THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Genetics)

DEGREE

Genetics	Genetics
FIELD	**DEPARTMENT**

TITLE: Identification of Genomic Locations of Quantitative Trait Loci for Leaf and Neck Blast (*Pyricularia grisea*) in Rice and Barley

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THESIS

IDENTIFICATION OF GENOMIC LOCATIONS OF QUANTITATIVE TRAIT LOCI FOR LEAF AND NECK BLAST (*Pyricularia grisea)* IN RICE AND BARLEY

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Genetics)
Graduate School, Kasetsart University
2009

Phinyarat Kongprakhon 2009: Identification of Genomic Locations of Quantitative Trait Loci for Leaf and Neck Blast (*Pyricularia grisea*) in Rice and Barley. Doctor of Philosophy (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Associate Professor Nitsri Sangduen, Ph.D. 91 pages.

Pyricularia grisae is the most destructive and cosmopolitan fungal pathogen of rice and it can also cause disease on other agriculturally important cereals. We determined the number, location, and interaction of QTL associated with resistance to *P. grisae* isolates obtained from rice (THL142 and THL222) and barley (TH16 and THL80) in leaf (LB) and neck blast (NB). We used mapping population of rice (IR64 x Azucena), barley (BCD47/Baronesse) and isogenic lines BISON. On rice IR64 was highly resistant, and Azucena was highly susceptible, to all four isolates. The numbers of resistant vs. susceptible progeny suggest that the resistance of IR64 is determined by two or three genes with additive effects in LB and NB. Five QTL were detected, one each on chromosomes 2, 8, 9, 11 and 12. On barley Baronesse was highly resistant, and BCD47 was susceptible, to all four isolates. The numbers of resistant vs. susceptible progeny suggest that the resistance of Baronesse is determined by one gene with additive effects in LB and two or three genes in NB. Four QTL were detected, one each on chromosomes 1H, 4H, and 7H. The results of the QTL analysis support interpretation of the phenotypic frequency distributions regarding the number of genes determining resistance to the four isolates in rice and barley population. We used isogenic lines BISON to validate resistance gene in barley. BCD12, BCD47, BISON1H, and BISON1H+4H+5H showed susceptibility to all blast isolates. Baronesse, BISON4H, BISON5H, BISON7H, BISON1H+4H, and BISON1H+5H showed resistance to all blast isolates. To compare the phenotypic data with the graphical genotype, we focused only on the region of chromosome 1H containing the major QTL. From previous studies reported this region coincident with *R* genes which confer resistance to many pathogens that causes disease to barley. In addition to we established co-linearity of gene order between 23.5 cM region of barley chromosome 1H containing *Mla* and a 510 kb region at the end of short arm of rice chromosome 5. These results indicate that a defined region on the short arm of barley chromosome1H, including *Mla*, harbors genes conferring qualitative and quantitative resistance to multiple pathogens.

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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 Advisor Dr. Nitsri Sangduen for her thoughtful guidance, friendship and consistent support to my studies and research in the last five years. Without her, it would be much harder if not impossible for me to make progress. I would like to express my cordial appreciation to Dr. Vipa Hongtrakul, Dr. Theerayut Toojinda, and Dr. Pattama Sirithunya, who took their precious time to serve on my committee and provide great guidance and assistance throughout my graduate program.

Life blessed me with the opportunity to meet Professor Patrick M. Hayes. I cannot overstate the importance of his involvement in my graduate career. I am thankful to him and Barley people, for support and encourage during my tenure at Oregon State University. My thanks also go to Dr. Alfonso Cuesta-Marcos, who always gave me his help and discussed on the use of all programs for QTL mapping.

I would like to send my special thanks to our collaborators at Rice Gene Discovery Unit: Dr. Tanee Sreevongchai, Dr. Nonglak Parinthawong, Mr. Sangchai Sriprakhon, Mr. Chatchai Kosawang and Miss Piyavadee Nasaree and the workers at Rice Gene Discovery Unit, BIOTEC, Thailand provided assistance with field work. Many thanks to the graduate students at Department of Genetics, Kasetsart University.

I wish to express my deep gratitude to my parents, and my brothers for their love, moral support and cordial encouragement. Thanks for believing in me. My final thanks go to my friends at OSU, who always gave me fight! to all situations.

Funding for this research was provided by Rice Gene Discovery Unit, BIOTEC, Thailand and Faculty of Science, Kasetsart University. The Commission on Higher Education has financed the study.

Phinyarat Kongprakhon

March, 2009

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LIST OF ABBREVIATIONS

AFLP	amplified fragment length polymorphism
BAC	bacterial artificial chromosome
BSR	barley stripe rust
CIM	composite interval mapping
cM	centi morgan
cm	centimeter
DH	doubled-haploid lines
DNA	deoxyribonucleic acid
DS	disease severity
EST	expressed sequence tags
IM	interval mapping
Kb	kilo base
LB	leaf blast
NB	neck blast
NBS-LRR	nucleotide binding site-luecine rich repeat
Mb	mega base
Mbp	mega base pair
MIM	multiple interval mapping
PCR	polymerase chain reaction
QTL	quantitative trait loci
R	resistance
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
S	susceptible
SNP	single nucleotide polymorphisms
SMA	single marker analysis
STS	sequence tagged sites
SSR	Simple Sequence Repeat
VNTR	variable number of tandem repeats

IDENTIFICATION OF GENOMIC LOCATIONS OF QUANTITATIVE TRAIT LOCI FOR LEAF AND NECK BLAST (*Pyricularia grisea*) IN RICE AND BARLEY

INTRODUCTION

Molecular marker based comparative genetic analyses have revealed extensive collinear relationship in gene orders, which is frequently referred to as synteny. In plants, it has been shown that the genomes of cereal grasses share colinearity in gene order as detected by probes that hybridize with DNA from various members across the grass family (Gale and Devos, 1998; Ahn and Tanksley, 1993). It has also been reported that some of the genes affecting seed mass, seed dispersal, and flowering time reside in corresponding chromosomal locations among the genomes of rice, sorghum and maize (Paterson *et al*., 1995). Alignment of cereal genomes identified candidate genes affecting dormancy from maize and dormancy-related quantitative trait loci (QTL) of rice that may be related to wheat QTL for preharvest sprouting (Gale *et al*., 2001). Thus identification of the syntenic relationship of important functional genes in different species may help gene discovery across species and help understanding the evolutionary processes that occurred to shape the genomes of the species (Chen *et al*., 2003).

Rice blast, caused by the fungus *Pyricularia grisea* Sacc., is the most destructive and cosmopolitan disease of rice (Puri *et al*., 2008). Blast has two commonly recognized phases: leaf blast and neck blast. Leaf blast occurs most often during the plant's vegetative stage. Spindle-shaped lesions appear on the leaf blade and necrotic lesions are found at the leaf collar. Although leaf infection is sometimes found at the reproductive and ripening stages, the most destructive symptom during these stages is neck blast (Bonman *et al*., 1989). Neck blast infections occur below the panicle, usually at the neck node, causing neck rot. If neck rot occurs early, the entire panicle dries prematurely and is sterile. Later infections may cause incomplete grain filling and poor milling quality. In farmer's fields, neck blast is often more destructive than leaf blast (Bonman *et al*., 1989). Leading to losses of up to 70 % (Puri *et al*., 2008). Although neck and leaf blast are caused by the same pathogen, there is no absolute relationship between the two diseases. Varieties susceptible to leaf blast have shown resistance to neck blast and vice versa (Bonman, 1992; Ou, 1985;

Puri *et al*., 2008). Blast disease on rice is a major threat to world food security; intensive farming practices, such as a greater use of fertilizers, will increase its occurrence (Valent, 2004). Of greater concern, however, is the fact that the fungus can also cause disease on other agriculturally important cereals including barley (Chen *et al*., 2003; Inukai *et al*., 2006; Sato *et al*., 2001); perennial ryegrass (Williams *et al*., 2001); Italian ryegrass (Miura *et al*., 2005); and wheat (Urashima *et al*., 2004). In the case of wheat, blast disease emerged as a significant disease in Brazil in 1985 and rapidly spread to other wheat growing regions.

This broad host range is of particular concern because cultural strategies, such as crop rotation, are simple and effective technique for blast disease control. If the crop that is grown in rotation with rice proves to be compatible with *P. grisea* then disease incidence will increase and a new alternative crop must be found. If two blast-susceptible crops are grown in rotation, there will be challenges too effectively deploying resistance genes in both crops. Likewise, in the face of continuous disease, dependence on fungicides will become increasingly costly and risky. A potentially positive aspect to broad host range is that the catalog of resistance genes available to plant breeders may be expanded and "non-host" resistance genes may prove to be more durable. In the case of blast, it is increasingly difficult to determine what is plant species are appropriately termed a host and which a non-host. The most studied of the novel hosts for blast is barley, where a range of responses to the pathogen are reported (Chen *et al*., 2003; Inukai *et al*., 2006; Sato *et al*., 2001).

Blast disease was most recently reported in barley (*Hordeum vulgare*) under field conditions in Thailand (Sato *et al*., 2001) and Japan (Inukai *et al*., 2006). A search for blast resistance genes in barley led to mapping QTL. Many of these QTL map to resistance gene-rich regions in barley (Chen *et al*., 2003; Inukai *et al*., 2006; Sato *et al*., 2001) and show some coincidence with syntenic resistance gene-rich regions in rice (Chen *et al*., 2003; Inukai *et al*., 2006). In addition to these QTL, a qualitative resistance gene (*PHR-1*) was reported by Yaegashi (1988). The dominant resistance allele at this locus was identified in two Japanese accessions 'Daisen Gold' and 'Miho Golden'. Since barley has been grown in Japan for thousand of years, and often in rotation with rice in areas where rice blast is endemic, some host: parasite co-evolution could be expected. In contrast, Sato *et al*. (2001), Chen *et al*. (2003), and Inukai *et al*. (2006) found quantitative and qualitative resistance

alleles in North American ("TR306") and European ("Baronesse") germplasm. Rice blast is not endemic in these regions and rice and barley are rarely grown in rotation. It is therefore surprising that barley germplasm from these areas would show reactions to a fungus with which it did not co-evolve that range from complete resistance to complete susceptibility.

The principal objective of this research was to determine the number, location, and interaction of genes determining blast resistance, in barley, using *P. grisea* isolates obtained from rice and barley grown in Thailand. We used a reference blast resistance mapping population of rice (IR64 x Azucena), the ORO population (BCD47 x Baronesse) to map resistance genes and a set of near-isogenic lines (BISON) for resistance gene validation. Moreover, we did identification of syntenic QTL for resistance to blast in rice and barley which should enhance the understanding of durable and wide spectrum resistance, which in turn may provide clues for formulating new strategies for improving disease resistance of rice and barley.

OBJECTIVES

1. To map the quantitative trait loci (QTL) for leaf and neck blast resistance on the molecular map of rice and barley.

2. To map the quantitative trait loci (QTL) for leaf and neck blast resistance on the molecular map of barley.

3. To find a possible syntenic relationship of loci for quantitative resistance to *P. grisea* in rice and barley.

LITERATURE REVIEW

Rice

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.

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.

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*., 1998). 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.

Barley

Cultivated barley, *Hordeum vulgare* L. subsp. *vulgare*, is a member of the tribe *Triticeae* in the grass family (Poaceae). The wild ancestor of cultivated barley is *Hordeum vulgare* L. subsp. *spontaneum* (Koch). Barley was domesticated about 10,000 years ago in the Near East Fertile Crescent (Nevo, 1992) Cultivated and wild barley has both winter and spring annual forms. The principal germplasm groups of cultivated barley, the lateral spikelets are sterile, while in six-rowed barley all spikelets are sterile, while in six-rowed barley all spikelets are fertile (Briggs, 1978). Barley is self-pollinated diploid (2n = 2x = 14) (Bothmer *et al*., 1992). The genome size of barley is approximately 5,000 Mbp, as Barley is primarily used as cattle feed, malt for beer, and human consumption in many parts of South American and Asia (ICARDA, 1995).

The barley genome

Barley is one of the most important and widely cultivated cereal crops in the world. There is also an extensive history of genetic, mutagenic and cytogenetic studies in barley, resulting in large numbers of mutants and quantitative traits and molecular markers have been mapped on the barley genome (Ramsy *et al*., 2000; Hayes *et al*., 2003) Since the end of the 20th century, several expressed sequence tag (EST) projects have generated very large amounts of barley EST data (Close *et al*. 2004). As of May 19, 2006, approximately 437,000 barley ESTs, derived from more than 85 cDNA libraries, were deposited in the "Expressed sequence tags" (dbEST) in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih. gov/ dbEST/dbEST_summary.html). The HarvEST:Barley project (http://harvest.ucr.edu/) provides the annotation of non-redundant barley ESTs. Conversion of non-redundant ESTs to DNA markers is an effective and useful strategy for marker generation, and several research groups have constructed high density linkage maps using markers derived from barley ESTs (Thiel *et al*., 2003; Sato *et al*., 2004). In addition, the Affymetrix 22K Barley1 GeneChip probe array is available for the biological expression analysis of approx. 22,000 barley genes (Close *et al*., 2004). Rostoks *et al*. (2005a) reported on a procedure to identify single-feature polymorphisms (SNPs) were identified as SFPs). Thus, despite the large genome size of barley, these resources have accelerated the advances of genetic and genomic studies in this crop species. In

addition to non-redundant and multiple types of genetic markers, the availability of large-insert genomic libraries is also crucial for genome analysis: for physical mapping, map-based gene and QTL cloning, and comparative genomic analyses between homologous chromosome (YAC) libraries had been constructed, and these contributed to the map-based cloning of three powdery mildew resistance loci: *Mlo*, *Rar1* and *Mla* (Buschges *et al*., 1997). However, it is generally recognized that YAC libraries cannot be easily handle. In recent years, bacterial artificial chromosome (BAC) libraries have been constructed and used for the genome analysis of many organisms, including crop plants. Several genomics resource centers have been established that distribute resources such as large-insert genomic libraries from model and/or crop plants (e.g. AGI [http://www.genome.arizona.edu/orders/], CUGI [http://genome.clemson./edu], GENEfinder Genomic Resource in TAMU [http://hbz7.tamu.edu/index.htm]). The BAC vector system has become an invaluable tool because of its ability to stably maintain large DNA fragments, and its ease of handling. In barley, two BAC libraries have been constructed using the North American six-rowed malting variety 'Morex'. Although the library reported by Yu *et al*. (2000) contained 313,344 griddle clones (6.3 haploid genome-equivalents), the library constructed by Laptitan *et al*. (1997) consisted of only 10,750 clones with an average insert size of 95 kb, and was estimated to be less than one genome-equivalent. Recently, Isidore *et al*. (2005) have constructed a barley cv. 'Cebada capa' BAC library, and adopted a pooling strategy for rapid, cost-reduced screening to obtain targeted clones. Despite these reports, progress in the construction of BAC libraries has been limited for species with large genome size, such as barley. The only barley libraries currently available with complete genome coverage and arrayed individual clones are derived from the variety 'Morex', and these are considered to be insufficient for an extensive analysis of the barley genome.

The pathogen: *P. grisea*

The fungus *P. grisea* (Hebbert) Barr (anamorph *P. 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). *P. 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 leave, nodes, and panicles.

The highly variable specific virulence of the fungus and its genetic plasticity make its control and management difficult. Thus, *P. 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).

Taxonomy and nomenclature

The taxonomic definition of the anamorph state of blast fungus is as follows:

Division: Eumycota
Subdivision: Ascomycotina
Class: Pyronomycetes
Order: Diaporthales
Family: Magnaporthaceae
Genus: Magnaporthe
Alexopolos (1996)

The fungus has two mating types. Each mating type produces its own specific pheromone. Compatibility for mating is governed by alternated alleles of the mating locus Mat1 (Yoder *et al*., 1986), with fertile interactions only possible between individuals of the opposite mating type (designated Mat1-1 and Mat1-2). They are two types of pathogen specificity. The first is plant species specificity (determining host species range) and the second is cultivar specificity (determining cultivar range within a given host species). Blast is wide spread pathogen of cereal and grasses. Over 30 genera of Graminea are being the host (Leburn *et al*., 1993). Three unstable genes for host specificity were found from rice pathogen and appear to mutate at high frequency. In order to infect the host, blast pathogen produces host specific toxin during spore germinated that determines the host specificity at plant species level. Individual isolates have a limited host range. The isolates derived from barley can infect wheat, oat, triticale, maize and rice. Only the isolates derived from rice and barley can be infected barley.

Blast symptoms and infection

Rice pathogenic isolate of the blast pathogen produces lesion on all parts of the rice plant; leaves, neck node, and panicles. The rice blast disease is characterized by examining these parts of the plant. When lesions appear on leaves, they are often white to gray-green with dark green or brown border (Fig. 1A). Their shape varies but lesions are characteristically spindle shapes. There are several types of lesions based on the resistance and plant age. Leaf reactions vary from pinpoint infections to large elliptical lesions up to 1.5 cm. Long, 0.3-0.5 cm. Broad. Large lesions without signs of plant resistance are referred to sometimes as acute lesions, while small lesions indicate a degree of resistance. Blast infection in early plant stages can cause stunting or death, depending on the severity of the attack and on the humidity and temperature during the infection period. Neck blast is seen as a gray brown lesion round the upper most nodes just below the panicle, causing the panicle to fall over (rotten neck) (Fig. 1B). Early attack at this point will result in no or very poor gain filling and high harvest losses. A later attack can be less damaging, although the grains will not completely develop (Tenjo and Hamer, 2002).

Conidia are produced on lesions on the rice plant about 5 to 7 days after infection. The productions of spore increase with increase in the relative humidity. Most of the spores

are produced and released during the night. After spore germination, an infection follows. Infection tubes are formed from the appressoria and later the penetration through the cuticle and epidermis. After entering the cell, the infection tubes forms a vesicle to give rise to hyphae. In the cell, the hyphae grew freely (Tenjo and Hamer, 2002).

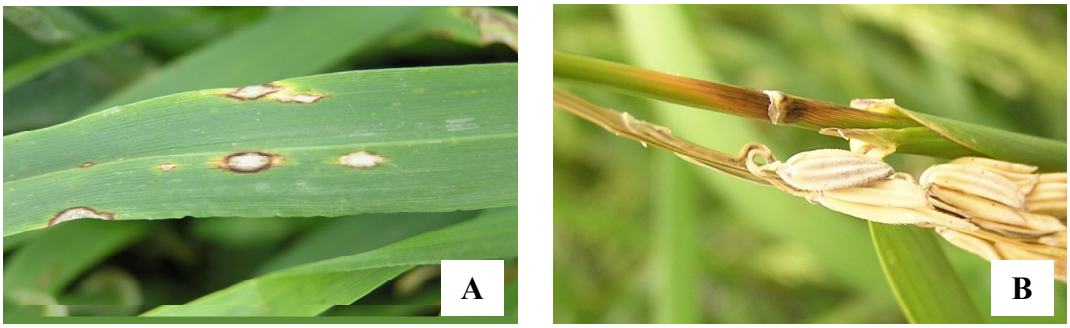


Figure 1 Typical spindle-shaped leaf A) and neck B) lesions caused by the rice blast fungus *P. grisea.*

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. 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.

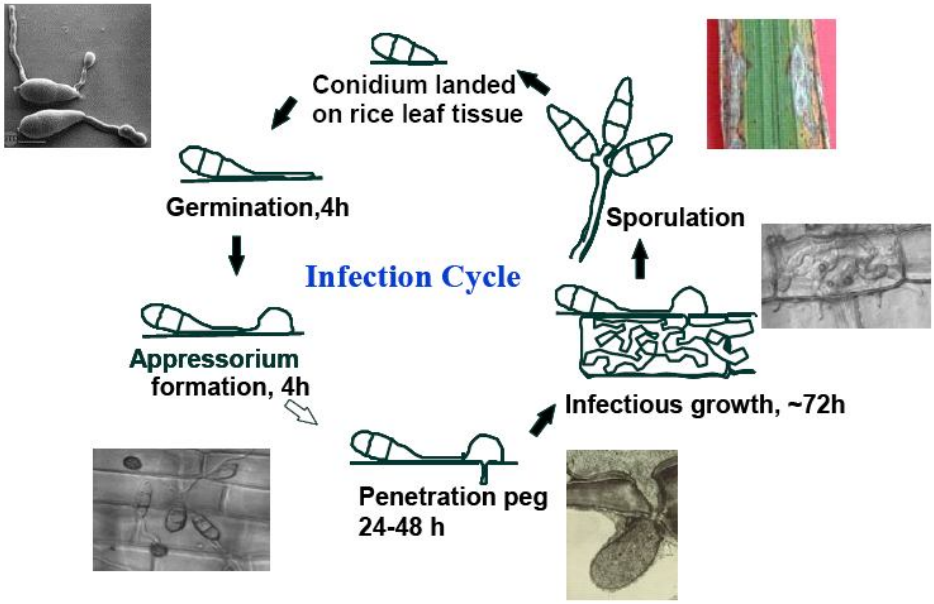


Figure 2 The life cycle infection-related morphogenesis of the rice blast fungus *P. grisea*. Modified from www.btny.purdue.edu/ Faculty/Xu/

Genetic of blast resistance in rice and barley

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-kh* (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 F2 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 *Pi-36*(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-kh* present in the rice cultivar Tetep. This *Pi-kh* gene is the third Pi gene cloned so far in rice. The *Pi-kh* 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-kh* 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 Blast resistance (*Pi*) genes identified in rice.

Gene	Chromosome	Markers	Reference
Pi-t	1		Kiyosawa, 1972
Pi-24	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	Tabein *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 and 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		Tabein *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
Pi-28(t)	10	RZ500	Sallaud *et al*., 2003

Table 1 (Continued)

Gene	Chromosome	Markers	Reference
Pi-a	11		Kiyosawa, 1967
Pi-f	11		Shinoda *et al*., 1971
Pi-k	11		Shinoda *et al*., 1971
Pi-k^{h}	11	RM2191	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		Tabein *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	Zheng *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 to *P. 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; Talukde *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 F2 family. In contrast, multiple genes could determine a quantitative trait and its value is continuous, such as plant height and human weight. Quantitative traits are very common and are important both in applied and theoretical studies. 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 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 (F2, 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.

Comparative Mapping

The idea of comparing the genetic maps of different species is an old one, especially in animals. In 1920, Dunn compared the linkage between the gene for albinism and the pink-eye gene in rat and mouse (Lyon, 1990). Several arrangements of the genes that are homologous between the maps of mammals have since been described. Some linkage groups are even conserved among the vertebrates, from fish to man. The possibility of using heterospecific probes, along with the relative facility of constructing maps in plants, has allowed numerous comparisons of the order of genes in species belonging to a single botanical family. In the solanaceae, Bonierbale *et al*. (1988), then Tanksley *et al*. (1992) show that the genomes of tomato and tomato were collinear, with the exception of 5 paracentric inversions. There is thus a very good conversation of synteny. (There is sytheny

when loci are carried on the same chromosome, whether or not they are genetically linked. Asynteny pertains to loci carried by different chromosomes.) Between tomato and pepper, which are phylogenetically not so close, rearrangements are more numerous (around 20), but locally the order of genes remains conserved (Tanksley *et al*., 1988; Lefebvre *et al*., 1995; Livingstone *et al*., 1999). In the Fabaceae, comparison of maps of lentil and pea reveal 8 well-conserved regions, which represent 40% of the genome. But it is the Poaceae that has been the focus of the most exhaustive studies. In the triticeae tribe, the order of marker is largely conserved between wheat, barley, and rye (Sharp *et al*., 1988; Devos *et al*., 1992; Wang *et al*., 1992), even if the rye genome seems to differ from that of wheat by many translocations. From a fundamental point of view, these results, once refined and extended to more species, could be used to analyses the evolution of the genome structure from the ancestral genome of the families of plants.

Even though the probes generally used in experiments are largely anonymous, the idea that the positional homology of genes is associated with functional homology has long been acknowledged. To take classical examples, let us cite the mutations that determine the loss of the ligule, mapped in maize and rice (Ahn *et al*., 1993a), or a major gene for frost tolerance and needs to vernalization found in rye, wheat, and barley (Plaschke *et al*., 1993). Comparative mapping can constitute a major tool for integrating research programs that are working in parallel lines in different species. This point was advocated in particular by Bennetzen and Feeling (1993) and Freeling (2001), who proposed that grasses be considered as “a single genetic system”. The following approaches may be possible:

- Markers from a species can be used on related species to saturate a given region. This could prove particularly useful in species with a high ploidy level, where mapping the entire genome is not feasible (Asnaghi *et al*., 2000).

- The gene homologous (orthologous) to the desired gene in related small-genome model species for which a YAC or BAC library is available can be cloned, and then the sequence obtained can be used to isolate the gene in the species studied (Robert *et al*., 1998).

Comparative methods (Laurie *et al*., 2004)

1. Gene homology

Before considering how comparative genetic methods are used, it is worth considering what they are and why they might be advantageous. Comparative genetics utilizes evolutionary conservation of individual genes, or conservation of gene order, in the chromosome of related species. The attraction of comparative genetics is that discoveries in one species may be transferable to others that are experimentally less tractable. Many aspects of plant biology are best understood in the model dicot arabidopsis which has well documented experimental advantages including a small genome, now completely sequenced and a large coordinated international effort devoted to developing resources for understanding gene function. In comparison, gene isolation has proven difficult in many crop species, including temperate cereals, and a major in this is the large sizes of their genomes [approximate haploid genome sizes in megabases (x 106 base pairs) are 130 for arabidopsis, 450 for rice (*Oryza sativa* L.), 2,500 for maize (*Zea mays* L.; about the size of the human genome), 5,400 for barley and 17,000 for bread wheat]. This makes positional (also known as map-based) gene isolation difficult. In contrast to maize, temperate cereals lack endogenous transposable elements suitable for gene isolation by insertional mutagenesis.

In essence, comparative methods offer the potential for ‘‘fast-track’’ isolation of genes, reducing cost and effort, by virtue of evolutionary conservation of gene function. A good example comes from work on the control of plant growth by gibberellic acid (GA). Analysis of dwarf mutants of arabidopsis allowed the isolation and characterization of the *GIBBERELLIC ACID INSENSITIVE* (*GAI*) gene (Peng *et al*., 1997). The *REDUCED HEIGHT 1* (*Rht1*) gene of wheat was then isolated on the basis of sequence homology. This showed that the genes encoded highly conserved proteins. Furthermore, phenotypically similar GA insensitive dwarf phenotypes were caused by equivalent mutations that involved deletion of a specific region in the respective proteins (Peng *et al*., 1999). Further work has shown that GAI is a member of a small gene family termed the DELLA group that act as growth repressors and whose action is opposed by GA through a mechanism that leads to targeted destruction of DELLA proteins by the proteasome (Fu *et al*., 2002). Auxin

signalling has also been shown to involve DELLA proteins, showing how the action of different hormone signals is integrated (Fu and Harberd, 2003). Clearly not all characters are conserved in this way. For example, several Brassica and temperate grass species have self-incompatibility systems, but the S locus system of the former is unrelated to the S and Z locus system of the latter (Hiscock and McInnis, 2003). This highlights an obvious problem with comparative approaches, which is that there is often no information on whether similar phenotypes are produced by variation in homologous genes or are the result of convergent evolution. However, with the increasing availability of DNA sequence information from many species it is now relatively easy to ask whether a gene cloned from arabidopsis, for example, has a counterpart in cereals. Direct searching of wheat or barley cDNA or genomic libraries using arabidopsis genes as hybridization probes is inefficient due to differences in nucleotide sequence of even the most highly conserved genes. Fortunately, the ability to search for wheat and barley homologues of genes from arabidopsis and rice has been greatly enhanced by the availability of the complete genomic sequence of rice and the development of large numbers of expressed sequence tags (ESTs) which are partially sequenced cDNA clones (currently over 280,000 for rice, 350,000 for barley and 540,000 for wheat; http://www.ncbi.nlm.nih.gov/dbEST/). Thus, much of the work can now be done by database searching using bioinformatic tools. Mapping using selected cereal sequences can then determine if the candidate gene cosegregates with the phenotype of interest. This emphasizes the importance of high quality genetic analysis of the target mutant or trait, even in cases where comparative approaches are used.

2. Comparative mapping

The section above deals with the conservation of individual gene function, but another important aspect is comparative mapping which aligns the genetic maps of different species using common markers, usually RFLP or polymerase chain reaction (PCR) markers based on the coding regions of evolutionarily conserved genes (Devos and Gale, 2000; Paterson *et al*., 2000; Feuillet and Keller, 2002; Laurie and Devos, 2002; Bowers *et al*., 2003). Comparative mapping has greatest benefit where one of the genomes is fully sequenced, and in the case of cereals this is rice. A high quality annotated sequence of rice is expected to be complete by the end of 2004, providing a great deal of information on the likely gene content of colinear regions in other species. Comparative mapping has to be

approached with care, as changes in gene order can complicate the relationship between different species (Sorrells *et al*., 2003). However, there is no doubt that this is an excellent way of generating new markers for particular regions and for identifying candidate genes. The properties of temperate cereal genomes confer intrinsic disadvantages when it comes to gene isolation. They are also relatively disadvantaged in terms of the available model species. In dicots, the evolutionary separation of Brassica species from arabidopsis is thought to have occurred 15–20 million years ago (Bowers *et al*., 2003). The dicot (arabidopsis/ Brassica) lineage is far removed from the monocot lineage, which diverged 150–180 mya or perhaps as much as 235 mya (Bowers *et al*., 2003) at one of the most basal branch points in the angiosperm phylogeny (Soltis *et al*., 1999). Temperate cereals are thought to have diverged from the ‘‘model’’ cereal rice about 60 million years ago. Thus, the power of model systems is proportionally reduced for the temperate cereals. Nevertheless, comparative approaches have great potential and the following sections discuss the experience of using arabidopsis and rice in the study of flowering in temperate cereals (Laurie *et al*., 2004).

MATERIALS AND METHODS

Materials

Plant materials

Three populations were used for studying the QTL to rice fungal blast.

1. The rice mapping population consisted of 111 doubled-haploid (DH) lines developed from the F1 of the cross between IR64 x Azucena. IR64 is an improved semi-dwarf *indica* cultivar developed by the International Rice Research Institute (IRRI); Azucena is an upland *japonica* cultivar from the Philippines. The population was kindly provided by Dr. Susan McCouch, Cornell University, USA. Two varieties were included as checks in the experiments: Black Tall (BT; a black rice with resistance to the blast isolates used in this work (see next section)) and Khao Dawk Mali (KDML; a traditional aromatic cultivar from Thailand that is susceptible to the same blast isolates).

2. The ORO population consists of 94 doubled-haploid (DH) lines developed from the F1 of the cross between BCD47 x Baronesse. BCD47 is a two-rowed, spring growth habit DH line, developed via marker-assisted selection for barley stripe rust resistance alleles at QTL on chromosome 4H and 5H (Castro *et al*., 2003a). Baronesse is a two-rowed, spring growth habit developed by Nordsaat and released in 1989 in Germany. The variety is grown extensively in the Pacific Northwest of the USA. The population was kindly provided by Professor Patrick M. Hayes at Oregon State University, USA

3. The BSR-BISON (Barley Stripe Rust Barley Isogenic) are a set of QTL isolines representing all possible one-way, two-way, and three-way introgressions of alleles at barley stripe rust resistance QTL on chromosomes 1H, 4H, and 5H (Fig. 3). The s*et al*so includes an isogenic line for a qualitative resistance gene on chromosome 7H. The BSR-BISON were developed by one cycle of MAS backcrossing of iBISON (intermediate BISON) lines to the recurrent parent Baronesse. The development of the iBISON is described in detail by Richardson *et al*. (2006). The BSR resistance QTL alleles on

chromosomes 4H and 5H trace to BCD47 and the QTL allele on chromosome 1H comes from BCD12. The MAS derivation of BCD12 and BCD 47 is described in detail by Castro *et al*. (2003). A "0 QTL" BSR-BISON (0-QTL) was selected from the same population as the other BSR-BISON lines, but was selected for the susceptibility alleles at each QTL region (1H, 4H, and 5H). D3-6/B23 was the source of the qualitative resistance gene on 7H (Castro *et al*. 2003). Forward and background MAS in the iBISON and BSR-BISON were performed using an array of marker types, principally SSRs. Detailed protocols are available at http://barleyworld.org/osubreeding/striperustmapping.php. Although the BSR-BISON were developed to validate BSR resistance QTL alleles, they are useful for validating blast resistance QTLs via a reverse genetics approach. Since Baronesse was resistant to *P. grisea* isolates from barley and rice (see Results and Discussion), introgression of a chromosome region containing a blast susceptibility allele (or alleles) should lead to susceptibility.

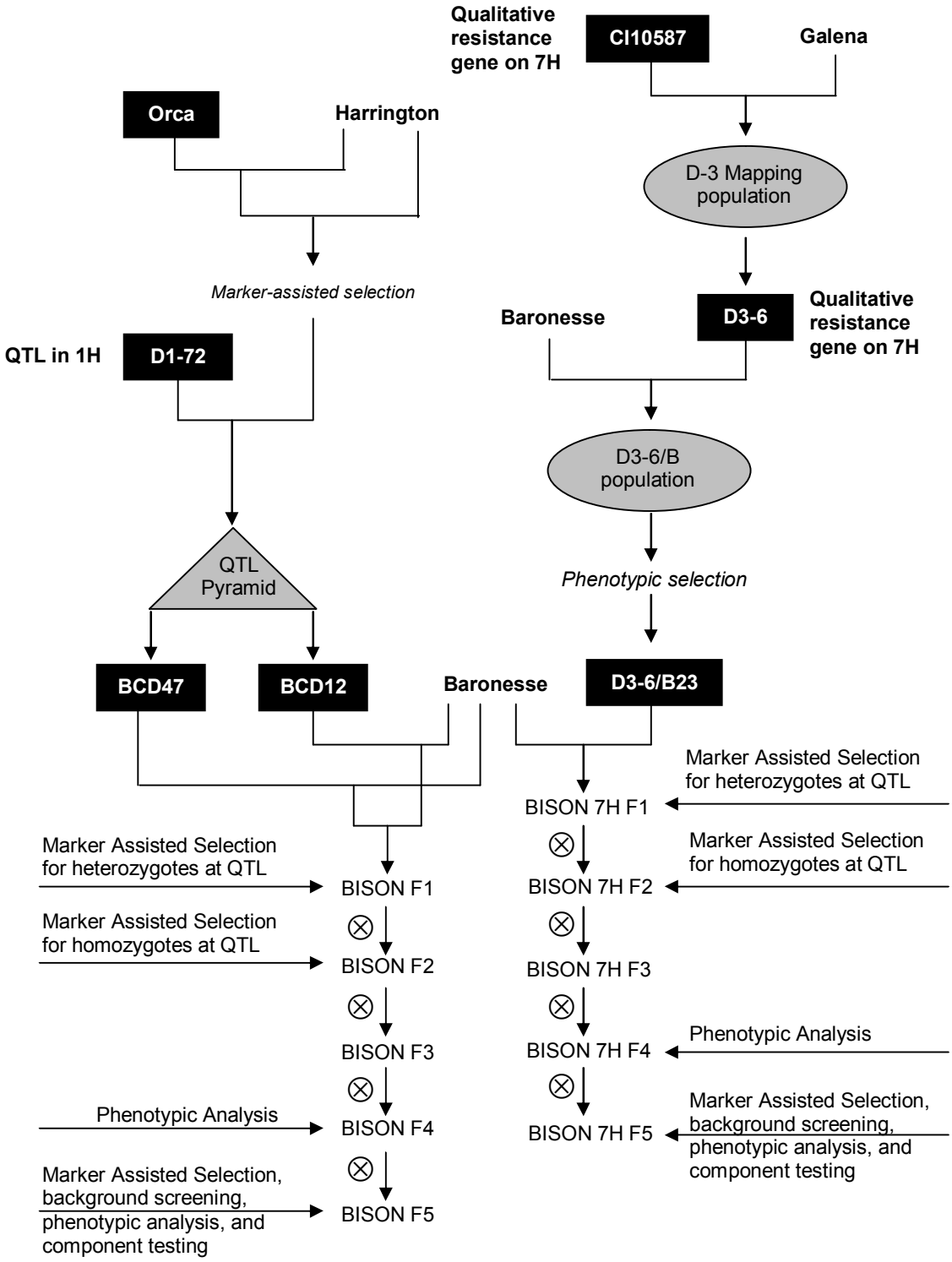


Figure 3 The pedigree and derivation of the BISON lines indicating generation when phenotypic and genotypic screenings were performed. Black squares indicate resistance allele donors. Modified from Richardson *et al*., 2006

***P. grisea* isolates and culture**

Four fungal isolates - TH16 (origin Khon Kaen, Thailand), THL80 (origin Khon Kaen, Thailand), THL142 (origin Chiang Mai, Thailand) and THL222 (origin Chiang Mai, Thailand) - were used. TH16 and THL80 were obtained from barley and THL142 and THL222 were obtained from rice. Additional detail on these isolates may be obtained from Dr. Pattama Sirithunya, Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. The isolates were grown on rice polished agar medium (20 g rice polish, 2 g yeast extract and 20 g agar/ l distilled water) at 24-25^{0}C using a 12 h photoperiod for 7-8 days. Spores were harvested by flooding the culture plates with 8 ml of sterile water.

Methods

Disease Assessments

For leaf blast (LB) phenotyping, rice plants were grown under greenhouse conditions (25 – 30°C continuous temperature with natural light) in 72-well plastic trays that measured 25.4 x 50.8 centimeters. In each well, five seeds of each DH line were sown. Two trays were needed to test the whole set of 111 DH lines, 58 in one tray and 53 in the other. Each tray also included three replicates of each of the two parents (IR64 and Azucena) and of each of the two checks (BT and KDML). Four experiments were performed to test the response to the four isolates (TH16, THL80, THL142 and THL222). Each experiment was replicated eight times. The clay soil was kept continually moist by adding tap water and once a week a commercial liquid fertilizer (16:16:16 N-P_2O_5-K_2O) was applied. Additional Nitrogen in the form of urea was applied (at a rate of 10 g/tray) at 10, 3 and 1 day prior to inoculation in order to increase susceptibility to blast.

Barley plants were grown under greenhouse conditions (25 – 30°C continuous temperature with natural light) in 72-well plastic trays that measured 25.4 x 50.8 centimeters. In each well, three seeds of each DH line were sown. Two trays were needed to test the whole set of 94 DH lines, 50 in one tray and 44 in the other. Each tray also included three replicates of each of the two parents (BCD47 and Baronesse) and of each of

the two rice checks (IR64 and Azucena). Four experiments were performed to test the response to the four isolates (TH16, THL80, THL142 and THL222).

For BSR-BISON lines, barley plants were grown under the same greenhouse conditions in 18-well plastic trays that measured 12.5 x 25.4 centimeters. Three seeds of each BSR-BISON line, BCD 12, D3-61B-23, CI10587, Baronesse, BCD47, IR64 and Azucena were sown per well. Each experiment was replicated four times. Once a week a commercial liquid fertilizer (16:16:16 N-P_2O_5-K_2O) was added. Additional Nitrogen in the form of urea was applied (at a rate of 10 g/tray for mapping population and 2.5 g/tray for BSR BSR-BISON lines) at 10, 3 and 1 day prior to inoculation in order to increase susceptibility to blast.

Seedlings were inoculated at the 1.5 – 2 leaf stage with each of the four isolates using 50 ml of 50,000 spore ml^{-1} suspensions, with 0.5 percent gelatin. Each tray of inoculated plants was then kept for one night in a controlled environment chamber at 24°C with 95 percent relative humidity. Trays were then returned to the greenhouse, which was kept at a continuous temperature of 25 – 30 °C with natural light. Three, five, and seven days post-inoculation, lesion types were scored using the seven lesion type categories described by Roumen *et al*. (1992). According to this scale, 0 = resistant, 1- 3 = intermediate and 4 - 6 = susceptible. Reaction types for each DH line were scored on a per well-basis (the score of the individual plant in each well with the highest score). Reaction types were highest seven days post inoculation. Therefore, the average scores across the eight replicates from this time point were used as the phenotypic values for QTL and correlation analyses.

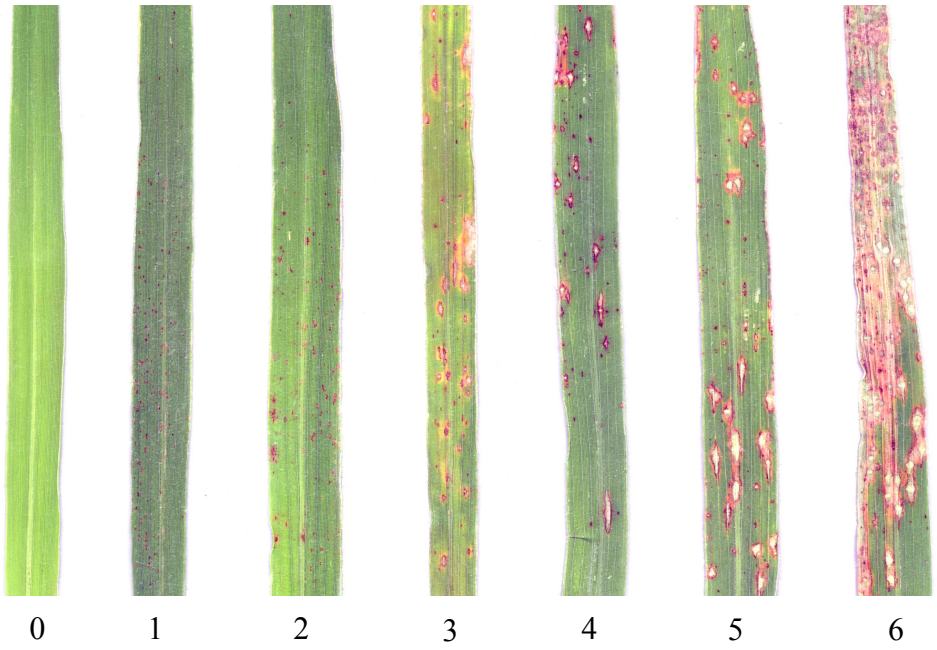


Figure 4 The disease severities index of leaf blast on rice with lesion 0-6 type.

Figure 5 The disease severities index of leaf blast on barley with lesion 0-6 type.

Table 2 Lesion type Score for leaf blast

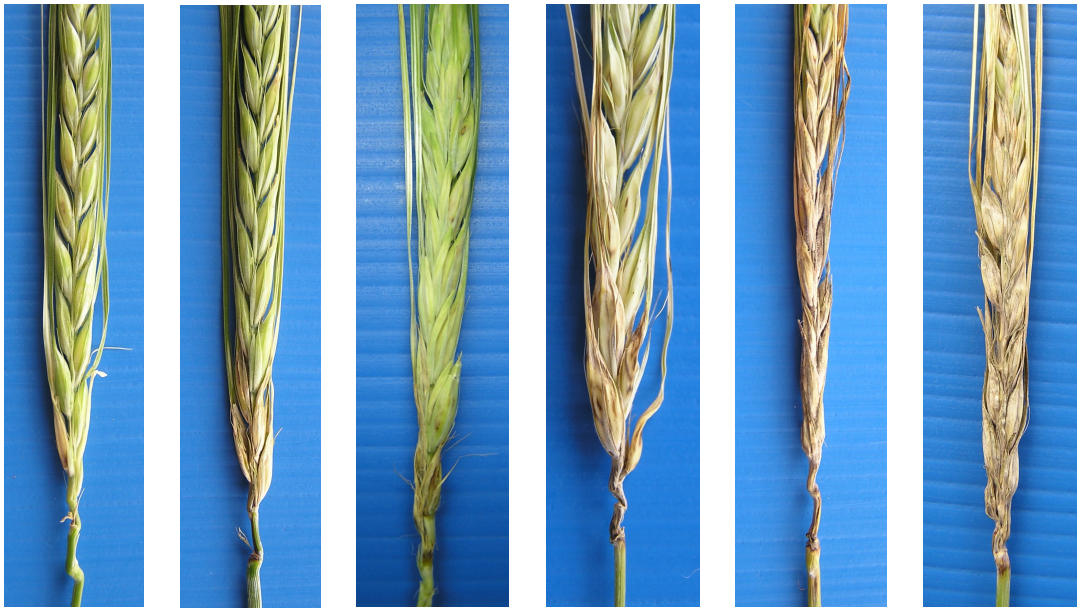
Lesion type	Symptoms
0	No evidence of infection
1	Brown pinpoint smaller than 0.5 mm., without sporulation
2	Brown pinpoint smaller than 0.5-1mm., without sporulation
3	Small eyespots about 1-3 mm, lesion capable of sporulation gray center
4	Small eyespots about 3 mm or more longs, lesion capable of sporulation gray center and dark margin.
5	Susceptible sporulating type, coalescence lesion without dark margin
6	Susceptible sporulating type, lesion without dark margin

For neck blast (NB) phenotyping, rice plants were grown under field condition in the middle August 2007 at Kamphaeng Saen Campus, Kasetsart University Thailand. For each individual DH line and the parents, the seed (about 50 grains) were sown in 150x50 cm plots. For barley, plants were sown under filed condition in early November 2007 at Kamphaeng Saen Campus, Kasetsart University Thailand. For each individual DH line and the parents, the seed (about 50 grains) were sown in 150x50 cm plots.

The experiment was performed with six repeats in case of rice and three repeats in case of barley. The injection method for field-grown material was performed in December for rice and the middle of January for barley. Inoculum (at the same concentration use for leaf blast resistance phenotyping) was injected into the leaf sheath at heading. Six heads per DH line were inoculated with each of the four isolates. Neck blast was scored at seven and fourteen days post-inoculation using a 0 to 6 scale based on the Standard Evaluation System for Rice (IRRI, 1996). According to this scale, 0 = resistant, 1- 3 = intermediate and 4 - 6 = susceptible.

Reaction types were scored on a per-plot basis (the score of the individual plant in each well/plot with the highest score). Reaction types were highest seven days post inoculation. Therefore, the average scores across the eight replicates from this time point were used as the phenotypic values for QTL and correlation analyses.

Figure 6 The disease severities index of neck blast on rice with lesion 0-6 type.



1 2 3 4 5 6

Figure 7 The disease severities index of neck blast on barley with lesion 0-6 type.

Table 3 Lesion type Score for neck blast

Lesion type	Symptoms
0	No visible lesion or observed lesions on analyze a few pedicels
1	Lesions on several pedicels
2	Lesions on secondary branches
3	Lesions on a few primary branches or the middle part of panicle axis
4	Lesion partially around the base (node) or the uppermost internode or the lower part of panicle axis near the base and less than 30% filled grains
5	Lesion completely around panicle base or uppermost internode or panicle axis near base with 30 -50 % of filled grains
6	Lesion completely around panicle base or uppermost internode or the panicle axis near the base with more than 50% of filled grains.

Statistical analysis, linkage map construction and QTL analysis

We calculated the Pearson correlation coefficients between traits and the significance of these correlations using the CORR procedure of SAS V9.1 (SAS Institute, Cary, NC, USA). The variance components were estimated using the VARCOMP procedure of SAS V9.1 with the restricted maximum likelihood method according to the following model:

$$Y_{ij} = \mu + E_i + R_{j(i)} + G_k + GE_{ik} + \varepsilon_{ijk}$$

where Y_{ij} was the infection score of the *k*th genotype (G) in the *j*th replicate (R) within the *i*th experiment (E), μ is the overall mean and *εijk* was the residual error. Experiments were considered as fixed effects, and genotype and replicates as random effects. Heritabilities (h^2) were calculated with the following formula:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2 / e + \sigma_\varepsilon^2 / re}$$

where σ^2_G represents the genotypic variance, σ^2_{GE} the genotype-by-experiment variance, σ^2_ε the error variance, *e* the number of experiments and *r* the number of replicates. Broad sense heritability on an entry mean basis was calculated with e=4 and r=8.

For rice mapping population, linkage map construction was performed using JoinMap 3.0 (Van Ooijen and Voorrips 2001). From the original marker data set, which consists of 239 markers and was kindly provided by Dr. Susan McCouch (Cornell University, USA) we deleted co-segregating markers, markers with more than 20 percent missing data and markers with poor goodness of fit. This left 152 markers, which generated 12 linkage groups at a minimum LOD score of 3.0. Map distances were calculated using the Kosambi function.

The genotypic data for the BCD47/Baronesse DH lines were reported by Rossi *et al*. (2006). Linkage map construction was performed using JoinMap 3.0 (Van Ooijen and Voorrips 2001) with Kosambi mapping function (Kosambi, 1944). The linkage map has 70 markers comprising eleven linkage groups at LOD threshold grouping value of 4.0. The

linkage groups were assigned to barley chromosome per Rossi *et al*. (2006). The map covers 611.8 cM, corresponding to an average density of 8.7 cM per marker.

The BSR-BISON lines were genotyped for 4608 SNPs under the auspices of the Barley CAP Project (http://www.barleycap.org/). The process of developing the high confidence SNP markers will be described in detail by Close *et al*. (unpublished). Briefly, SNPs were used to design three Illumina 1536-plex pilot Oligonucleotide Pool Assays (pilot OPAs; POPA1, POPA2, POPA3). The three pilot OPAs (POPAs) were used to genotype the "CAP Core germplasm array, which included the BSR BISON, BCD12, and BCD47 using the Illumina GoldenGate BeadArray SNP detection platform. The genotyping assays were conducted at the Southern California Genotyping Consortium at the University of California, Los Angeles using DNA samples provided by the Close lab. Data on the CAP Core germplasm array is available at (http://www.barleycap.org/). SNPs are designated by their pilot OPA numbers (e.g. 1_1311), where 1 = the POPA number (POPA1 in this case) and the subsequent four digits correspond to the SNP order in the corresponding POPA. The locus designations can be directly referenced to assembly #35 unigene numbers by referring to the barley SNP consensus map at HarvEST (http://harvest.ucr.edu and www.harvest-web.org). For example, 1_1311 maps to the short arm of chromosome 1H and corresponds to assembly #35 unigene 5087. A spreadsheet translating POPA to barley (BOPA) locus names, provided by Close, is available at THT (http://www.hordeumtoolbox.org/).

QTL analysis was performed using composite interval mapping (CIM) (Zeng, 1994) implemented in Windows QTL Cartographer 2.5 (Wang *et al*., 2005). Cofactors for CIM were chosen using a forward-selection backward-elimination stepwise regression procedure with a significance threshold of 0.10. The maximum number of cofactors used was six. The scan window was set in 10 cM. Experiment-wise significance (alpha=0.05) likelihood ratio test (LR) thresholds for QTL identification were determined with 1000 permutations and expressed as LOD (LOD = 0.217 LR). For every significant QTL we calculated individual R^2 (proportion of phenotypic variance explained by the individual QTL) and additive effect (expressed as one half of the difference between the two allelic classes). Negative values indicate that the parent line contributing the resistance allele was IR64. Epistatic interactions between QTL were tested by means of Multiple Interval Mapping (MIM, Zeng *et al*., 1999) using QTL Cartographer and the Bayesian Information Criteria (BIC-M0). In

each experiment, we also calculated the R^2 of the multilocus model that included the QTL detected using CIM and their significant interactions detected using Multiple Interval Mapping (MIM). We also calculated the average TR^2, which is the proportion of variance explained by the QTL conditioned on the cofactors in CIM obtained with EQTL (QTL Cartographer v. 2.5).

For sequence analysis, standard nucleotide-nucleotide (blastn; Altschul *et al*. 1997) searches were performed against the NCBI nr database (http://www.ncbi.nlm. nih.gov/BLAST/) and the TIGR Rice Genome Annotation database(http://www.tigr. org/tdb/e2k1/osa1/) (Inukai *et al*, 2006).

RESEARCH LOCATION AND PERIOD OF STUDY

Location

Rice Gene Discovery Unit, Kasetsart University, Kamphaengsaen Campus, Nakornpathom, Thailand

Period of study

December 2006 – April 2008

RESULTS AND DISCUSSION

Results

Mapping population of IR64/Azucena

IR64 was highly resistant, and Azucena highly susceptible, to all four isolates for LB and NB. This reaction pattern corresponds to prior reports (Ghesquiere *et al*., 1996; Bagali *et al*., 2000). The phenotypic frequency distributions of disease severity (DS) in the IR64 x Azucena DH lines support quantitative inheritance (Fig. 8 and 9). For all four isolates, the distributions of DS were skewed towards resistance. The numbers of resistant vs. susceptible progeny suggest that the resistance of IR64 is determined by two or three genes with additive effects, or to a single gene with large effects and several lesser-effect QTL. The h^2 of DS on an entry mean basis was 89%, confirming the high level of repeatability of the DS data and providing a sound basis for mapping the resistance genes (Toojinda *et al*., 1998).

In order to determine, based on phenotype, if the same or different genes determined resistance to the four isolates, we calculated correlations between DS in response to inoculation with the four isolates (Table 4). The correlation coefficients for all pairwise comparisons are high, suggesting that most of the same QTL confer resistance to the different isolates. However, correlations were highest between barley isolates (TH16 and THL80) and between rice isolates (THL142 and THL222), suggesting that there may be some minor differences in the genetic basis of resistance to blast isolates from the two different crops.

The number, allele phase, and interaction of the blast resistance QTL identified in response to inoculation with each of the four isolates are presented in Table 5, 6 and Fig. 12. A total of five QTL were detected. The QTL located on chromosomes 2, 8, 9, 11 and 12 had LOD scores ranging from 2.9 to 8.7 (Table 5 and 6). IR64 contributed resistance alleles at three of the QTL (chromosomes 2, 8, 9 and 12). Azucena contributed the resistance allele at the QTL on chromosome 11 in response to inoculation with isolate THL142 (obtained from barley). Allele main effects were, in general, larger than epistatic interactions. As detailed

in the subsequent sections, there are prior reports of blast *R* genes and/or QTL forming resistance gene clusters (Miyazaki *et al*., 2001) at each of the four genomic regions.

Five QTL analyzed of LB resistance with four blast isolates. One QTL was identified on chromosome 2 was in the region of the long arm of chromosome and in the interval between RG256 and RZ123, effected resistance to four isolates (Table 5 and Figure 12). This locus controlled 9.9, 9, 19.6 and 10.8 % of the phenotypic variance in the resistance to TH16, THL80, THL142 and THL222, respectively. This region has been mapped to specific *R* gene; *Pib* in previous study was previously mapped closed to the SSR marker RM 208 (Wang *et al*., 1999).

One QTL was identified on chromosome 8 was in the interval between RM223 and RM80, effected to three isolates, TH16, THL80 and THL222 (Table 5 and Fig. 12). This locus controlled 15.3, 22.9, and 13.3 % of the phenotypic variance in the resistance to TH16, THL80, and THL222, respectively. This region has been mapped to *R* gene; *Pi-zh*, *Pi-33*, *Pi-11(t)* and *Pi-29* in previous studies were mapped closed between markers RZ617 and RZ323 (Causse *et al*. 1994; Berruyer *et al*. 2003; and Sallaud *et al*. 2003). The *Pi-33* gene has been shown to confer resistance to blast isolates from Columbia (Correa-Victoria *et al*., 2004).

Furthermore, we were identified one loci was affecting resistance to isolate THL142 on chromosome 9. This loci was located near the centromere between markers G103 and RZ492 (Fig. 12) and the phenotypic variance explained by the locus was 7.8 %. At least two genes, *Pi-15* and *Pi-5* (*Pi-3*) conferring high levels of blast resistance have been mapped in this region (Pan *et al*., 2003; Jeon *et al*., 2003). One locus on chromosome11 was contributed to resistance to isolate THL222, this locus was located between marker RM209 and RZ536 and the phenotypic variance was 8.1 %. At least six *R* genes have been mapped around this locus and one of them, *Pi-k*^{h}, was recently cloned (Sharma *et al*., 2005). One locus on chromosome12 was affected resistance to isolate THL142; this locus was identified between markers RG457 and RG901. This QTL accounted for 8.8 % of phenotypic variance. 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 (Bryan *et al*., 2000) (Fig. 12).

Two QTL analysis of NB resistance with four blast isolates, TH16, THL80, THL142 and THL222, were located on chromosome 8 and 12 which carried of resistance allele from IR64 (Table 6). One locus was identified on chromosome 8 was in the interval between TGMS1-2 and RM223, effected to three isolates, TH16, THL80 and THL222 (Table 6 and Fig. 12). This locus controlled 10.7, 11.9, and 7.7 % of the phenotypic variance in the resistance to TH16, THL80, and THL222, respectively. One locus on chromosome12 was affected resistance to isolate THL142; this locus was identified between markers RM247 and RG457. This QTL accounted for 12.4 % of phenotypic variance. We found that rice leaf blast resistance alleles which inoculated by TH16, THL80, THL222 on chromosome 8 and the resistance alleles of neck blast with the same isolates were located in the same region on chromosome 8, these chromosome region know as resistance gene *Pi-zh* (Fig. 12). Those QTLs carried resistance allele from IR64.

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. 12). This is consistent with the idea that Azucena/IR64 DHL population 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.

Table 4 Pearson correlation coefficients between disease severities observed in the IR64 x Azucena mapping population inoculated with four rice blast isolates (TH16, THL80, THL142 and THL222).

	LBTHL80	LBTHL142	LBTHL222
LBTH16	0.76^{***}	0.65^{***}	0.64^{***}
LBTHL80		0.68^{***}	0.64^{***}
LBTHL142			0.84^{***}

Significant at $^{***}p<0.001$

Mapping population of BCD47/ Baronesse

Baronesse and BCD47 showed contrasting disease reactions to inoculation with all four blast isolates in LB and NB. Baronesse had intermediate resistant reaction and BCD47 showed susceptible reaction to all four blast isolates in both LB and NB (Fig. 10 and 11).

In order to investigate the inheritance of blast resistance in Baronesse, The phenotypic frequency distributions of disease severity (DS) in the BCD47/Baronesse DH lines have no transgressive and do not support mendelian inheritance. For LB, isolates TH16 and THL80, the distributions of DS were bimodal, suggesting that the number of resistant vs. susceptible progeny of Baronesse is determined by one gene. For isolate THL142 and THL222 were skewed towards resistance and NB, the distribution of DS of all blast isolates were skewed towards susceptible. The numbers of resistant vs. susceptible progeny suggest that the resistance of Baronesse is determined by two or three genes with additive effects, or to a single gene with large effects and several lesser-effects QTL. The h^2 of DS on an entry mean basis of LB and NB was 89 and 86%, respectively, confirming the high level of repeatability of the DS data and providing a sound basis for mapping the resistance genes.

The number, allele phase, and interaction of the blast resistance QTL identified in response to inoculation with each of the four isolates in LB and NB are presented in Table 7, 8 and Figure 2. A total of four QTL were detected. The QTL located on chromosomes 1H, 4H and 7Hb had LOD scores ranging from 2.8 to 22.6 (Table 7 and 8). Baronesse contributed resistance alleles at two of the QTL (chromosomes 1H, and 7Hb). BCD47 contributed the resistance allele at the QTL on chromosome 4H. As detailed in the subsequent sections, there are prior reports of *R* genes and/or QTL forming resistance gene clusters at each of the four genomic regions (Chen *et al*., 2003; Inukai *et al*. 2006; Sato *et al*., 2001).

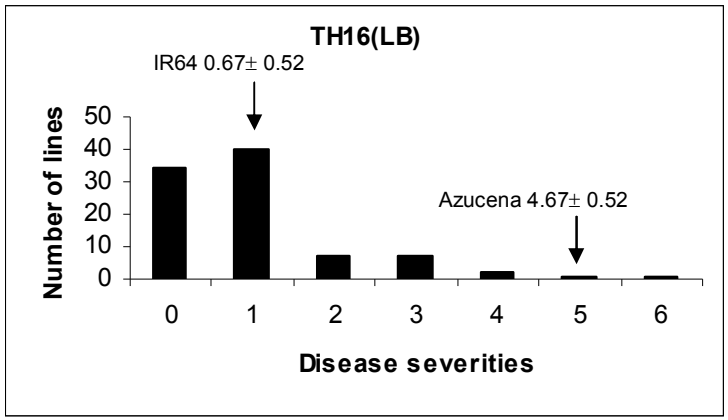
A QTL on chromosome 1H (k04435 – Bmac399) was associated with resistance to all four isolates in LB and NB. For LB, alleles at this QTL determined 62, 59, 47 and 42 % of the phenotypic variance in resistance to TH16, THL80, THL142 and THL222, respectively. For NB, alleles at this QTL determined 71, 63, 12 and 26 % of the phenotypic variance in resistance to TH16, THL80, THL142 and THL222, respectively. The position of this QTL is coincident with *Mla*, a gene that confer resistant to powdery mildew (*Blumeria graminis* f. sp. *Hordei*). From previous study was reported Baronesse carry *Mla3* resistance, based on reaction to inoculation with differential isolates (Dreiseitl, 2003; Hovmøller *et al*., 2000)

Two QTL on chromosome 4H (HVMO3-Bmac030B and EBmac701-k07229) were identified with isolates THL222 and were detected only in LB (Table 7 and Fig. 13). Those QTL accounted for 6 and 3 % of the phenotypic variance in resistance to THL222. Baronesse is the resistance allele contributor for both QTL. The position of QTL that located between markers EBmac701 and k07229 is coincident with *Mlo*, a gene that confers broad spectrum resistance to powdery mildew

One QTL on chromosome 7Hb, accounting for 13 and 10% of the phenotypic variance, was associated with resistance to isolate TH16 and THL80, respectively (Table 8 and Fig. 13). This QTL was detected only in NB. BCD47 was contributed resistant alleles. Inukai *et al*. (2006) mapped a QTL in BCD47/Baronesse to this same location, which is also coincident with the positions of *Mlf*, a gene that confer resistant to powdery mildew. In addition to we detected this QTL only inoculated with the isolated obtained from barley.

The results of the QTL analysis support our interpretation of the phenotypic frequency distributions regarding the number of genes determining resistance to the four isolates in LB and NB in this population. The resistance allele at the chromosome 1H QTL was sufficient for resistance to all four isolates in LB and NB. This QTL may be an effect of the *Mla* gene, or of another tightly linked gene, or genes, in this apparent gene cluster. This genomic region in Baronesse is an important source of broad-spectrum resistance to many diseases such as powdery mildew and stripe rust which obtained from barley.

Our results are novel in adding the new phase of rice blast screening in two phase; LB and NB and adding blast isolates from barley to the catalog of pathogen specificities to which a gene, or genes, from Baronesse confer resistanc. If only this region of Baronesse was associated with resistance, we would have observed a bimodal phenotypic distribution of resistance and susceptibility. In addition to we found minor specific QTL only on chromosome 7H in NB the inoculated with isolates obtained from rice. The resistance allele at the chromosome 4H QTL, which traced to the susceptible parent, is not alone sufficient to confer resistance but when combined with another resistance allele, it is. The allele from Baronesse associated with resistance to the rice blast isolate could be of scientific interest from the standpoint of non-host resistance and of practical importance in cases where barley and rice are grown in rotation.



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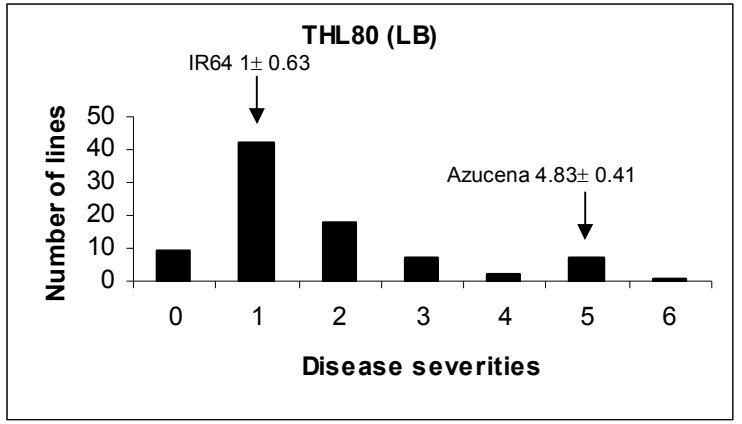

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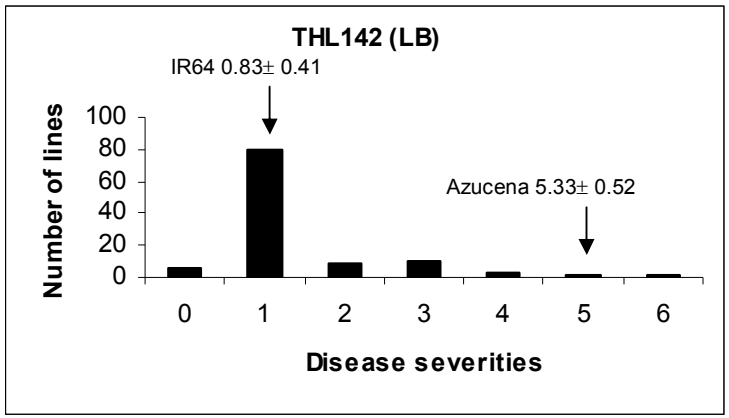

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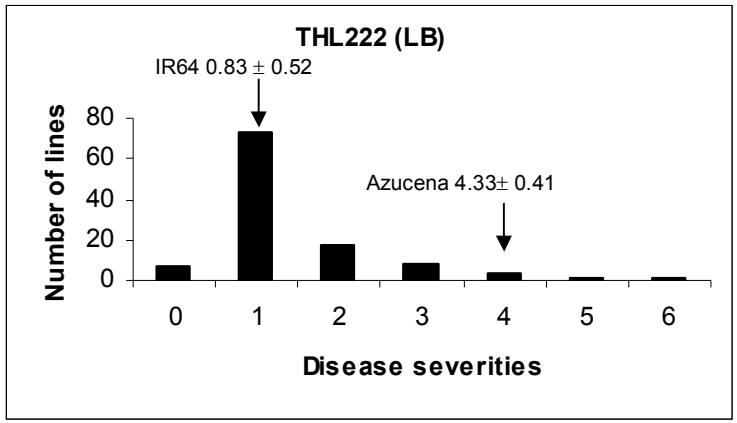

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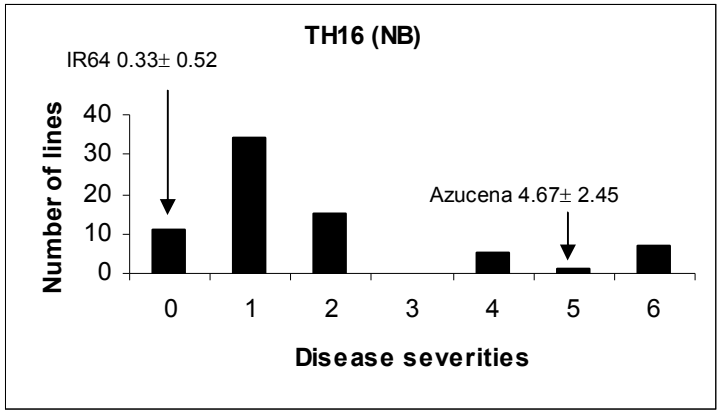
Figure 8 Phenotypic distributions for rice leaf blast disease severity.



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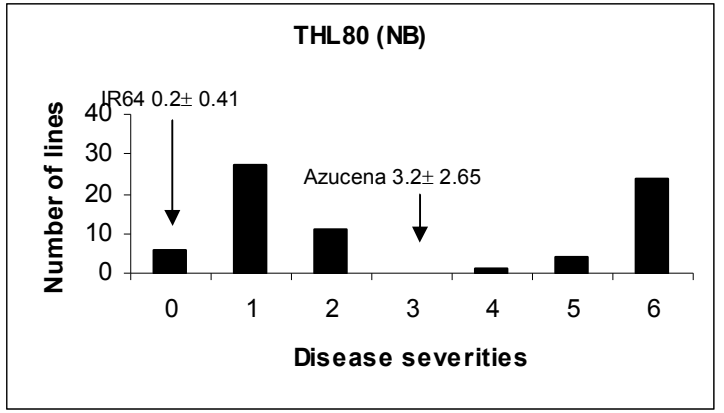

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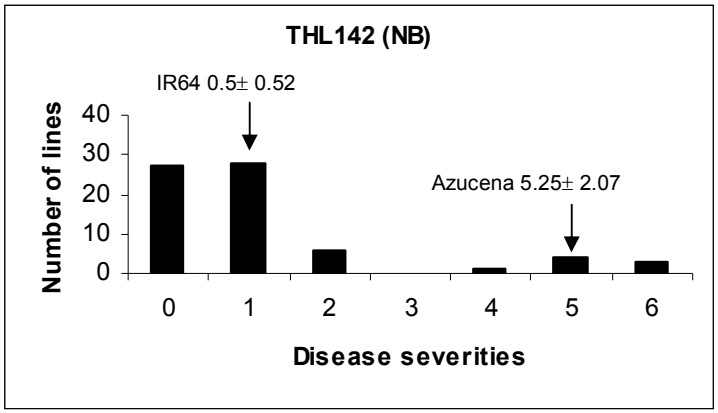

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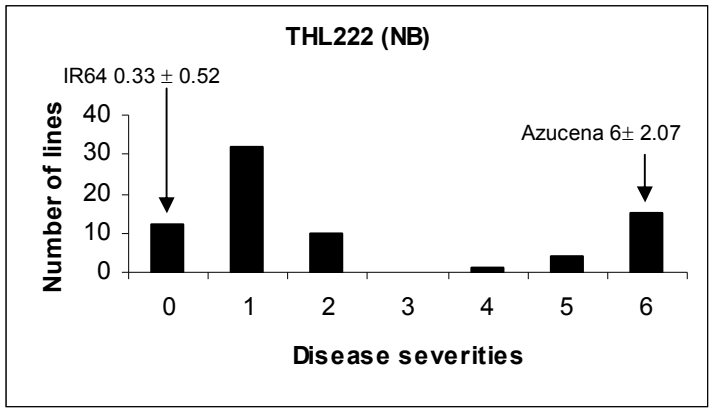

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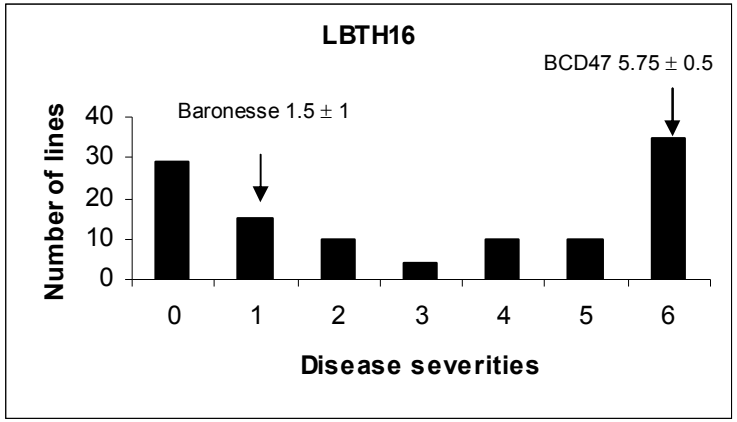
Figure 9 Phenotypic distributions for rice neck blast disease severity.



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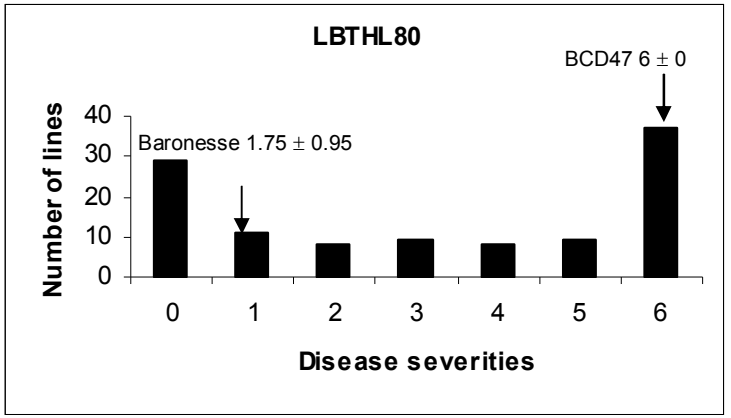

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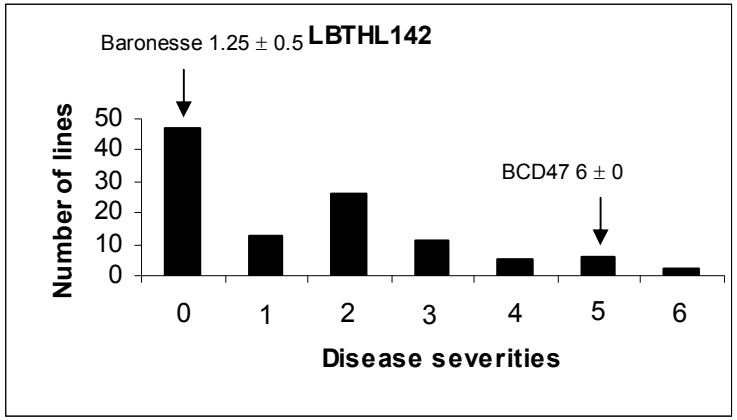

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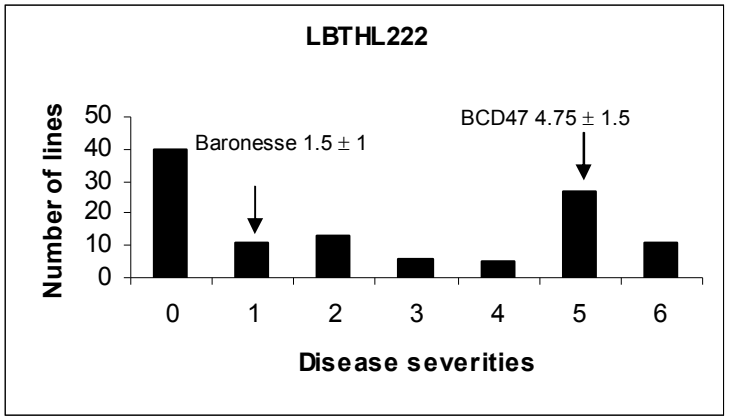

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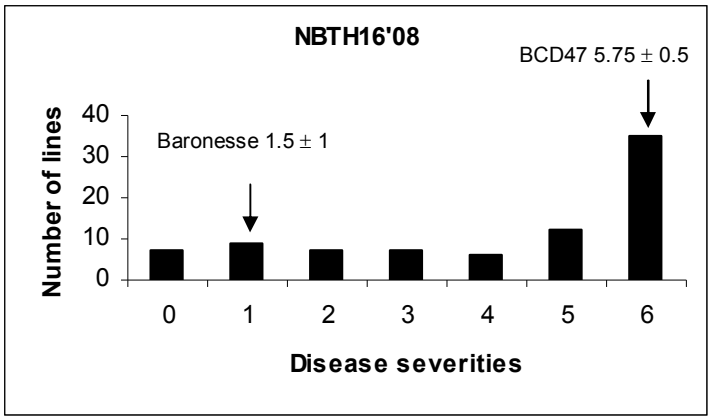
Figure 10 Phenotypic distributions for barley leaf blast disease severity.



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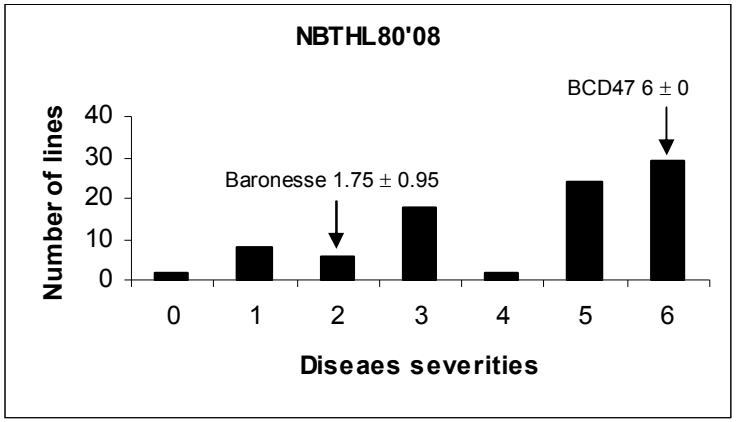

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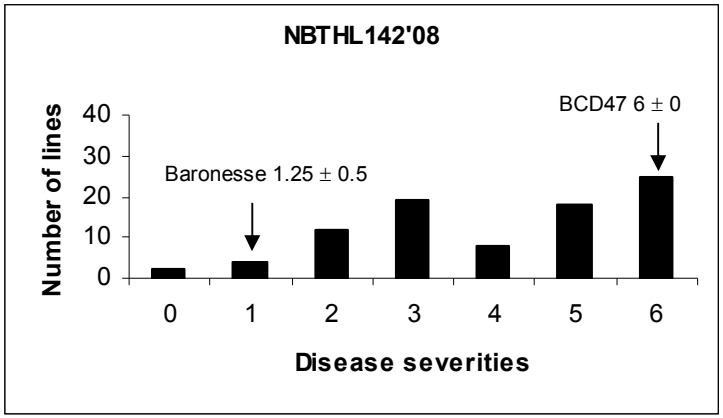

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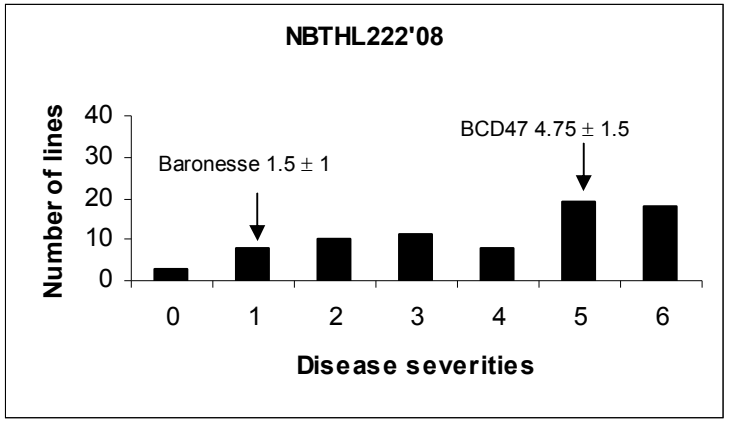

D

Figure 11 Phenotypic distributions for barley neck blast disease severity on 2008.

Table 5 Four Loci identified for leaf blast resistance to four blast isolates by QTL approach in DHLs from Azucena/IR64 population.

Traits	QTL No.	Chrom.	Peak position (2 LOD conf. interv.)	Closet marker	LOD Score	Additive effect	R^2	Resistance contributor
LBTH16	1	2	74.2 (56.9-74.2)	RZ123	3.88	-0.57	9.9	IR64
	2	8	69.2 (28.9-93.5)	RG1	5.96	-0.45	15.3	IR64
LBTHL80	1	2	74.2 (50.4-74.2)	RZ123	2.91	-0.62	9	IR64
	2	8	73.2 (65.2-73.2)	RG1	5.73	-0.79	22.9	IR64
LBTHL142	1	2	74.2 (47.7-74.2)	RZ123	8.7	-0.45	19.6	IR64
	2	9	109 (37.8-109)	RM215	3.53	-0.29	7.8	IR64
	3	12	66.8 (61.6-66.8)	Sdh-1	3.5	-0.29	8.8	IR64
LBTHL222	1	2	73.1 (64.1-73.1)	RZ213	4.22	-0.35	10.2	IR64
	2	8	69.2 (61.1-69.2)	RG1	5.23	-0.38	13.3	IR64
	3	11	49.8 (49.8-138.1)	RM260	3.14	0.32	8.1	Azucena

Table 6 Two Loci identified for neck blast resistance to four blast isolates by QTL approach in DHL from Azucena/IR64 population.

Traits	QTL No.	Chrom.	Peak position (2 LOD conf. interv.)	Closet marker	LOD Score	Additive effect	R^2	Resistance contributor
NBTH16	1	8	51.5 (39.5-51.5)	RZ617	3.93	-0.56	10.7	IR64
NBTHL80	1	8	53.5 (47.9-53.5)	RZ617	3.31	-0.89	11.9	IR64
NBTHL142	1	12	48.5 (37.3-48.5)	AF6	4.86	-0.48	12.4	IR64
NBTHL222	1	8	47.7 (47.7-53.5)	A10K250	3.15	-0.6	7.7	IR64

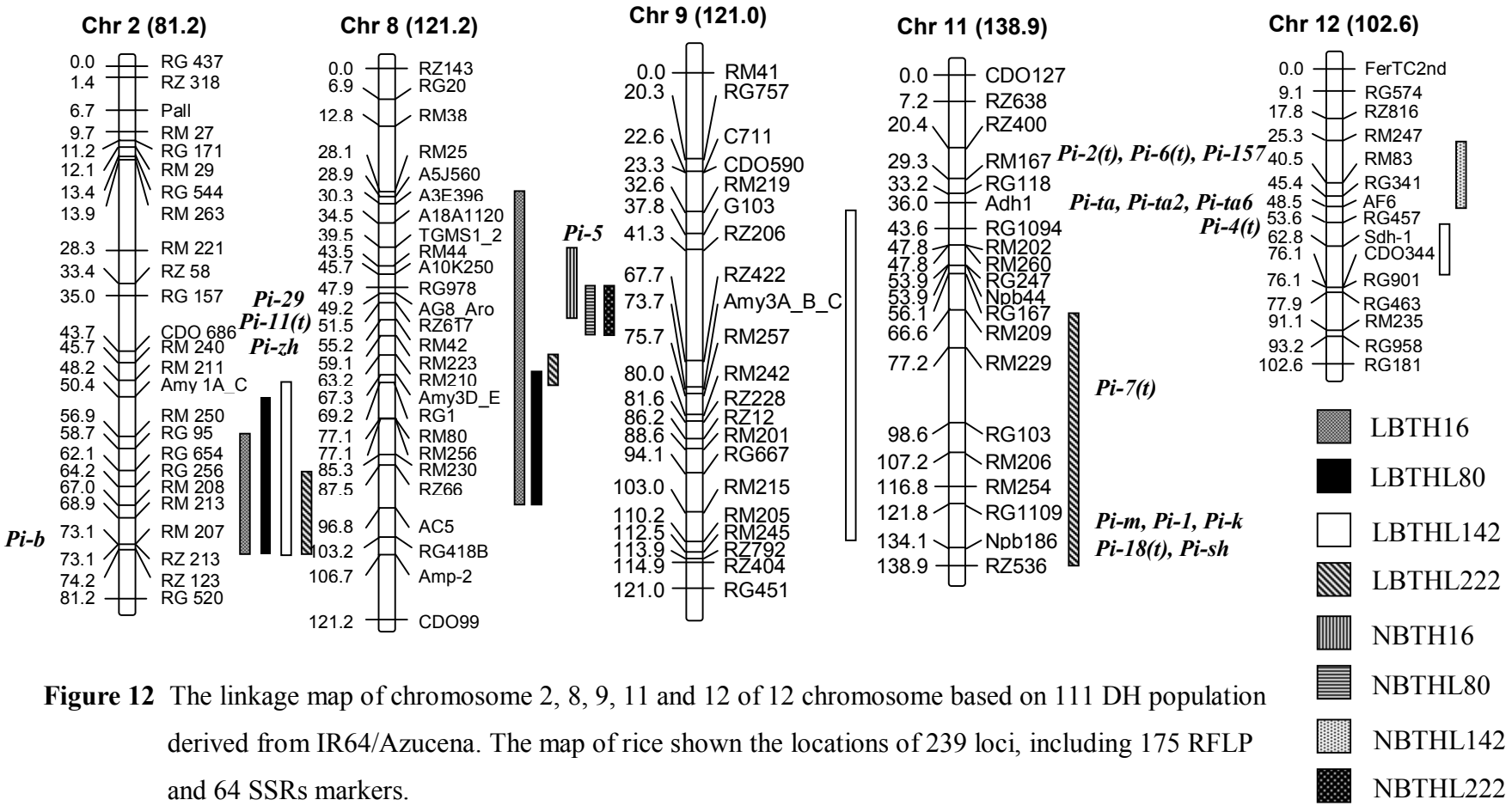

Figure 12 The linkage map of chromosome 2, 8, 9, 11 and 12 of 12 chromosome based on 111 DH population derived from IR64/Azucena. The map of rice shown the locations of 239 loci, including 175 RFLP and 64 SSRs markers.

Table 7 Three loci identified for leaf blast resistance to four blast isolates by QTL approach in DHLs from Baronesse/BCD47 population.

Traits	QTL No.	Chrom.	Peak position (2 LOD conf. interv.)	Marker interv.	LOD Score	Additive effect	R^2	Resistance contributor
LBTH16	1	1H	9.1 (0.2-48.8)	GMS21	22.4	1.7	0.65	Baronesse
LBTHL80	1	1H	9.1 (0.1-50.6)	GMS21	26.5	2.0	0.69	Baronesse
LBTHL142	1	1H	9.1 (2.0-44.9)	GMS21	15.8	1.1	0.51	Baronesse
LBTHL222	1	1H	9.1 (1.4-50.6)	GMS21	27.0	1.2	0.71	Baronesse
	2	4H	44.6 (36.6-54.6)	Bmac030B	5.6	-0.4	0.06	BCD47
	3	4H	105.3 (89.6-116.9)	K03352	2.9	-0.2	0.03	BCD47

Table 8 Two loci identified for leaf blast resistance to four blast isolates by QTL approach in DHLs from Baronesse/BCD47 population.

Traits	QTL No.	Chrom.	Peak position (2 LOD conf. interv.)	Marker interv.	LOD Score	Additive effect	R^2	Resistance contributor
NBTH16	1	1H	9.1 (0.3-46.2)	GMS21	9.7	1.2	0.42	Baronesse
	2	7Hb	103.6 (93.5-104.0)	Bmac135	3.6	0.6	0.13	Baronesse
NBTHL80	1	1H	24.9 (6.4-46.9)	Bmac399	8.0	1.2	0.37	Baronesse
	2	7Hb	95.6 (78.8-101.3)	Bmac156	3.1	0.5	0.10	Baronesse
NBTHL142	1	1H	7.3 (1.6-23.4)	k08302	4.1	0.6	0.19	Baronesse
NBTHL222	1	1H	34.9 (6.9-52.3)	Bmac399	6.1	1.1	0.48	Baronesse

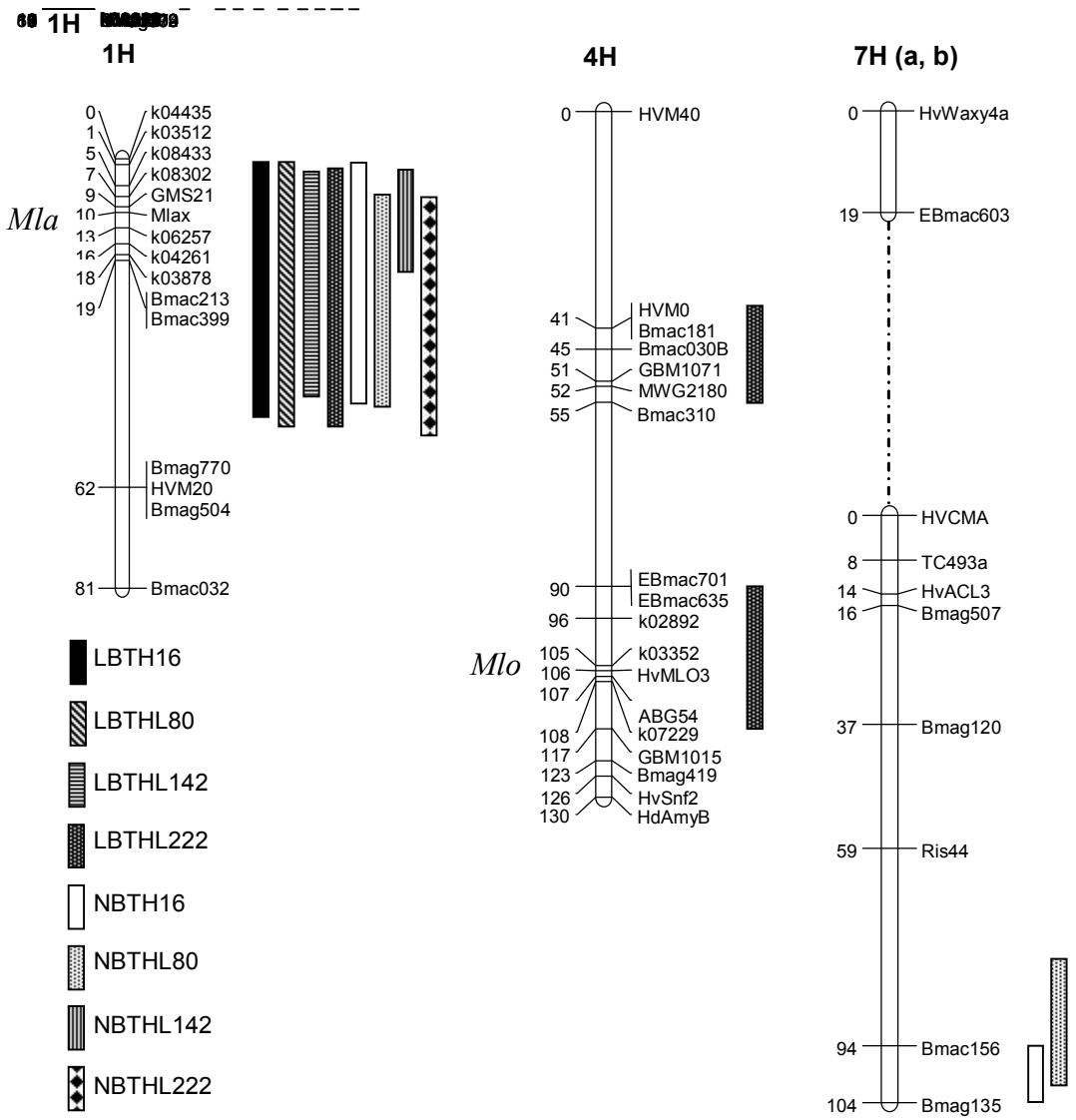

Figure 13 Linkage maps of chromosomes 1H, 4H, and 7Hb in the BCD47/Baronesse mapping population. Distances are in Kosambi cM. The bars to the right of each linkage group indicate 2-LOD confidence intervals for leaf blast(LB) and neck blast (NB) resistance QTL detected in response to inoculation with each of four isolates (TH16, THL80, THL142, and THL222).

Disease assessment for Isogenic lines

We used isogenic lines for BSR QTL to validate the effect of blast resistant genes in barley. BCD12, BCD47, BISON1H, and BISON1H+4H+5H showed susceptibility to all blast isolates. Baronesse, BISON4H, BISON5H, BISON7H, BISON1H+4H, and BISON1H+5H showed resistance to all blast isolates (Table 9).

Table 9 Barley blast disease severity for BISON lines and parents

Accession	Resistant alleles	TH16	THL80	THL142	THL222
BCD12	1H	S	S	R	R
D3-61B23	7H	R	R	R	R
CI10587	7H	R	R	R	R
Baronesse	2H, 5H, 7H	R	R	R	R
BCD47	3H, 4H, 5H, 6H	S	S	S	S
BISON 0-QTL	control	R	R	R	R
BISON1H	1H	S	S	S	S
BISON4H	4H	R	R	R	R
BISON5H	5H	S	R	R	R
BISON7H	7H	R	R	R	R
BISON1H+4H	1H, 4H	R	R	R	R
BISON1H+5H	1H, 5H	R	R	R	R
BISON4H+5H	4H, 5H	R	R	R	R
BISON1H+4H+5H	1H, 4H, 5H	S	S	S	S

Association between haplotype and phenotype of BISON lines

The genotype of BSR BISON lines focused only on the region of chromosome 1H containing the major QTL showed in Table 10. Baronesse and BCD47 are the parents. BISON1H, we found introgression from BCD12 in Baronesse background. BISON4H, BISON5H, BISON7H, and BISON4H+5H have no introgression on the region of chromosome 1H, so in this region those lines have the Baronesse background. BISON1H+4H has introgression from BCD12 which the same fragment that we found in BISON1H. BISON1H+5H, we found only one introgression in the region of marker

2_0749 that from BCD12. BISON1H+4H+5H, we found introgression from BCD12 only in the genetic distance 0.0 cM to 23.9 cM.

To compare the phenotypic data with the graphical genotype, we focused only on the region of chromosome 1H containing the major QTL. From previous studies reported this region coincident with *R* genes which confer resistance to many pathogens that causes disease to barley such as stripe rust and powdery mildew (Castro *et al*., 2003; Inukai *et al*., 2006.) The graphical genotype of BISON1H and BISON1H+4H+5H that showed Baronesse is genetic back ground, we found introgressions from BCD12 that has susceptible alleles to blast isolates can cause them susceptible to the blast disease. Apparently, BISON4H, BISON5H, BISON7H, and BISON1H+5H have no introgressions or susceptible alleles from BCD12 in this region. So they showed resistance to all blast isolates. From this result support interpretation of the QTL result on chromosome 1H that Baronesse is the resistance allele contributor.

BISON1H+4H is a special case, since the graphical genotype shows introgression from BCD12 which should make it susceptible to blast isolates. However, this line showed resistant to all blast isolates. The explanation for this could be that the genes causing resistance or resistance alleles are located in an interval where no markers are available, and despite the fact that this line shows introgressions from BCD12. Perhaps we have not detected that region containing the resistance alleles, or this line could be still carrying the resistance alleles from Baronesse or we have to consider on another chromosome that have fragment that can cause resistance to blast isolates for this line.

Table 10 Graphical genotype of Isogenic lines focused on chromosome 1H

Chromosome	centrimogan	Close_index	Baronesse	BISON1H	BISON4H	BISON5H	BISON7H	BISON1H+5H	BISON1H+4H	BISON4H+5H	BISON1H+4H+5H	BCD12	BCD47
1H	0.8	2_1354	A	B	A	A	A	A	B	A	B	B	A
1H	1.5	2_0502	A	B	A	A	A	A	B	A	B	B	A
1H	1.5	2_0836	A	A	A	A	A	A	A	A	A	A	B
1H	3.8	1_0419	A	A	A	A	A	A	A	A	A	A	B
1H	6	3_0933	A	B	A	A	A	A	B	A	B	B	B
1H	8.3	2_1174	A	A	A	A	A	A	A	A	A	A	B
1H	8.8	2_1226	A	B	A	A	A	A	B	A	B	B	A
1H	10.7	3_0817	A	B	A	A	A	A	B	A	B	B	A
1H	10.7	3_0919	A	A	A	A	A	A	A	A	A	A	B
1H	11.4	3_0951	A	A	A	A	A	A	A	A	A	A	B
1H	11.4	3_0952	A	B	A	A	A	A	B	A	B	B	A
1H	15.3	3_0955	A	B	A	A	A	A	B	A	B	B	B
1H	15.4	1_0332	A	B	A	A	A	A	B	A	B	B	A
1H	17.3	2_0749	A	B	A	A	A	B	B	A	B	B	A
1H	18.1	1_0030	A	A	A	A	A	A	A	A	A	A	B
1H	18.6	3_0876	A	B	A	A	A	A	B	A	B	B	-
1H	20.8	2_0712	A	B	A	A	A	A	B	A	B	B	B
1H	20.9	1_0873	A	B	A	A	A	A	B	A	B	B	B
1H	23.9	2_0767	A	B	A	A	A	A	B	A	B	B	B
1H	26.1	1_0744	A	B	A	A	A	A	B	A	A	B	B
1H	31.2	2_1233	A	A	A	A	A	A	B	A	A	B	B
1H	34.8	1_0760	A	A	A	A	A	A	B	A	A	B	B
1H	35.5	1_0814	A	A	A	A	A	A	B	A	A	B	B
1H	37	3_1177	A	BB	A	A	A	A	B	A	A	B	B
1H	41	1_0764	A	A	A	A	A	A	B	A	A	B	B
1H	41.8	3_0336	A	-	A	A	A	A	B	A	A	B	B
1H	42.5	2_0514	A	-	A	A	A	A	B	A	A	B	B
1H	47.5	1_0259	A	A	A	A	A	A	B	A	A	B	B
1H	50	2_0427	A	A	A	A	A	A	B	A	A	B	B
1H	51.2	1_1484	A	A	A	A	A	A	B	A	A	B	B
1H	51.7	2_1357	A	A	A	A	A	A	B	A	A	B	B
1H	52.5	1_0833	A	A	A	A	A	A	B	A	A	B	B
1H	53.2	3_0522	A	A	A	A	A	A	B	A	A	B	B
1H	54.7	1_1359	A	A	A	A	A	A	B	A	A	B	B
1H	54.7	2_1217	A	A	A	A	A	A	B	A	A	B	B
1H	55.5	2_0798	A	A	A	A	A	A	B	A	A	B	B

Candidate genes for the QTL conferring resistance to the rice blast isolates, TH16, THL80, THL222 and THL142

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 2, 8, 9, 11 and 12 that confer partial resistance against rice blast isolates, TH16, THL80, THL142 and THL222 in LB and NB were examined for possible candidate genes.

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 located in the interval between RM250 and RZ123 (~17.3 cM.) on chromosome 2, included three NBS-LRR gene (Table 11). The second QTL mapped in the interval between RM25 and RM256 (~ 117 Mb.) on chromosome 8 which included ten NBS-LRR and receptor-like kinase Xa21-binding protein 3-like (Table 11). The QTL interval on chromosome 9 contained two NBS-LRR proteins and three defense genes (Table 11). The QTL on chromosome 11 harbors 27 predicted NBS-LRR proteins, one defense gene and one JAMyb-family transcription factor (Table 11). Finally, the QTL on chromosome 12 in the interval between RM247 and Sdh-1 included 18 predicted NBS-LRR proteins (Table 11).

Table 11 Candidate NBS-LRR and defense genes in the QTL regions conferring resistance to rice blast isolates, TH16, THL80, THL142 and THL222 of *P. grisea* on chromosomes 2, 8, 9, 11 and 12. The SSR markers used in this study are also shown.

Marker	Chr.	bp	Gene	Protein
RM207	2	17544000		
57310	2	35112900	LOC_Os02g57310	NBS-LRR
RZ213	2	35367861		
57960	2	35495167	LOC_Os02g57960	NBS-LRR
58030	2	35527222	LOC_Os02g58030	NBS-LRR
RM25	8	6744000		
14830	8	8920104	LOC_Os08g14830	NBS-LRR
14850	8	8933136	LOC_Os08g14850	NBS-LRR
14950	8	9010613	LOC_Os08g14950	NBS-LRR
15840	8	9627924	LOC_Os08g15840	receptor-like kinase
15880	8	9665037	LOC_Os08g15880	NBS-LRR
16070	8	9792238	LOC_Os08g16070	NBS-LRR
16120	8	9818284	LOC_Os08g16120	NBS-LRR
16450	8	10065520	LOC_Os08g16450	NBS-LRR
16460	8	10071081	LOC_Os08g16460	NBS-LRR
RM44	8	10512000		
19694	8	11791873	LOC_Os08g19694	NBS-LRR
24380	8	14588483	LOC_Os08g24380	NBS-LRR
RM256	8	18504000		
RM257	9	16248000		
RM242	9	17544000		
10232	9	19814069	LOC_Os09g34150	NBS-LRR
10233	9	19819867	LOC_Os09g34160	NBS-LRR
RM201	9	20688000		
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
RM206	11	25728000		
10329	11	25831137	LOC_Os11g43700	NBS-LRR
10330	11	26406046	LOC_Os11g44580	NBS-LRR
10331	11	26672305	LOC_Os11g44960	NBS-LRR
10332	11	26684649	LOC_Os11g44970	NBS-LRR
10333	11	26711543	LOC_Os11g45050	NBS-LRR
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

Table 11 (Continued)

Marker	Chr.	bp	Gene	Protein
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
RM206	11	28032000		
10355	11	28169486	LOC_Os11g47780	NBS-LRR
10864	11	28179212	LOC_Os11g47810	metallothionein-like protein
RZ539	11	33336000		
10390	12	5499710	LOC_Os12g10390	NBS-LRR
10400	12	5500645	LOC_Os12g10400	NBS-LRR
10410	12	5514002	LOC_Os12g10410	NBS-LRR
10460	12	5544299	LOC_Os12g10460	NBS-LRR
10710	12	5751976	LOC_Os12g10710	NBS-LRR
RM247	12	6072000		
11860	12	6446148	LOC_Os12g11860	NBS-LRR
11930	12	6505399	LOC_Os12g11930	NBS-LRR
12010	12	6582236	LOC_Os12g12010	NBS-LRR
12120	12	6646206	LOC_Os12g12120	NBS-LRR
12130	12	6649801	LOC_Os12g12130	NBS-LRR
13550	12	7599451	LOC_Os12g13550	NBS-LRR
RM83	12	9720000		
17090	12	9784163	LOC_Os12g17090	NBS-LRR
17140	12	9809880	LOC_Os12g17140	NBS_LRR
17410	12	9982060	LOC_Os12g17410	NBS-LRR
17420	12	9985325	LOC_Os12g17420	NBS-LRR
17430	12	9993859	LOC_Os12g17430	NBS-LRR
17480	12	10011703	LOC_Os12g17480	NBS-LRR
17490	12	10018771	LOC_Os12g17490	NBS-LRR
Sdh-1	12	15072000		

Syntenic relationship of loci for quantitative resistance to *P. grisea* in rice and barley

Based on the QTL mapping results of four blast isolates (TH16, THL80, THL142 and THL222), the QTL peak position of all isolates false in marker interval GMS21 to Bmac399 on chromosome 1H (LOD score = 22.56, 18.52, 13.19 and 13.1, respectively) and Baronesse is the resistant contributor, that region cover with the sequence Tagged Site (STS) marker Mlax – based on the published sequence of *Mla*7- a gene which confers resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*)(Inukai *et al*, 2006). Inukai and associates (2006) was designated the race-specific resistance gene in Baronesse to rice blast isolates with co-segregate with Mlax as *RMo*1 (Resistance to Magnaporthe oryzae 1). From the Blast homology search of the marker, k04230, that co-segregated with Mlax showed corresponds to the barley gene 711N16.16 (e-value=0) which is reported to be 190kb from *Mla* (Wei *et al*., 2002). This establishes the position of k04320 as lying within, or near, the *Mla* - 711N16.16 interval (Inukai *et al*, 2006). Baronesse is reported to carry *Mla*3 resistance, based on reaction to inoculation with differential isolates (Hovmøller *et al*. 2000; Dreiseitl 2003). *Mla7* and *Mla3* are members of the *Mla* complex locus. Based on linkage data, *RMo*1 cosegregates with *Mla* (Inukai *et al*, 2006).

In order to established a basis for synteny comparison with rice. We used this information to explore the syntenous region in rice, based on the TIGR Rice Genome Annotation database (http://www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice/). Five barley EST markers in the vicinity of Mlax showed high homology to sequences on rice chromosome 5. There is co-linearity of gene order between the 19 cM region extending from k04435 to k03878 in barley chromosome 1H and the 510 kb region from the gene Os05g01300 to Os05g02220 in the end of rice chromosome 5 (Figure. 14). According to the annotation data in TIGR rice genome database, there are one NBS-LRR genes and eight genes that for proteins commonly induced in defense reactions in this region (Table12): a bacterial blight resistance gene *xa*5 (Os05g01710), which encodes the gamma subunit of transcription factor IIA (TFIIAγ) (Iyer and McCouch 2004). TFIIA, a component of the RNA polymerase II holoenzyme complex, facilitates binding of the polymerase to the TATA-box binding protein (Gill 2001). Although none of the basal transcription factors, such as TFIIa, are known to be involved in disease response they have been shown to be targets of signal transduction pathways that regulate genome

expression in other eukaryotic organisms. In addition, several other transcription factors from the WRKY (Deslandes *et al*., 2002) and EREBP families (Sessa and Martin 2000) are important for plant disease resistance. The other five putatively identified candidate genes included a hypothetical protein, Drought induced 19 protein (Di19), an ATP-binding cassette protein (ABC transporter), a tRNA pseudouridine synthase, ABC transporters are a large family of proteins found in a diverse array of organisms such as bacteria, humans and plants (Davies and Coleman 2000). They transport a variety of molecules across biological membranes and are typically composed of two ATP binding domains and two transmembrane domains, which can be found in 1–4 peptides. The putative transporter described here was similar to the Arabidopsis PXA1 peroxisomal membrane protein; however, this gene in rice does not appear to contain any transmembrane domains.

Table 12 Candidate NBS-LRR and defense genes in the syntenous regions in rice

Gene	Protein
LOC_Os05g01300	CHCH domain containing protein, expressed
LOC_Os05g01360	expressed protein
LOC_Os05g01700	PXA1, putative, expressed
LOC_Os05g01710	TFIIA gamma chain, putative, expressed
LOC_Os05g01720	hypothitical protein
LOC_Os05g01730	Drought induced 19 protein (Di19)
LOC_Os05g01750	tRNA pseudouridine synthase B
LOC_Os05g01990	ATP binding protein, putative, expressed
LOC_Os05g02220	amino acid binding protein, putative, expressed

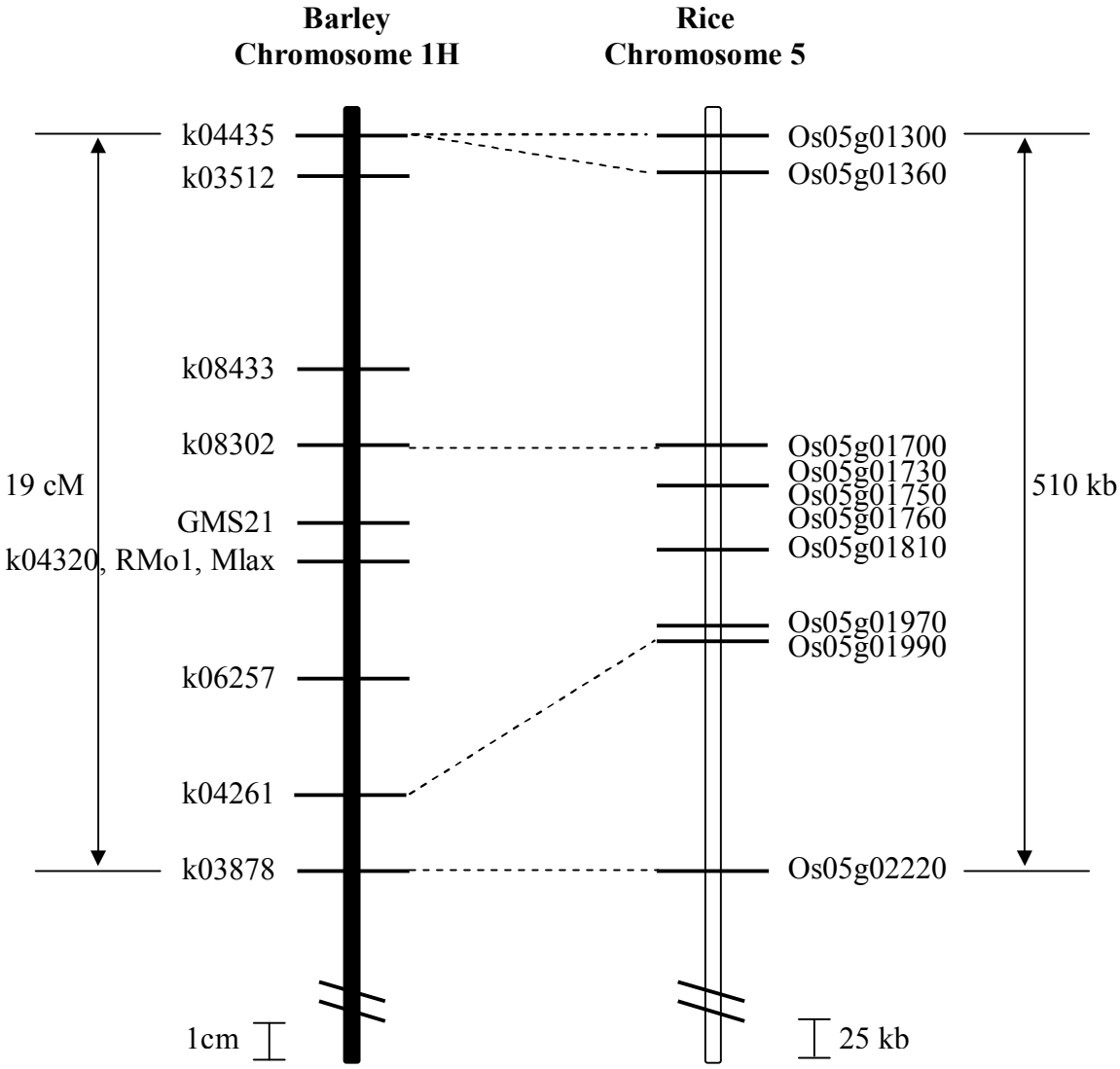

Figure 14 Synteny between the region of barley chromosome 1H and the subtelomeric region of rice chromosome 5 is indicated. The genetic map of barley chromosome 1H and the physical map of rice chromosome 5 are aligned. In the barley genetic map, the k – loci is barley EST-based markers. Mlax is the Sequence Tagged Site (STS) marker based on the published sequence of *Mla*7 and GMS21 is a microsatellite marker (Vales *et al*., 2005). Five EST markers showed high homology to genes in the subtelomeric 510kb region of rice chromosome 5, and the order of these genes is conserved.

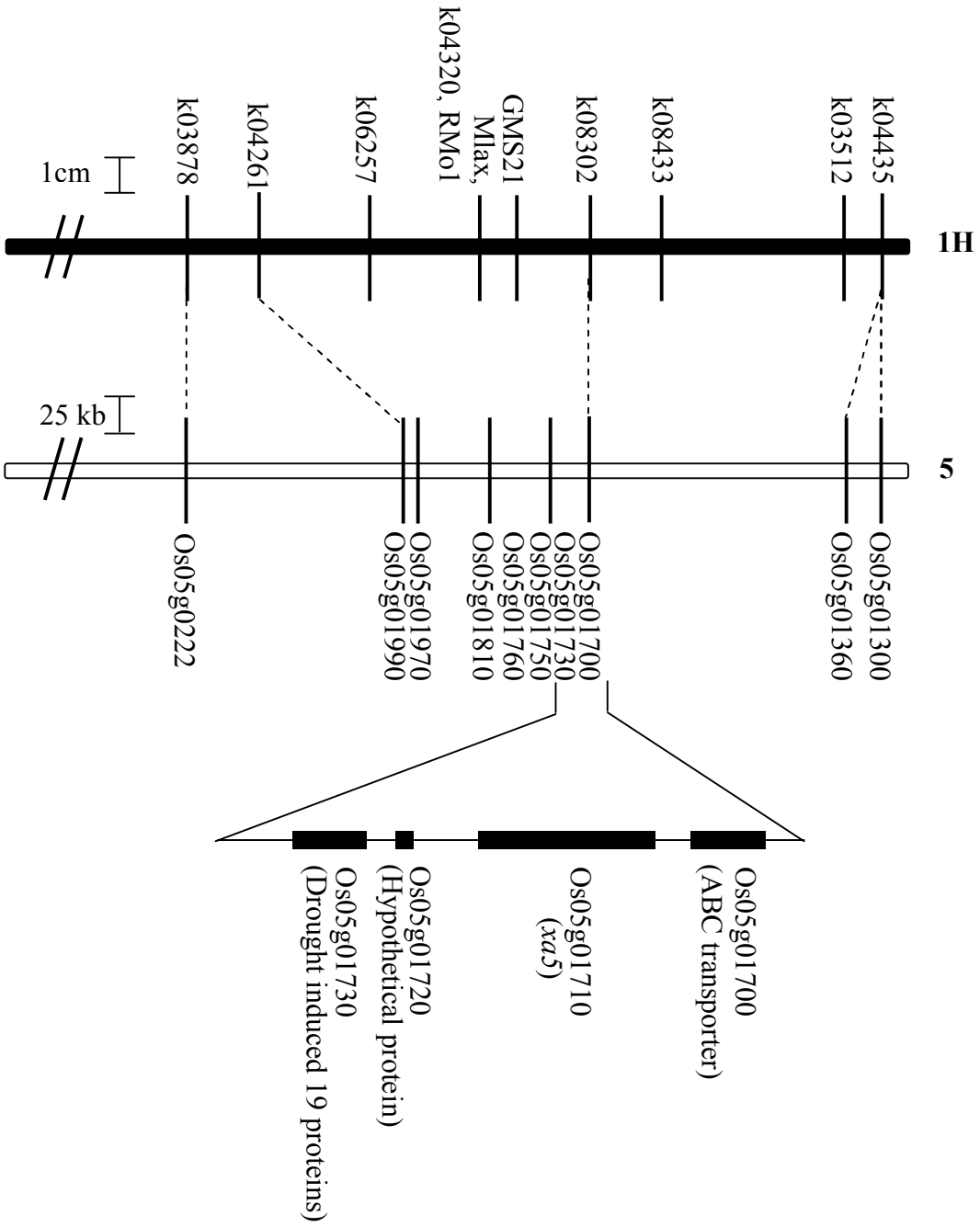

Figure 15 Genome structure of the rice region contained genes corresponding to barley EST markers k08432 and CA027247

Discussion

Blast resistance in the Azucena/IR64 DHL population was found to have very complex inheritance. Five and two resistance QTLs were identified on LB and NB, respectively that were statistically significant in 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. $R^2 < 10\%$). 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 stages 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; Rossi *et al*., 2006).

Many of QTLs on rice LB and NB identified in this study mapped to regions where blast resistance traits had previously been mapped. Seven QTLs that were detected in both stages 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 Azucena/IR64 DHL population and are therefore good candidates for the genes controlling the resistance. QTL had been previously mapped to most of the other QTL identified in this population; including putative QTL that only had significant effects in one stage. These regions include: QTL on chromosome 9 and 11 are located in the same regions as QTL for resistance to rice blast identified in a cross between cultivars Zhenshan 97 and Minghui 63 (Chen *et al*., 2003). Four QTLs 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 QTLs were also identified in a double haploid (DH)

population derived from an IR64 by Azucena cross (Sallaud *et al*., 2003). The QTL on chromosome 12 occurred at the same positions as QTL for blast resistance in a RIL derived from a Bala x Azucena cross (Talukder *et al*., 2004). Two QTLs (on chromosome 9 and 12) occurred at the same locations as the four blast resistance QTL mapped in a Nipponbare x Owarihatamochi cross (Fukuoka and Okuno, 2001). One QTL on chromosome 2 coincided with the QTL mapped in a cross between Zhong 156 and Gumei 2 (Wu *et al*., 2005).

The results of the QTL analysis support our interpretation of the phenotypic frequency distributions regarding the number of genes determining resistance to the four isolates in this population. The resistance allele at the chromosome 2 QTL was sufficient for resistance to all four isolates. This QTL may be an effect of the *Pib* gene, or of another tightly linked gene, or genes, in this apparent gene cluster. This genomic region in IR64 is an important source of broad-spectrum resistance to blast isolates obtained from rice. Our results are novel in adding blast isolates from barley to the catalog of pathogen specificities to which a gene, or genes, from IR64 confer resistance. If only this region of IR64 was associated with resistance, we would have observed a bimodal phenotypic distribution of resistance and susceptibility. The skewness of the phenotypic frequency distributions toward resistance may be attributed to the main and combined effects of the smaller-effect QTL on chromosomes 8, 9. The resistance allele at the chromosome 10 QTL, which traced to the susceptible parent, is not alone sufficient to confer resistance but when combined with another resistance allele, it is. For example, there was a significant positive interaction between alleles at the chromosome 2 and chromosome 10 loci (Table 5). The apparent isolate specificities of these three lesser-effect QTL alleles may have a biological host: pathogen basis or they may be an artifact due to the relatively small size of the mapping population (Vales *et al*., 2005). In any event, three genome regions in IR64 are useful targets for introgression into Thai rice varieties in order to achieve resistance to the spectrum of rice and barley blast represented by the four isolates. The allele from Azucena associated with resistance to the barley blast isolate could be of scientific interest from the standpoint of non-host resistance and of practical importance in cases where barley and rice are grown in rotation.

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 Azucena/IR64 DHL population. Most of the experiment- specific QTL in the present study were not significant in both stages and all blast isolates even when the statistical criterion was relaxed (e.g. $P < 0.01$ to $P< 0.05$). 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 (Wang *et al*., 1994; Jeon *et al*., 2003). Race specific genes like *Pi-1*, *Pi-2*, *Pi-9*, and *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), the Korean cultivar Suweon 365 (Ahn *et al*., 2000) and the Chinese cultivar Sanhuangzhan 2 (Liu *et al*., 2004). 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 Azucena/IR64 DHL population 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.

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 seven genomic regions conferring resistance to four isolate in both stages were examined, NBS-LRR genes were found in all seven regions, leading the idea that *R* genes would be involve in the QTL observed. In fact the QTL region on chromosome 11 contains 23 NBS-LRR in a genomic region of ~ 1.8 Mb and several races 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 a 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.

The barley cultivar Baronesse shows nearly complete and race-specific resistance to the rice blast fungus. This resistance is determined by a single dominant gene located on chromosome 1H at position of *Mla*. This gene co-segregates with *Mla* and 711N16.16 which is ~ 190kb from *Mla* according to Wei *et al*. (2002). These authors reported that 15 of the 32 predicted genes in the 261 kb region sequenced around *Mla* are associated with defense responses to pathogens. These genes were classified into five structurally unique, non cross-hybridizing families. These included (1) three CC-NBS-LRR *RGH* families, one of which includes *Mla*; (2) one chymotrypsin inhibitor 2 (Cl2) family; and (3) one chemically-induced family. Three of the five families' likely function in at least two dependent pathways associated with powdery mildew defense response, including the *Mla* gene-for-gene pathway and an induced systematic resistance pathway.

The results of the QTL analysis support our interpretation of the phenotypic frequency distributions regarding the number of genes determining resistance to the four

isolates in LB and NB in this population. The resistance allele at the chromosome 1H QTL was sufficient for resistance to all four isolates in LB and NB. This QTL may be an effect of the *Mla* gene, or of another tightly linked gene, or genes, in this apparent gene cluster. This genomic region in Baronesse is an important source of broad-spectrum resistance to many diseases such as powdery mildew and stripe rust which obtained from barley.

Our results are novel in adding the new phase of rice blast screening in two phase; LB and NB and adding blast isolates from barley to the catalog of pathogen specificities to which a gene, or genes, from Baronesse confer resistanc. If only this region of Baronesse was associated with resistance, we would have observed a bimodal phenotypic distribution of resistance and susceptibility. In addition to we found minor specific QTL only on chromosome 7H in NB the inoculated with isolates obtained from rice. The resistance allele at the chromosome 4H QTL, which traced to the susceptible parent, is not alone sufficient to confer resistance but when combined with another resistance allele, it is. The allele from Baronesse associated with resistance to the rice blast isolate could be of scientific interest from the standpoint of non-host resistance and of practical importance in cases where barley and rice are grown in rotation.

We established co-linearity of gene order between 23.5 cM region of barley chromosome 1H containing *Mla* and a 510kb region at the end of rice chromosome 5. The 510 kb syntenous region contains the bacterial blight resistance gene *xa*5. According to the one of the models proposed by Iyer and McCouch (2004), TFIIAγ- the product of *xa*5 – functions as a virulence target via interaction with the Avrxa5 protein. The complex of the two molecules is then detected by a third factor, such as R gene, and this initiates a signaling pathway that leads to bacterial blight resistance. A similar scenario - which is based on the 'guard hypothesis' (van der Biezen and Jones, 1998) - can be applied to the case of *Mla*. However, since we detected no barley ortholog of *xa*5 in the published 261 kb sequence of the short arm of barley 1H, additional experiments will be needed to the candidacy of this gene for *RMo1*.

Four blast resistance QTLs were identified in Baronesse and BCD47. Interestingly, the largest-effect QTL, where Baronesse contributed the favorable allele, was detected at the same position as *Mla*. Two of the other QTLs, where BCD47

contributes the resistance allele, were also coincident with the positions of mildew resistance genes: the *mlo* locus on chromosome 4H and the *Mlf* locus on chromosome 7H. In total, at least four blast resistance QTLs map to the same genomic regions as mildew resistance genes. Previously researchers reported the association of mildew resistance loci and barley blast resistance QTLs for *Mlg, mlo* and *Mlhb* in the Harrington/TR306 mapping population (Sato *et al*., 2001; Chen *et al*., 2003). *Mla* is a CC-NBS-LRR R gene, it is co-located with other R gene family members and with *RMo*1(Inukai *et al*., 2006). Blast resistance major genes, QTLs and NBS-LRR R gene clusters are also reported in rice (Wisser *et al*., 2005). Thus, members of the NBS-LRR R gene cluster could differentiate as major genes and as QTL alleles for blast resistance. The 4H blast resistance QTL is coincident with *Mlo*, which functions as a negative regulator of broad spectrum disease resistance to powdery mildew and spontaneous leaf cell death (Büschges *et al*., 1997). Homozygous mutations in *Mlo* confer race non-specific resistance to the powdery mildew, and enhanced susceptibility to the rice blast fungus (Jarosch *et al*., 1999). The basis of the contrasting responses to different pathogens is not known. It has been suggested that *Mlo* functions as a key element of basal pathogen resistance and the durable resistance to the powdery mildew fungus due to the *mlo* mutations are exceptional events that alter this basal response (Jarosch *et al*., 2003). In the same BCD47/ Baronesse population, we have detected a large effect QTL associated with stripe rust (incited by *Puccini*a *striiformis* f.sp. *hordei*), and this QTL is coincident with the rice blast resistance QTL and *Mlo* (Vales *et al*., 2005). For both diseases, BCD47 confers the resistance allele.

The structure and function of *Mlg*, *Mlhb* and *Mlf* have not been determined, but the linkage relationship of these mildew resistance genes with other quantitative and qualitative resistance genes has been reported. For instance; a QTL for powdery mildew resistance was detected at the same location as *Mlg* (Spaner *et al*., 1998) and *Mlhb* maps to the same position as *Rph.Hb*, a major gene conferring leaf rust resistance (Pickering *et al*., 1998). Therefore, these facts possibly suggested that *Mlg*, *Mlhb* and *Mlf* are members of resistance gene complexes, similar to *Mla* region. Therefore, at this point, it is not possible to determine if the blast resistances QTL we report are pleiotropic effects of the mildew resistance genes or the effects of linked genes.

CONCLUSION AND RECOMMENDATION

Conclusion

1. The 111 DHLs of the cross IR64/Azucena were used for mapping blast resistance genes using four isolates, TH16, THL80, THL142 and THL222. Five genomic blocks located on chromosomes 2, 8, 9, 11 and 12 were detected to be associated with LB and two genomic blocks located on chromosome 8 and 12 were detected with NB resistance with LOD score ranging from 2.9 to 8.7. IR64 showed resistant reaction against all blast isolates. Azucena showed resistant reaction against 1 blast isolates, THL222, on chromosome 11. The genomic blocks on chromosomes 2 and 8 were detected using all isolates; these blocks were located in the vicinity of the mapped *Pi-b* on chromosome 2 and *Pi-29*, *Pi-11(t)* and *Pi-zh* loci on chromosome 8 in IR64. The genomic block on chromosome 9, 11 and 12 were detected only using THL142 and TH222, respectively. These genomic blocks located close to previously mapped *Pi-5* on chromosome 1, *Pi-7(t)*, *Pi-m*, *Pi-1*, *Pi-k*, *Pi-18(t)*, and *Pi-sh* on chromosome 11 and *Pi-2(t), Pi-6(t), Pi-157, Pi-ta, Pi-ta2, Pi-ta6*, *and Pi-4(t)* on chromosome 12.

2. QTL analyses using the ORO population were performed on greatest value of DS for blast isolates, TH16, THL80, THL142 and THL222 on barley LB and NB. One QTL was identified with LB from each isolates, the QTL peak position of all isolates false in marker interval GMS21 to Bmac399 on chromosome 1H (LOD score = 22.56, 18.52, 13.19 and 13.1, respectively). The resistance alleles came from Baronesse. This QTL had the largest effect, Baronesse allele reduced disease severity to TH16, THL80, THL142 and THL222 by 61.9, 58.7, 46.8, 42.4 percentage points. The genomic blocks on chromosomes 1H, this region cover with the STS marker Mlax which confers resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*). Using NB data, we found QTL were located in the same region as LB on chromosome 1H, this QTLs carried resistance allele from Baronesse. Furthermore we found the QTL located on chromosome 4H with THL80 on 2006 and THL222 on 2008. The resistant allele came from BCD47. This QTL is coincident with *Mlo*, which functions as a negative regulator of broad spectrum disease resistance to powdery mildew and spontaneous leaf cell death. The 7H QTL was identified

with THL222, this QTL was coincident with *Mlf* are members of resistance gene complexes, similar to *Mla* region.

3. To further explore the association between the graphical genotype and the phenotype, we found on chromosome 1H, marker 2_0712 at position 33.23 cM. revealed a SNP allele tracing to Baronesse that was presented in BISON4H, BISON5H, BISON7H, BISON1H+5H, and BISON4H+5H. On chromosome 4H, we found marker 2_0762 at position 96.87 cM. performed a SNP allele tracing to BCD47 was presented in all BISON with 4H introgression revealed resistance to all blast isolates, accepted BISON1H+4H+5H that has 1H and 4H introgression alleles from BCD47 showed susceptible to all blast isolates suggesting that at 1H allele has the largest effect than 4H allele.

4. We established co-linearity of gene order between 23.5 cM region of barley chromosome 1H containing *Mla* and a 510 kb region at the end of short arm of rice chromosome 5. The 510 kb syntenous region contains the bacterial blight resistance gene *xa*5. TFIIAγ- the product of *xa*5 – functions as a virulence target via interaction with the Avrxa5 protein.

Recommendation

Information on the blast resistance genes in barley is not currently of economic importance because barley is usually not cultivated in rotation with rice and in the moist, warm conditions conducive to blast disease development. However, if barley acreage were to expand, or shift, in response to global warming or other factors, the location and linkage relationships of these genes could be of significant importance. Likewise, information on these genes could be of value to rice breeders and geneticists.

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