores in the parental cultivars.

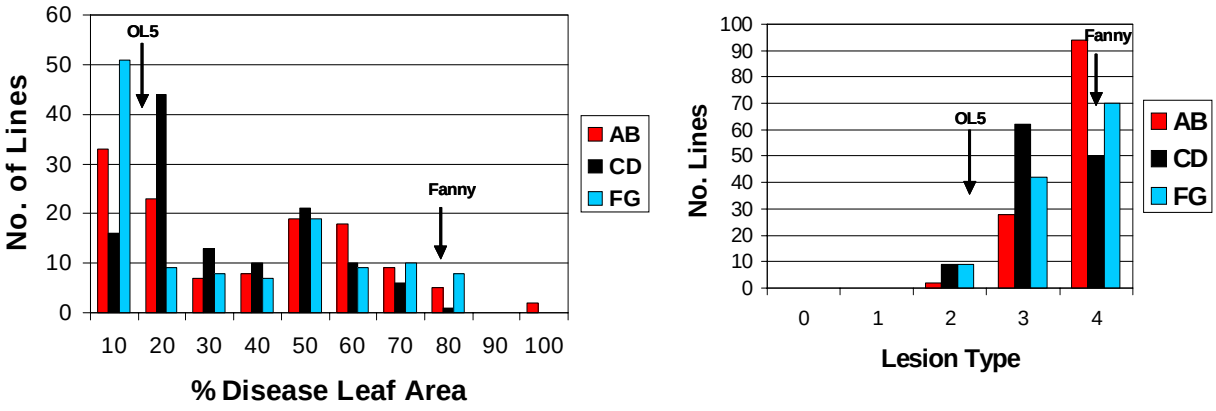
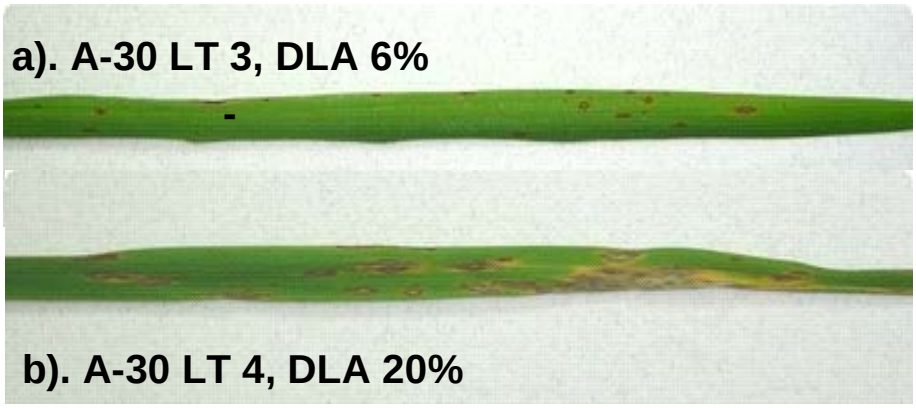


Figure 3-7 Lesion type and DLA in two seedling leaves from a single plant from the A-30 BC_2F_3 family after inoculation with blast isolate FL440. (a). Lesion type scores of 3 and DLA of 6%. (b). Scores of LT of 4 and DLA of 20%. The average score for the entire family was LT of 3 and DLA of 20%.



MAPPING SSR MARKERS ON BC_2F_2 FAMILIES

The genetic maps resulting from the segregation of 120 progeny from each of the five selected BC_2F_2 populations (AB, CD, FG, HI and K) using SSR markers are shown in Fig. 3-8. Four of the five populations were made by combining two families derived from the same BC_2F_1 families which were predicted to carry the same QTL as follows: population AB included families A and B and both segregated for *qrbr-1.2*; population CD included families C and D segregating for *qrbr-11.3*; population FG included families F and G segregating for *qrbr-9.2*, population HI included families H and I segregating for *qrbr-11.1* and family K segregated for *qrbr-4.2*. The families J and E were not included in the mapping study because they carry the same QTL as the HI and CD populations, respectively. Thus, 120 progenies from population AB were analyzed with six SSR markers covering the *qrbr-1.2* QTL on chromosome 1. These markers covered a region of ~13 cM. A total of five SSR markers covering ~ 8 cM of the *qrbr-11.3* QTL were mapped in the CD population. Seven SSR markers covering ~ 14 cM around the *qrbr-9.2* QTL were mapped in population FG. Five SSR covering the QTL *qrbr-11.1* were mapped using the HI population and finally six SSR markers were mapped around the QTL *qrbr-4.2* using the K population. For all of the SSR markers evaluated in the BC_2F_2 families, the three expected genotypes were observed (Fig 3-9) and their segregation ratios were approximately of 1:2:1 as expected (data not shown).

Figure 3-8. Genetic map of the five chromosomal regions derived from the BC_2F_2 populations, containing the five QTL.

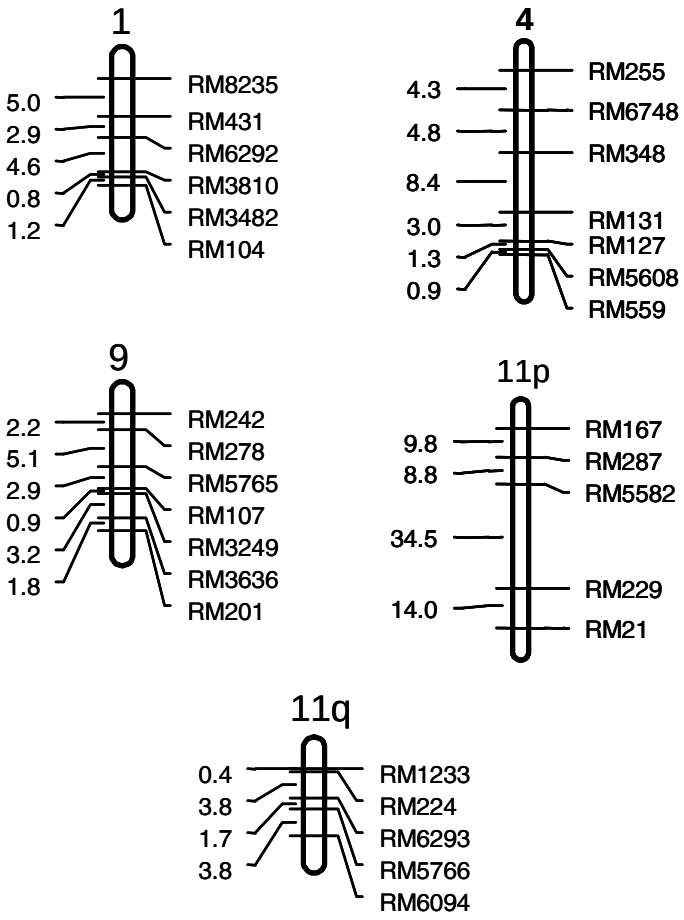
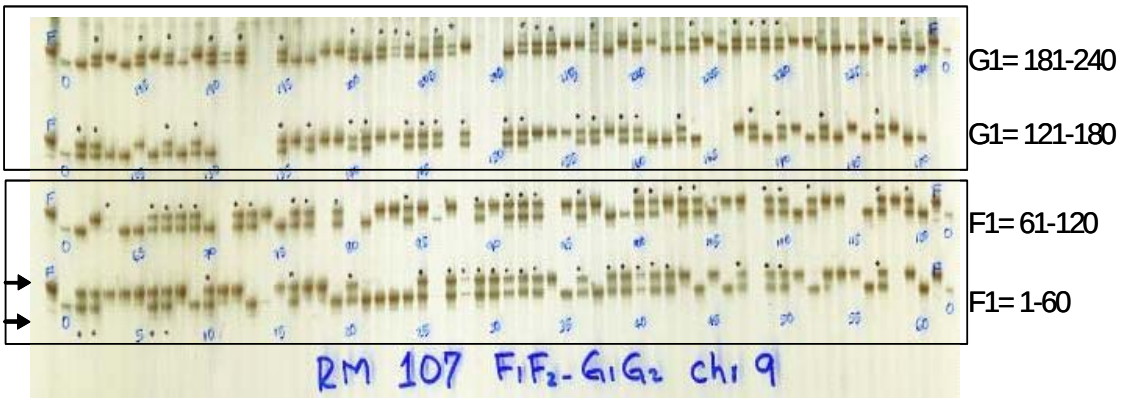


Figure 3-9. PCR products stained with silver nitrate for 120 BC_2F_2 lines from family F and 120 lines from family G amplified with the rice SSR marker RM107 near the *qrbr-9.2* QTL. The expected genetic ratios were ~1:2:1 for Fanny, Fanny/OL5 and OL5 alleles. The arrows indicated the Fanny and OL5 parents' bands.



Note: Because of the differences in flowering time for F and G families, only 120 were used for QTL mapping and segregation analysis.

QTL ANALYSIS

QTL analysis of three target regions showed that only one QTL had a statistically significant effect on the resistance traits examined. Only the chromosome 11 QTL had an LOD score above 2.4, the empirically determined significance threshold equivalent to $P < 0.01$ for interval mapping (Fig 3-10, Table 3-3) determined by a permutation test. This QTL was mapped for the resistance traits DLA and LT on chromosome 11 using the CD population and it explained 12.4% and 8.0% of the phenotypic variance, respectively. The QTL detected by IM (Q Gene) and CIM (QTL Cartographer) corresponded well (data not shown). This QTL coincided with the same location as the previously mapped QTL *qrbr-11.3*. The results confirm that the *qrbr-11.3* QTL is stable across different genetic backgrounds. Its effect is small, accounting for less than 15% of genetic variation in the BC_2F_3 families. It was surprising that it did not account for more of the variation in these families than it did among the original RIL population. Part of the reason may be due to the fact that the BC_2F_3 families were still segregating for resistance and approximately half the families were still segregating for the QTL.

No QTL could be verified in the AB and FG populations. This result was not expected for QTL *qrbr-9.2* since it was significant in both RIL populations in the previous study. Similarly, the chromosome 1 QTL (*qrbr-1.2*) represented in the AB population accounted for 17% of the phenotypic variance in one of the previous experiments, although its effect was not observed in the other experiment.

Figure 3-10 Location of the QTL *qrbr-11.3* on chromosome 11 for the blast resistance traits DLA and LT. A in the BC_2F_3 population CD above the threshold (LOD 2.4) using IM (Qgene) with FL440 blast isolate.

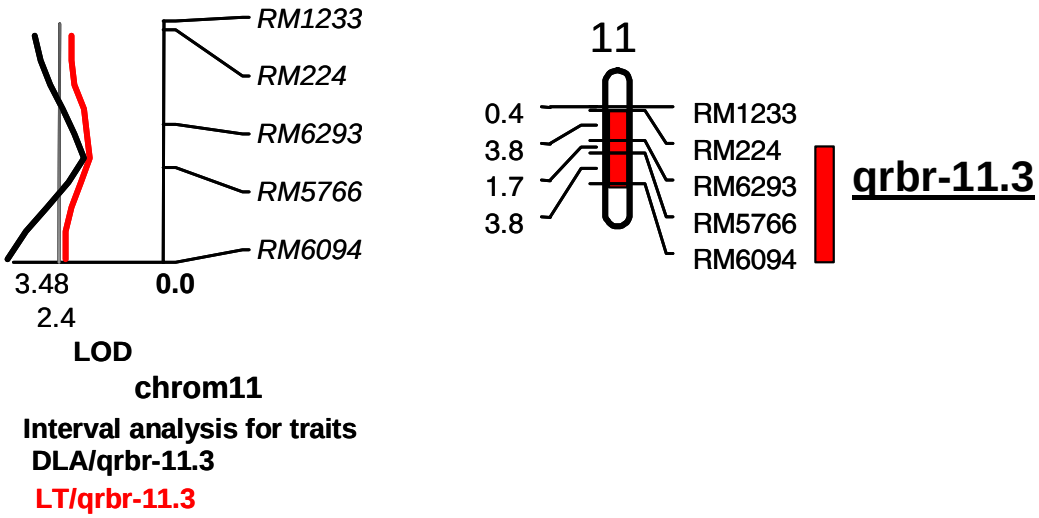
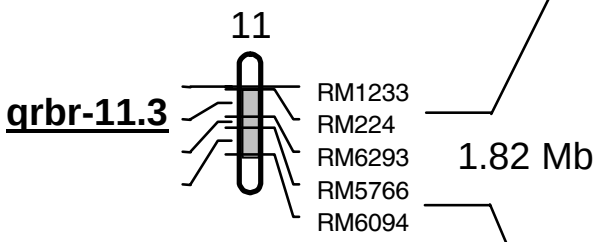


Table 3-3 Main effects QTL detected for the resistance trait DLA in population CD based on interval markers analysis. Note that three of the five SSR markers (bold) have LOD values above of the significance threshold (LOD > 2.4).

Marker	Chrom	Source	F	RSq	LOD	P	Add
RM6094	**11**	**A**	**8.36**	**0.1241**	**3.48**	**0.0004**	**10.31**
RM224	**11**	**A**	**6.82**	**0.1036**	**2.87**	**0.0016**	**10.16**
RM1233	**11**	**A**	**6.22**	**0.0954**	**2.63**	**0.0027**	**9.95**
RM6293	11	A	4.74	0.0744	2.03	0.0105	8.46
RM5766	11	A	3.99	0.0633	1.72	0.0210	7.44

Figure 3-11. Candidate NBS-LRR and defense genes in the *qrbr-11.3* locus



Marker	*Chr.*	*bp*	*Gene*	*Protein*	*QTL*
10329	11	25831137	LOC_Os11g43700	NBS-LRR	
G181	11	26132682			
10330	11	26406046	LOC_Os11g44580	NBS-LRR	
RM1233	11	26456128			
10331	11	26672305	LOC_Os11g44960	NBS-LRR	
10332	11	26684649	LOC_Os11g44970	NBS-LRR	
10333	11	26711543	LOC_Os11g45050	NBS-LRR	
RM224	11	27123770			
10334	11	26720036	LOC_Os11g45060	NBS-LRR	
10335	11	26732682	LOC_Os11g45090	NBS-LRR	
10336	11	26769392	LOC_Os11g45130	NBS-LRR	
10337	11	26785664	LOC_Os11g45160	NBS-LRR	
10338	11	26792478	LOC_Os11g45180	NBS-LRR	
10339	11	26800570	LOC_Os11g45190	NBS-LRR	
10340	11	26878226	LOC_Os11g45330	NBS-LRR	
10341	11	27056210	LOC_Os11g45620	NBS-LRR	
10863	11	27123771	LOC_Os11g45740	JAMyb Oryza sativa	
10342	11	27140738	LOC_Os11g45750	NBS-LRR	
10343	11	27141649	LOC_Os11g45760	NBS-LRR	
10344	11	27157662	LOC_Os11g45790	NBS-LRR	
10345	11	27185021	LOC_Os11g45840	NBS-LRR	
10346	11	27243159	LOC_Os11g45920	NBS-LRR	
10347	11	27244227	LOC_Os11g45930	NBS-LRR	
10348	11	27268880	LOC_Os11g45970	NBS-LRR	
10349	11	27270758	LOC_Os11g45980	NBS-LRR	
10350	11	27335825	LOC_Os11g46070	NBS-LRR	
10351	11	27370513	LOC_Os11g46130	NBS-LRR	
10352	11	27379180	LOC_Os11g46140	NBS-LRR	
10353	11	27434046	LOC_Os11g46200	NBS-LRR	
10354	11	27435146	LOC_Os11g46210	NBS-LRR	
RM5766	11	28225397			
10355	11	28169486	LOC_Os11g47780	NBS-LRR	
RM144	11	28158704			
10864	11	28179212	LOC_Os11g47810	metallothionein-like protein	
RM6094	11	28284056	117.9		

DISCUSSION

An AB-QTL strategy and marker assisted selection was used to introgress selected genomic regions at five loci carrying QTL with minor resistance effects into the blast-susceptible cultivar Fanny. One of the QTL regions, designated *qrbr-11.3* was associated with partial resistance to isolate FL440 in an advanced population. This QTL affected both DLA and LT and explained a modest amount 12.4% (DLA) and 8.0% (LT) of the phenotypic variance, indicating that the gene affects both traits. The gene(s) controlling the *qrbr-11.3* resistance could be a typical *R* gene that acts in a gene-for-gene interaction but with a very small effect on resistance. The resistance may also be the effect of a defeated major gene that is also involved in gene-for-gene interactions with other isolates, or it might be another type of defense gene. A very large number of NBS-LRR genes lie in this chromosomal region. The NBS-LRR genes are the biggest class of resistance genes and are typically involved in gene-for-gene interactions, including rice interactions with blast and bacterial blight pathogens (Chen *et al*. 2003, Ramalingam *et al.* 2003, Sun *et al*. 2003). It therefore seems likely that one, or a combination, of these may be causing the resistance effect. Certain combinations of NBS-LRR genes in the *Rp1* complex of maize are thought to provide nonspecific resistance to rust fungi (Hu *et al.* 1997). Similarly, some of the bacterial blight resistance genes (*Xa* genes) in rice provide high levels of resistance to some pathogen isolates, presumably in a gene-for-gene type of interaction, but also are thought to provide a small level of resistance to 'virulent' isolates (Li *et al.* 1999). At least five major *Pi* genes (*R* genes to blast) have been mapped at that locus including *Pi-1, Pi-k, Pi-sh, Pi-f, Pi-18(t),* (reviewed in Sallaud *et al.* 2003 and Tabien *et al.* 2002). Another type of possible candidate would be a pathogen defense-type gene, coding for a protein whose expression is induced after pathogen recognition. Although there are not many defense genes in this chromosomal region, gene predicted to encode a metallothionein-like

protein (Degenhardt *et al.* 2005) and a JA-regulated transcription factor (Lee *et al.* 2001) are present at that locus (www.tigr.org; Fig. 3-11). Blast resistance QTL affecting DLA and lesion number traits also have been mapped to this locus in previous studies (Ramalingam *et al.* 2003). Therefore, the consistencies of the results present in those studies, plus the results in this study confirm that this locus carries genes with major and minor effects on blast resistance.

The *qrbr-11.3* QTL on the bottom of the chromosome 11 is consistently detectable and presents the possibility that the minor gene responsible for the partial resistance to FL440 isolate might be molecularly characterized. Positional cloning approaches could be performed by fine mapping with all of the lines in families C, D and E, which also carry the same QTL, and by identifying additional recombinants and markers in the QTL region. Additional fine mapping experiments could determine which of the candidate genes in the region, such as the 27 NBS-LRR genes, one defense gene and one transcription factor (Fig 3-11), were the best candidates. One important question concerning the minor effects of *qrbr-11.3* (R^2 < 15%) is whether it is a race-specific or nonspecific effect. In the previous study, this QTL affected resistance to only three of the eight isolates in a statistically significant manner. However, the number of different resistance factors segregating could have obscured its effects on the other isolates. Part of the reason may be due to the fact that the BC_2F_3 families were still segregating for resistance and approximately half the families were still segregating for the QTL.

The molecular nature of the gene responsible for the minor effect observed in *qrbr-11.3* is currently unknown, but the advanced BC_2F_3 population developed in this study could be used in tests to determine its specificity. The population consists of approximately 850 lines (combining families C, D and E) and could be used for fine mapping and eventual cloning of the *qrbr-11.3* locus.

No resistance effects were associated with the other two predicted QTL regions in our advanced populations. The expression of the QTL could be affected by environment, or genetic background, or that the magnitude of its effect is sufficiently small that larger populations are needed to detect it. Only 120 families were analyzed and half of the individual families were segregating for the QTL region under analysis. If the expression of these genes is dependent on genetic background, they may require other factors from the OL5 parent for them to provide noticeable amounts of resistance. A larger sample of families, or possibly advancing the population to greater homozygosity should enhance the detection of QTL with smaller effects (Zeng, 1994). It is also possible that the QTL, especially the QTL on chromosome 1 which was only significant in one experiment, is not a real genetic effect and that the original association of the locus with resistance is due to chance.

Why those QTL are not stable across different experiments would be also important to consider in further studies. In consistency of the QTL across years using the same population have been reported previously (Talukder *et al*. 2005; Fanizza *et al*. 2005). It is not surprising that the *qrbr-1.2* was not detected in the analysis of the advanced population, since its effect was only significant in experiment 1 in the previous study of RI lines. However, other studies have also reported QTL conferring blast resistance at the same locus (Tabien *et al*. 2002, Chen *et al.* 2003) so the *qrbr-1.2* QTL probably does contribute to resistance.

Similar analyses with the FL440 isolate and the advanced BC_2F_3 populations carrying the *qrbr-4.2* and *qrbr-11.1* QTL should be conducted. These putative QTL needed to be confirmed in a large BC_2 population. The QTL *qrbr-4.2* was significant in both QTL mapping populations (Chapter 2) but it had a small effect ($R^2 < 10\%$), similar to *qrbr-11.3*. Analysis of phenotypic segregation for resistance to the FL440 blast isolate in a population carrying the *qrbr-4.2* locus is in progress.

The segregations of resistant and susceptible progenies in the different BC_2F_3 populations were incompatible with a 3:1 ratio in the three populations analyzed indicating that a single QTL could not be scored as a Mendelian factor in these populations. The development of large BC_3 populations may help to reduce other genetic variation from segregating, but it is not clear if the poor heritability of the individual QTL loci was due to the segregation of other genes in these families or due to other factors, like environmental effects.

CONCLUSIONS

The study confirms that the QTL *qrbr-11.3* confers partial resistance to the isolate FL440 and that it would be a good candidate for fine mapping and positional cloning studies. The most likely candidate gene for this QTL might be one or more of the NBS-LRR genes since 27 of them are predicted to reside at that locus. The advanced backcross populations generated in this study segregating for *qrbr-11.3* would be useful for fine mapping the locus and scrutinizing these candidate genes. The QTL that were not detected in advanced populations, such as *qrbr-1.2* and *qrbr-9.2*, but have been mapped to regions of known QTL may still be useful for crop improvement by marker-assisted transfer.

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OVERALL CONCLUSIONS

Resistance to blast in OL5 is very complex and is composed of a combination of *Pi* genes as well as some unknown genes that appear to contribute to partial resistance to some blast isolates. Most of the QTL identified in OL5 are race-specific with major effects in blast resistance. Other QTL with minor effects conferring resistance to blast isolate FL440 could be nonspecific.

Most of the QTL identified mapped to region of known *Pi* genes except the *qrbr-9.2,* which mapped to a locus where previous QTL for blast resistance have been reported. The *qrbr-9.2* QTL may be a minor gene.

The *qrbr-11.3* QTL has a small but consistent effect on resistance to isolate FL440 through three different experiments. Its effect on resistance always accounted for less that 15% of the total of the phenotypic variation but it was detectable in all three mapping populations analyzed. It had no detectable effect on several blast isolates in experiments where many resistance factors were segregating but it is possible that its' effect was obscured by major genes. Its effect on these other blast isolates could be examined using the A and B BC_2F_3 families.