AN ABSTRACT OF THE THESIS OF

Kelley L. Richardson for the degree of Doctor of Philosophy in Crop Science presented on September 25, 2006.

Title: Quantitative Resistance to *Puccinia striiformis* f. sp. *hordei* in Barley Near-Isogenic (BISON) Lines.

Abstract approved:

Patrick M. Hayes

Quantitative resistance (QR) to disease is usually more durable than qualitative resistance, but its genetic basis is not well understood. We used the barley/barley stripe rust pathosystem as a model for the characterization of the QR phenotype and associated genomic regions. As an intermediate step in the preparation of near-isogenic lines representing individual QTL alleles and combinations of QTL alleles in a homogeneous genetic background, we developed a set of QTL introgression lines. These intermediate barley near-isogenic (i-BISON) lines represent disease resistance QTL combined in one-, two-, and three-way combinations in a susceptible background. In the first described experiment, we measured four components of disease resistance on the i-BISON lines: latent

period, infection efficiency, lesion size, and pustule density. The greatest differences between the target QTL introgressions and the susceptible controls were for the latter three traits. On average, however, the QTL introgressions also had longer latent periods than the susceptible parent (Baronesse). There were significant differences in the magnitudes of effects of different QTL alleles. The 4H QTL allele had the largest effect, followed by the alleles on 1H and 5H. Pyramiding multiple QTL alleles led to higher levels of resistance in terms of all components of quantitative resistance except latent period. In the second experiment, we measured the response to inoculation with the pathogen, as either infection type or percent disease severity, on the i-BISON lines at the seedling and adult plant stages, in controlled and field environments, with varying races of the pathogen, and combinations there of. The i-BISON QTL allele introgression effects are consistent across controlled and field testing environments and across the Mexico and Washington locations. Stripe rust resistance QTL alleles have consistent effects across varying races but show interactions with growth stage, mainly due to magnitude of response. The data do not conclusively support a benefit to pyramiding multiple resistance alleles; yet, the potential durability may justify their construction.

Quantitative Resistance to *Puccinia striiformis* f. sp. *hordei* in Barley Near-Isogenic (BISON) Lines

by
Kelley L. Richardson

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Dean of the Graduate School

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Kelley L. Richardson, Author

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CONTRIBUTION OF AUTHORS

Dr. Patrick M. Hayes assisted with all aspects of this project including formulating hypotheses, experimental design, and writing. Dr. M. Isabel Vales assisted with experimental design and writing of Chapter 2. Dr. Jennifer G. Kling assisted with interpretation and statistical analysis of data of Chapters 2 and 3. Dr. Chris C. Mundt was involved with experimental design, specifics of inoculation and screening of response to the pathogen, statistical analysis, and writing of Chapter 2. Dr. Xianming Chen performed barley stripe rust screening under controlled and field conditions in Washington, USA for Chapter 3. Dr. Marieke Johnston performed barley stripe rust screening under controlled conditions in Montana, USA for Chapter 3. Dr. Flavio Capettini performed barley stripe rust screening under field conditions in Toluca Valley, Mexico for Chapter 3.

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GENERAL INTRODUCTION

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. s*pontaneum* (Koch). Barley was domesticated about 10,000 years ago in the Near East Fertile Crescent (Nevo, 1992). Cultivated and wild barley have both winter and spring annual forms. The principal germplasm groups of cultivated barley are two-row and six-row, which refers to spike morphology. In two-rowed barley, the lateral spikelets are sterile, while in six-rowed barley all spikelets are fertile (Briggs, 1978). Barley is a self-pollinated diploid (2n = 2x = 14) (Bothmer *et al*. 1992). The genome size of barley is approximately 5,000 Mbp, as compared to wheat (17,300 Mbp), rice (450 Mbp) and Arabidopsis (125 Mbp). Barley is primarily used as cattle feed, malt for beer, and human consumption in many parts of South American and Asia (ICARDA, 1995).

Barley stripe rust and *Puccinia striiformis* f. sp. *hordei*

On a worldwide basis, diseases are one of the principal constraints to barley production. Plant diseases can proliferate and cause epidemics, which

reduce yield and affect grain quality. Fungi are, if not the most abundant plant pathogens, one of the most important and well characterized. The rust fungi cause some of the most significant diseases of barley (Line, 2002; Qi *et al*. 1998) and genetic resistance to rusts has been an area of intensive research in the *Triticeae*. Barley stripe rust, caused by *Puccinia striiformis* f. sp. *hordei*, is an important disease all over the world. The pathogen is closely related to the wheat stripe rust fungus, *Puccinia striiformis* (Adams and Line, 1997). The disease is sometimes called yellow rust (Adams and Line, 1997). In 2000, about 6% (372,900 bushels), 5% (442,100 bushels), and 2% (700,700 bushels) of yield losses caused by barley stripe rust were estimated for the states of California, Oregon, and Washington, respectively (Chen and Line, 2000). Losses to stripe rust would have been much greater, but many fields planted with susceptible cultivars were sprayed with fungicides (Long *et al*., 2000). Barley stripe rust has caused severe yield losses in many regions of the west and has a high potential for causing major yield losses in the future (Chen and Line, 2000). Whenever the disease shows up and is not controlled, it causes significant damage. When severe, yield losses of 50% are not uncommon (Comis and Wood, 1999). Studies in South America have shown that mountains and deserts are not sufficient barriers to prevent continental dispersal of the windborne pathogen. Some local movement may be due to transport of plant materials by animals or vehicles (Dubin and Stubbs, 1986). Even though barley stripe rust has the potential to become a severe disease in the United States, host resistance in winter barley and fungicides can effectively minimize yield loss

(Marshall and Sutton, 1995). Because the *Puccinia striiformis* f. sp. *hordei* population changes very rapidly, non-race specific, durable resistance should be identified, characterized, and used in breeding programs (Chen and Line, 2000). Genetic resistance is the most successful, efficient, and economical means to control rusts in cereals and it should be used for the control of barley stripe rust (Castro *et al*., 2002).

History of the disease

Historically, barley stripe rust has occurred in parts of Western Europe, the Middle East, south Asia, and east Africa for many years (Marshall and Sutton, 1995). The first reported case in the Americas was in 1975 in the savannan area near Bogotá, a principal area for growing malting barleys in Colombia (Dubin and Stubbs, 1986). The *Puccinia striiformis* f. sp. *hordei* found in Colombia was probably introduced from Europe (Marshall and Sutton, 1995). From Colombia the disease spread south into Chile and Argentina (Adams and Line, 1997). Barley stripe rust then quickly spread throughout South America (Marshall and Sutton, 1995). Within seven years, *Puccinia striiformis* f. sp. *hordei* and its variants moved 6,000 km and infected almost all major commercial barley areas in South America. There have been no known reports of *Puccinia striiformis* f. sp. *hordei* in Brazil, presumably due to unfavorable climate (Dubin and Stubbs, 1986). The northward movement of *Puccinia striiformis* f. sp. *hordei* from Colombia was slower, probably due to a scarcity of compatible hosts (Marshall and Sutton,

1995). Barley stripe rust was first found in North America on April 18, 1991 on barley plots in a Texas A&M University small grain-breeding nursery in Uvalde, Texas (Marshall and Sutton, 1995). The disease was differentiated from wheat stripe rust because the stripe rust was severe on the barley, but absent on the adjacent plots of wheat (Marshall and Sutton, 1995). From Texas, the disease moved to Montana and southern Idaho in 1993, to California in 1994, and to Oregon and Washington in 1995 (Adams and Line, 1997). It took twenty years for barley stripe rust to move from Colombia to Oregon (ICARDA, 1995). In 1996, barley stripe rust spread rapidly through barley fields in central Washington causing crop loss (Adams and Line, 1997). Spread of the pathogen can be explosive and cause significant loss, especially in the Pacific Northwest where cool, wet weather greatly favors the disease development (Smiley and Ocamb, 2002). *Puccinia striiformis* f. sp. *hordei* has not been reported in the eastern states, the Great Plains states north of Oklahoma, nor Canada (Marshall and Sutton, 1995). The spread of barley stripe rust into these areas will depend largely on the amount of *Puccinia striiformis* f. sp. *hordei* overwintering in fall-sown barley (Marshall and Sutton, 1995). Because cattle consume most of the fall and winter plant growth, it is unlikely that Texas barley would serve as a major inoculum source for *Puccinia striiformis* f. sp. *hordei* on barley growth to the north (Marshall and Sutton, 1995). Barley stripe rust infected plants of *Hordeum jubatum*, *Hordeum leporinum*, and *Hordeum muticum* have been found and have

the potential to serve as sources of inoculum (Dubin and Stubbs, 1986; Marshall and Sutton, 1995).

Biology of the pathogen

Puccinia striiformis f. sp. *hordei* is a basidiomycete fungus. It is biotrophic, obligately parasitic, and very host-specific (Adams and Line, 1997). *Puccinia striiformis* f. sp. *hordei* is a polycyclic fungus. Uredospores, also known as urediniospores, are the asexual spores and the only inoculum source for *Puccinia striiformis* f. sp. *hordei* (Adams and Line, 1997). In the summer, they are found on barley leaves, and sometimes heads, as a yellow-orange and powdery material (Adams and Line, 1997). As the disease progresses the yellow-orange spores grow into stripes of rust pustules between the leaf veins, giving the leaves a striped, rust appearance (Adams and Line, 1997; Comis and Wood, 1999). The fungus may rapidly cover the leaves and head and effectively suck the plant dry (Comis and Wood, 1999). The rust causes rapid water loss, creating a drought for the plant no matter how much water is available (Comis and Wood, 1999). Late in the summer, black teliospores develop as linear black pustules on the leaves. Basidia and basidiospores are produced from the teliospores. These spores serve no purpose because no alternate host for the pathogen is known to exist (Adams and Line, 1997). Since there is no known alternate host for *Puccinia striiformis* f. sp. *hordei* variability in the fungus is largely determined by mutation and somatic recombination (Marshall and Sutton, 1995). To survive the winter, spores from

last year's crop must find a suitable host in which to over winter (Adams and Line, 1997). Warm, wet winters with mild temperatures or plenty of snow cover favor survival of the rust fungus as mycelium in the leaves of winter barley, volunteer barley, and other hosts. Over wintering on volunteer barley or rye, certain wild barleys, wheat, and numerous perennial grass species has been reported (Smiley and Ocamb, 2002). Cold dry winters destroy the infected leaves, reducing the amount of rust available to spread the disease in the spring (Adams and Line, 1997). The weather in the Pacific Northwest and California is tailor-made for the barley stripe rust fungus (Comis and Wood, 1999). *Puccinia striiformis* f. sp. *hordei* uredospores require about eight hours of moisture on the plant leaves to germinate and infect the host. Without dew or rain, new infections cannot take place, flag leaves remain healthy, and crop loss does not occur (Adams and Line, 1997). An increase of stripe rust on winter barley in eastern Washington was slowed by dry weather in early and mid-May (Long *et al.*, 2000). Under lab conditions, inoculated plants are placed in a dew chamber at 10^0C for 24-48 hours to promote infection, then placed in a growth chamber with 16 hours of light at 15^0C and 8 hours of dark at 12^0C (Marshall and Sutton, 1995). The disease begins from a very small number of infections that are difficult or impossible to detect in the field (Smiley and Ocamb, 2002). The spores blow in the wind from plant to plant and from field to field (Adams and Line, 1997). The latent period, infection frequency, infection period, and spore production are highly affected by temperature, host age, and host and pathogen genotypes (Sandoval-Islas *et al*.,

2002). Severity percentage and infection type decrease as temperature increases (Sandoval-Islas *et al*., 2002). It has been suggested that the epidemiological fitness of the fungus decreases as temperature increases (Sandoval-Islas *et al*., 2002). The host's genetic variability and its interaction with the environment could result in variable resistance expression (Sandoval-Islas *et al*., 2002). Stripe rust severity is usually measured as the visual percentage of leaf surface covered, for example, 70% covered in rust pustules (Marshall and Sutton, 1995). Infection types are measured on a scale of 0-9: 0-3 is a resistant reaction, 4-6 is an intermediate reaction, and 7-9 is a susceptible reaction (Marshall and Sutton, 1995).

Races of *Puccinia striiformis* f. sp. *hordei*

For many years barley stripe rust was known as Race 24 of wheat stripe rust and was identified by its ability to attack specific wheat and barley varieties referred to as differential varieties (Adams and Line, 1997). As of 2000, 52 races of *Puccinia striiformis* f. sp. *hordei* have been detected in the United States (Chen and Line, 2000). Certain races will survive on wild barley, wheat, and volunteer rye (Adams and Line, 1997). *Puccinia striiformis* f. sp. *hordei* could attack a wide range of barley varieties and easily create new races that might attack new resistant varieties that are developed (Comis and Wood, 1999). Results indicate that the *Puccinia striiformis* f. sp. *hordei* population in the U.S. has become less complex and races with few virulence factors have become more prevalent. The changes

from a population with numerous races to a population with few races and from a population with many virulence factors to a population with few virulence factors may have resulted from selection pressure (Chen and Line, 2000). The major spring barley varieties grown are susceptible to barley stripe rust (Adams and Line, 1997). Some resistance may occur in winter barley varieties, yet resistant cultivars have not been widely used in commercial production. Therefore, resistant cultivars may not have provided enough selection pressure to account for the changes in the rust population. One hypothesis is that races with more virulence factors may have less fitness or aggressiveness (Chen and Line, 2000).

Cultural and chemical methods of control

There are several cultural and chemical methods used for controlling barley stripe rust. The number of initial infections can be reduced if spring barley is planted as early as possible, which gives the plant a head start on *Puccinia striiformis* f. sp. *hordei* (Adams and Line, 1997). The same result is achieved if winter barleys are planted as late as possible (Smiley and Ocamb, 2002). Using cultivar mixtures or multilines may slow down the rate of *Puccinia striiformis* f. sp. *hordei* epidemic buildup (Smiley and Ocamb, 2002). This practice is common in Texas where barley is predominantly planted for fall and winter grazing pastures for beef and dairy cattle (Marshall and Sutton, 1995).

There are numerous chemical methods available for the control of barley stripe rust. Seed treatment is a popular method of chemical control. It is generally

cheaper than later foliar applications done by airplanes (Adams and Line, 1997). Yet, windblown spores could still attack the crop later in the season making another application of fungicide necessary (Adams and Line, 1997). Baytan treated seed can delay the onset of an epidemic by preventing early buildup of the disease on seedlings (Adams and Line, 1997). Seed treatments are recommended for use in areas where barley stripe rust was detected the previous year (Smiley and Ocamb, 2002). Baytan is often used at 1.5 fl. oz. /100 pounds of seed (Smiley and Ocamb, 2002). While Baytan prevents early disease buildup it also may reduce seedling emergence (Smiley and Ocamb, 2002). Foliar fungicide application is also helpful at lowering disease severity. There are many fungicides on the market that lower the barley stripe rust disease effects. Tilt can be used to protect a crop after the disease is found in the planting and crop loss is imminent (Adams and Line, 1997). Tilt is often used at a dilution of 4 fl. oz. in no less than 15 gallons of ground water (Smiley and Ocamb, 2002). Tilt is applied at the flag leaf emergence, but cannot be used after (Adams and Line, 1997; Smiley and Ocamb, 2002). Fungicides should be applied if stripe rust severity is greater than 5% at the late tillering stage (Adams and Line, 1997). Applications made after flowering may not be economical (Smiley and Ocamb, 2002). By 1996, the fungicide Folicur became available to growers under an emergency authorization by the U. S. environmental protection agency (EPA). Folicur protects barley late into the season throughout all of its vulnerable stages (Comis and Wood, 1999).

Flusilazole is another fungicide reported to be effective at lowering disease severity in barley plots (Marshall and Sutton, 1995).

Genetic resistance

Flor (1955) proposed the gene-for-gene theory, based on his results on genetics of resistance in flax rust, in which successful infection depends on genetic factors in both the host and the pathogen. Host and pathogen interact in an evolutionary process. In this genetic interaction the pathogen has a clear advantage due to shorter life cycle and greater reproductive capacity (Schumann, 1991). For this reason cultivars with single gene resistance typically do not show durable resistance. In the gene-for-gene model, resistance (incompatibility) is a recognition process between an active gene product from the resistant host and an active gene product from the avirulent pathogen (Knott, 1989).

Distinctions between different types of resistance can be made on the basis of reaction type, race specificity, level of resistance, stage of expression during the plant life cycle, and the type of gene involved (major vs. minor) (Parlevliet, 1997). Qualitative and quantitative are two types of host resistance that exist in barley for barley stripe rust. Qualitative, or hypersensitive, was the first type of resistance to *Puccinia striiformis* f. sp. *hordei* recorded in barley (Osman-Ghani and Manners, 1985). It has been shown that this type of resistance is monogenic (Osman-Ghani and Manners, 1985). Only a small number of loci for qualitative resistance to different races of *Puccinia striiformis* f. sp. *hordei* have been discovered in the

barley genome (Chen and Line, 1999). Scientists in India discovered eight loci with multiple resistance alleles (*Ps1* to *Ps8*) for barley stripe rust resistance (Chen and Line, 1999). European scientists discovered four genes (*yr*, *yr2*, *yr3*, and *yr4*) for resistance (Chen and Line, 1999). Although there are examples of single gene durable resistance –such as the *Rpg1* gene that conferred resistance to all barley stem rust pathotypes for more than 50 years (Jin *et al*. 1994)- quantitative resistance has been considered the optimum strategy for prolonging the effectiveness of host resistance (Parlevliet, 1997). Quantitative resistance tends to be comprised of multiple, minor, additive genes conferring a reasonable level of resistance that is considered likely to be durable (Osman-Ghani and Manners, 1985). Several mechanisms confer quantitative resistance including resistance to spore deposition, to spore germination, to penetration, and to growth within the plant, as well as low sporulation rates and long generation times (Osman-Ghani and Manners, 1985). Latent period and infection frequency are the most important quantitative resistance components (Sandoval-Islas *et al*., 2002). Their genetics seem to be controlled by at least two to three genes (Sandoval-Islas *et al*., 2002). The resistance in some cultivars increases with higher temperatures because infection type decreases. This type of resistance is labeled high temperature resistance in mature plants (Sandoval-Islas *et al*., 2002). Quantitative trait loci (QTL) have been found on almost every chromosome in the barley genome (Chen *et al*., 1994; Toojinda *et al*., 2000; Vales *et al*., 2005). Current research focuses on dissecting the QTL of barley stripe rust resistance to better understand the

importance of each resistance locus. This provides the possibility for new cultivars with efficient and durable resistance to *Puccinia striiformis* f. sp. *hordei*.

Marker assisted selection

Molecular breeding tools promise to facilitate selection for quantitative resistance by shifting the basis of selection from phenotype to genotype. QTL and linkage map information can be used to design marker assisted selection (MAS) schemes for resistance breeding. The efficiency of MAS can be increased by using markers flanking the target gene instead of a single linked marker (Peng *et al*. 1988). Markers bracketing the gene reduce the probability of losing the resistant QTL by recombination and minimize linkage drag. Toojinda *et al*. (1998) introgressed two resistance QTL for barley stripe rust (BSR) into the variety "Steptoe", obtaining the first barley variety selected using MAS. Tagged resistance genes can be moved more rapidly from one varietal background to another and also provide a tool for pyramiding diverse genes in single cultivars (Castro *et al*., 2003a). MAS can be used as an adjunct for any breeding technique, such as backcrossing for variety conversion or recurrent selection for allele accumulation where several parents are combined in the process (Lindhout, 2002; Castro *et al*. 2003a).

Molecular breeding is extensive for stripe rust resistance in barley. A doubled haploid population developed using an ICARDA/CIMMYT parent (Calicuchima –sib) with quantitative resistance and a backcross derivative of

Bowman, the susceptible parent, was used to map the first two QTL conferring resistance to barley stripe rust (Chen *et al*. 1994). Subsequent work revealed a cluster of quantitative and qualitative resistance genes at the same region on chromosome 1H. Three adult plant resistance QTL were mapped in the Bleheim/E224/3 population on chromosomes 7H, 1H, and 5H (Thomas *et al*., 1995). The QTL on chromosome 1H had the largest effect and may be related with the *Yr4* locus. This was inferred by the ancestral relationship between Blenheim and Deba Abed, a cultivar with the *Yr4* resistance. Toojinda *et al*. (2000) mapped a QTL in the same region in Shyri x Galena. Toojinda *et al*., (1998) in an example of marker-assisted selection, introgressed adult plant resistance QTL from chromosome 4H and 5H in a new genetic background. An example of a resistance gene pyramiding breeding strategy was developed by Castro *et al*. (2003a, 2003b) who combined qualitative and quantitative resistance genes.

PYRAMIDING AND DISSECTING DISEASE RESISTANCE QTL TO BARLEY STRIPE RUST

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Abstract

Quantitative resistance (QR) to disease is usually more durable than qualitative resistance, but its genetic basis is not well understood. We used the barley/barley stripe rust pathosystem as a model for the characterization of the QR phenotype and associated genomic regions. As an intermediate step in the preparation of near-isogenic lines representing individual QTL alleles and combinations of QTL alleles in a homogeneous genetic background, we developed a set of QTL introgression lines in a susceptible background. These intermediate barley near-isogenic (i-BISON) lines represent disease resistance QTL combined in one-, two-, and three-way combinations in a susceptible background. We measured four components of disease resistance on the i-BISON lines: latent period, infection efficiency, lesion size, and pustule density. The greatest differences between the target QTL introgressions and the susceptible controls were for the latter three traits. On average, however, the QTL introgressions also had longer latent periods than the susceptible parent (Baronesse). There were significant differences in the magnitudes of effects of different QTL alleles. The 4H QTL allele had the largest effect, followed by the alleles on 1H and 5H. Pyramiding multiple QTL alleles led to higher levels of resistance in terms of all components of quantitative resistance except latent period.

Keywords

Hordeum vulgare subsp. *vulgare*, *Puccinia striiformis* f. sp. *hordei*, quantitative trait loci, components of resistance, pyramiding

Introduction

Host plant genetic resistance is typically the most cost-effective and sustainable approach to the control of plant diseases. Plant resistance to biotic stresses can be classified as qualitative or quantitative. Generally speaking, these terms refer to the resistance phenotype and its inheritance. Qualitative resistance shows simple, "major" gene inheritance, i.e. progeny of resistant x susceptible crosses fall into discrete resistant and susceptible classes. Quantitative resistance shows more complex, usually polygenic, inheritance. Progeny of resistant x susceptible crosses show a range, often continuous, of phenotypes. Parlevliet (1979) more explicitly defined the terms in the context of resistance to cereal rusts (incited by *Puccinia* species). Strictly speaking, qualitative resistance is race-specific and involves gene-for-gene interactions between the host and pathogen. It is usually complete, or nearly complete, in that there is little or no spore production. Quantitative resistance is race-nonspecific with no gene-for-gene interaction between the host and pathogen. Quantitative resistance is incomplete: host plants are infected but spore production is reduced. The qualitative- quantitative resistance debate is of importance because of the probable durability of disease resistance. Durability is an attribute that can only be defined in an historical context: durable resistance

remains effective while a cultivar possessing it is widely cultivated (Johnson 1981). In theory, quantitative resistance has a higher probability of being stable and durable (Niks and Rubiales 2002), although there are examples of durable qualitative resistance genes, such as *Rpg1* of barley that confers resistance to stem rust (Brueggeman *et al*. 2002).

Though quantitative disease resistance is highly valued for its higher probability of durability, the genetic basis and underlying mechanisms are not as well understood as in the case of qualitative resistance, where a number of genes have been cloned and characterized (Pflieger *et al*. 2001). Quantitative resistance may be due to uncharacterized classes of resistance genes or to the presence of alternative alleles at loci where other alleles correspond to known classes of resistance genes (NBS-LRR for example) (Lefebvre and Chevre 1995; Qi *et al*. 1998; Li *et al*. 1999; Gebhardt and Valkonen 2001). According to Wisser *et al*. (2005), about half of the genetically defined rice genome is involved in quantitative disease resistance. The coincidence of disease resistance quantitative trait loci (QTL) with qualitative resistance gene clusters may indicate functional and evolutionary relationships or simply association due to linkage disequilibrium.

The availability of whole genome sequences from plant model systems and tools for positional cloning in large-genome species will ultimately allow us to determine the genetics of quantitative and qualitative resistance. In the interim, quantitative resistance to stripe rust (incited by *Puccinia striiformis* Westend. f.sp. *hordei*) in barley (*Hordeum vulgare* subsp. *vulgare*) provides a model system for

characterizing the quantitative resistance phenotype and association with genomic regions (Toojinda *et al*. 2000; Castro *et al*. 2002; Castro *et al*. 2003a; Castro *et al*. 2003b; Castro *et al*. 2003c; Vales *et al*. 2005). The quantitative resistance phenotype used in the preceding citations was disease severity - the area of plant tissue affected by disease, expressed as a percentage of the total area assessed (Parlevliet 1979). With polycyclic diseases such as stripe rust, in the absence of qualitative resistance genes, disease severity is the cumulative result of several component mechanisms conferring partial resistance (Osman-Ghani and Manners 1985). For example, Broers (1997) showed that quantitative resistance to wheat stripe rust (caused by *Puccinia striiformis* Westend. f.sp. *tritici*) could be dissected into mechanistic components: latent period, infection efficiency, lesion size, and pustule density. Accordingly, a logical next step in our quantitative resistance research is the assignment of components of disease severity to QTL. Unfortunately, the time required for phenotyping each of the components precludes conducting the experiment at the population level: research shows that a large ($n \geq 300$) mapping population is needed to detect the most barley stripe rust resistance QTL with the least bias (Vales *et al*., 2005). Our alternative was to develop a set of lines, of known disease severity QTL allele architecture, and to measure the components on this smaller set of germplasm.

Our long-term goal is to develop a set of near-isogenic lines (NILs) representing resistance alleles at individual QTL and combinations of QTL. NILs not only provide a better estimate for the effect of single QTL alleles, but also

provide a better insight into QTL x pathogen and QTL x environment interactions (van Berloo *et al*. 2001). Furthermore, QTL-NILs provide a starting point for positional cloning of quantitative resistance gene candidates. As an intermediate step between the QTL allele introgression lines described by Castro *et al*. (2003a), which represent resistant alleles at individual QTL and QTL combinations in variable genetic backgrounds, and a set of QTL-NILs, previously described, we developed a set of QTL resistance allele introgression lines in a more homogenous genetic background. These lines contain resistant alleles at disease severity QTL that were mapped in different backgrounds and then combined in one-, two-, and three-way combinations in an elite agronomic background. In this report, we describe the results of an experiment in which we measured the components of disease severity on these lines (the intermediate barley near-isogenic lines; i-BISON). Our goals were to (i) determine if the disease components are QTL-specific, and (ii) if pyramiding resistance alleles at multiple QTL leads to higher levels of resistance.

Materials and methods

Germplasm development

A set of intermediate QTL resistance allele introgression lines, i-BISON, was developed by molecular marker assisted introgression of barley stripe rust (BSR)

resistance QTL alleles into a BSR susceptible background, the variety "Baronesse." Baronesse is a two-rowed, spring growth habit, feed barley developed by Nordsaat in Germany from the cross Mentor/Minerva//mutant of Vada////Carlsberg/Union///Opavsky/Salle//Richard/////Oriol/6153 P40. The variety was introduced into the United States by Western Plant Breeders, Inc. in 1991 and is grown extensively in the Pacific Northwest of the USA. Based on repeated tests in Mexico, Baronesse is susceptible to BSR, although under less disease pressure in the Pacific Northwest it is not as susceptible as other varieties (Vales *et al*. 2005). The donors of the resistance alleles were BCD47 and BCD12. These are two-rowed, spring growth habit doubled-haploid (DH) experimental lines developed via marker-assisted selection (MAS) for BSR QTL resistance alleles. BCD47 contains resistance alleles at the QTL on chromosomes 4H and 5H, and BCD12 on 1H (Castro *et al*. 2003a). The i-BISON lines (Table 2.1) were derived from the cross of BCD47/Baronesse, F1//BCD12/Baronesse, F1 (Figure 2.1). Resistance alleles at QTL on three chromosomes (designated as targets 1H, 4H, and 5H) were tagged for introgression. In addition, we developed two controls (i) a "0 –QTL" line selected for the susceptible (Baronesse) alleles at the 1H, 4H, and 5H targets and (ii) a qualitative resistance gene i-BISON containing a major gene on chromosome 7H, derived from the experimental line D3-6/B23 (Castro *et al*. 2003a). These controls were developed as checks to test the effectiveness of MAS and to compare the effects of resistance alleles at QTL in a susceptible background.

Other approaches to the development of near-isogenic QTL would have been more efficient if the breeding program did not have other objectives. Other objectives of our breeding program at that time were to accumulate all possible BSR QTL resistance alleles into single, agronomically favorable genotypes and to combine the high yield of Baronesse with the Barley Yellow Dwarf resistance of BCD12 and the malt quality profile of BCD47. Further, the initial crosses from which the i-BISON lines were eventually derived were made prior to the availability of detailed information on BSR resistance QTL location and effect.

DNA extraction and genotyping

DNA was extracted from 30-50 mg of young leaf tissue harvested from greenhouse-grown plants using a Qiagen/Retsch MM300 mixer Mill and the Qiagen DNeasy 96 Plant Kit (Qiagen Inc, Valencia, CA). Simple Sequence Repeat (SSR) markers (Liu *et al.* 1996; Ramsay *et al.* 2000) were amplified by polymerase chain reaction (PCR) using a fluorescently-tagged reverse primer and a non-labeled forward primer. Twelve SSRs linked to the target regions were used for foreground screening and MAS (Figure 2.2). For the F1 i-BISON generation, one to three PCR products, with non-overlapping sizes, were analyzed simultaneously with an internal size standard using ABI PRISM DNA sequencers equipped with Genescan® and Genotyper® software (PE Biosystems, Foster City,

CA). PCR products for generations F2-F5 were analyzed on 6% polyacrylamide gels (Wang *et al*. 2003).

Marker assisted selection

Germplasm in the F1 generation was screened with the SSRs GMS021, Bmac0399, Bmac0213, and Bmac0032 spanning the chromosome 1H QTL; EBmac0701, EBmac0635, EBmac0788, and HvMLO3 spanning the chromosome 4H QTL; Bmac0096, Bmag0323, and Bmag0337 spanning the chromosome 5H QTL; and Bmag0120, Ris44, and Bmac0156 flanking the major gene on chromosome 7H to identify and select heterozygotes for the resistance alleles at one, two, or three of the target QTL resistance allele introgressions and heterozygotes for the resistance allele at the major gene. For example, at the F1 generation a line selected for the 1H QTL was heterozygous for the 1H QTL and either heterozygous or homozygous for the susceptible allele at the 4H and 5H QTL. All SSRs were described by Ramsay *et al*. (2000) except for GMS021, which was first described by Struss and Plieske (1998). Ris44 is a size polymorphism based STS (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=probe;name=RIS44). The same SSRs were used to screen lines in the F2 generation to identify and select homozygotes for the resistance alleles at one, two, or three of the target QTL resistance allele introgressions and homozygotes for the resistance allele at the major gene. The

lines identified at the F2 were only selected if, besides being homozygous for the resistance allele at the target QTL, they were also homozygous for the susceptible allele at the other target QTL. The i-BISON lines were again screened with molecular markers at the F5 generation. During the time between the F2 and F5 generations, a new and more concise map (Vales *et al*. 2005) was generated. Based on this map we selected markers that more closely bracketed each QTL. The F5 generation of the i-BISON lines were screened using GMS021, k06267 (an EST from the Research Institute for Bioresources, Okayama University, Japan), Bmac0213, and Bmac0399 spanning the chromosome 1H QTL; EBmac0679, EBmac0788, and HvMLO3 spanning the chromosome 4H QTL; Bmag0337 and GBM1039 spanning the chromosome 5H QTL; and Bmag0120, Ris44, and Bmac0156 flanking the major gene on chromosome 7H. In addition to these twelve markers in the QTL target regions, 34 additional markers were used to screen the background genome of the F5 generation i-BISON lines (Figure 2.2). These markers are described in detail by Vales *et al*. (2005).

Phenotyping components of disease severity

We used a randomized complete block design. Treatments consisted of the QTL target introgressions (1H, 4H, 5H, 1H+4H, 1H+5H, 4H+5H, and 1H+4H+5H), two controls (0-QTL and the 7H major gene introgression), and the three parents (Baronesse, BCD12, and BCD47). There were variable numbers of lines

representing each QTL target introgression, with a total of 28 genotypes (Table 2.1). Three separate Percival MB-60B growth chambers (Percival Scientific, Inc., Iowa) were used as blocks (replications). Each genotype was grown in a single pot in each growth chamber, with ten individually labeled seedlings per pot. Plants were grown at 15^0C with a 16 hour light (245 µmol m^{-2} s^{-1})/ 8 hour dark photoperiod. Seedlings were inoculated at the third leaf stage. The 28 pots per replication were divided in two groups for inoculation in order to meet the size limitations of the inoculation chamber. For each replication, 14 pots at a time were placed in an inoculation chamber (45.7 cm long x 45.7 cm wide x 61 cm high) and the 140 seedlings were inoculated with a powdered mixture of 0.36 mg fresh *Puccinia striiformis* f.sp. *hordei* (race PSH-31) spores in 0.58 g talc powder using a DeVilbiss powder blower (Model 119) (Sunrise Medical, Australia) held at the top of the chamber. The powder blower was rotated around the top of the chamber to ensure uniform coverage with the inoculum. Each experimental unit received 26 µg spores per 41 mg talc powder. Inoculated plants were placed in a dew chamber at 13^0C and 100% relative humidity for 16 hours. The 28 pots per replication were then transferred back to the growth chamber at 15^0C with 16 hours light (245 µmol m^{-2} s^{-1})/8 hours dark per 24 hour period.

The second leaf of each plant in each pot was examined daily until the end of the experiment (20 days post inoculation). The number of sporulating lesions present on the second leaf of each plant was counted on a daily basis until there was no further increase in lesion number. These data were used to calculate *latent period*

as the number of days post inoculation when the median number of sporulating lesions occurred (based on the day the first sporulating lesion appeared until the day when there was no further increase). The highest number of sporulating lesions was used as the estimate of *infection efficiency*. At approximately one week after lesion appearance, the length and width of three isolated lesions present on the second leaf of each plant were measured to determine *lesion size* (cm^2). At 18 days post-inoculation, digital images were taken of the second leaf of each plant. Each digital image included a 1 cm^2 guide and based on these images the *pustule density* was determined as the number of pustules per cm^2.

Statistical analyses

Analysis of variance (ANOVA) was performed using latent period, infection efficiency, lesion size, and pustule density data (Table 2.2). *F*-tests were performed using the pooled error term as the denominator. The SAS GLM and Mixed procedures (SAS institute 2001) were employed and produced similar results. Levene's test was performed to confirm the assumptions of ANOVA. We partitioned the Genotype variation (24 degrees of freedom (df)) into four sources of variation: Parents (2 df), Introgressions (7 df), a Parents versus Introgressions contrast (1 df), and Lines within Introgressions (14 df). We compared the percentage of Introgressions sums of squares, with respect to the total genotype sums of squares, to the percentage of Lines within Introgressions sum of squares.

This allowed us to determine if there was more variation between introgressions than among the lines within introgressions. The additivity of resistance allele pyramiding was tested using SAS GLM procedure with a set of contrasts to determine if the effect of number of QTL on latent period, infection efficiency, lesion size, and pustule density was linear, quadratic, or cubic based on polynomial coefficients. We used the PROC CORR procedure in SAS to determine the correlations between latent period, infection efficiency, lesion size, and pustule density.

Results

The ANOVA revealed significant differences between the introgressions for latent period, infection efficiency, lesion size, and pustule density (Tables 2.2 and 2.3). BCD12, the donor of the resistance allele on 1H, had a significantly lower infection efficiency, lesion size, and pustule density than the susceptible parent Baronesse, but its latent period was not significantly different (Figure 2.3). BCD47 was significantly different from Baronesse for all components (Figure 2.3). The 7H qualitative resistant introgression conferred complete immunity and showed no disease development. Consequently, we did not include the 7H introgressions in the statistical analyses.

When comparing introgressions with the same QTL allele architecture as the resistance donor parents, e.g. 4H+5H vs. BCD47 and 1H vs. BCD12, there

were no significant differences for latent period, infection efficiency, lesion size, and pustule density. In cases where there are multiple lines per introgression target (1H, 4H, 5H, 1H+4H, 1H + 5H, and 4H + 5H) the variation among lines was always less than the variation between introgression targets.

Individual introgression targets had effects of different magnitudes on the components of resistance (Figure 2.3). The 4H introgression had the largest effect on the four components of resistance. Latent period, infection efficiency, and pustule density all exhibited significant differences for pair-wise comparison of alleles (i.e. 1H vs. 4H, 1H vs. 5H, and 4H vs. 5H). The order of magnitude of effects for all three phenotypes was 4H > 1H > 5H. For lesion size, there was no significant difference. The four components of resistance were highly correlated (Table 2.4). There were significant negative correlations between latent period and infection efficiency, lesion size, and pustule density. There were significant positive correlations between infection efficiency and lesion size and pustule density and between lesion size and pustule density.

Increasing the number of QTL resistance alleles in single genotypes led to more resistant infection efficiency, lesion size, and pustule density, but it did not significantly increase latent period (Figure 2.4). However, even the most resistant pyramids did not reach the zero symptom level of the immune 7H qualitative resistance gene introgression.

Forty-six markers were surveyed in the case of the quantitative resistance allele targets (1H, 4H, and 5H). Twelve markers were used for resistance allele

introgression and the remaining for background characterization (Figure 2.2). There are three possible alleles per locus: Baronesse, BCD12, and BCD47. At five loci for background characterization, identification of the three possible alleles was possible. At the remaining 12 foreground and 29 background loci, alleles from two of the three could not be distinguished. For the eight of the 12 foreground markers it was possible to differentiate the resistance allele from the susceptible alleles. BCD12 and BCD47 were most often identical (29 loci), followed by BCD12 and Baronesse (11) and BCD47 and Baronesse (1). In the case of the 7H introgression, there are only two allele possibilities. Of the 46 markers used for the quantitative resistance introgression, 31 were polymorphic between D3-6/B23, the source of the qualitative resistance gene, and Baronesse (3 flanking and 28 background). Due to the occurrence of identical alleles from two parents in the quantitative resistance allele introgressions, the percentage Baronesse in each i-BISON line can only be estimated. To calculate the percentage background Baronesse for each line, a value of 1 was assigned to each non-target locus if the allele could be identified as originating from Baronesse and 0.5 if it could have originated from Baronesse or one of the two resistance donor parents. These scores were summed and divided by the total number of background markers. The number of background markers varied from 37 to 44, depending on the number of target QTL resistance alleles. In the case of the 7H introgression, the percentages of Baronesse background were all based on the number of Baronesse alleles at 43 non-target loci. Estimates of the percentages of background loci at which

Baronesse alleles are fixed ranged from 29% to 76% in the quantitative resistance introgressions and from 42% to 61% for the 7H introgression (Figure 2.5). Considering all the i-BISON, residual heterozygosity at the F5 was observed at 2% of the loci surveyed.

Discussion

We observed a range of resistance phenotypes in the materials tested. The occurrence of some disease symptoms in BCD12 and BCD47 and all QTL allele introgressions confirms that quantitative resistance is present in this germplasm. The 7H major gene control was immune to infection, as expected. Under intense field epidemic conditions in Mexico, we have observed limited symptom development (i.e. trace) at the adult plant stage in CI10587, the line contributing this major gene (Castro *et al*. 2003a).

We were able to dissect the BSR disease severity QTL reported in previous studies (Toojinda *et al*. 2000; Castro *et al*. 2002; Castro *et al*. 2003a; Castro *et al*. 2003b; Castro *et al*. 2003c; Vales *et al*. 2005) into four components and found that the most notable differences were for infection efficiency, lesion size, and pustule density. On average, the QTL allele introgression lines had longer latent periods than the susceptible parent, Baronesse, but these differences were greater for the other three components. These differences may be due in part to the resistance alleles present in the parental sources; BCD47 had a significantly longer latent

period than Baronesse, but BCD12 and Baronesse were not different. These results are similar to those of Parlevliet (1975), who used the barley: *Puccinia hordei* pathosystem and found that there were smaller differences in latent period between resistant and susceptible cultivars than for infection efficiency. The epidemiological importance of latent period was confirmed in studies that showed that changes in latent period produced greater changes in the rate of disease increase than did changes of similar magnitude in other components of resistance (Leonard and Mundt 1984).

We confirmed that marker assisted introgression of resistance alleles is effective. The introgression of the major resistance gene on chromosome 7H gave more clear-cut results (immunity) than the introgression of the QTL with resistance alleles. The introgressed resistance alleles at the target QTL generally led to superior levels of one or more components of resistance, but particularly with some of the single allele introgressions, these effects were modest. There are indications that resistance alleles other than the targets were also introgressed: the 0-QTL control did not always equal Baronesse, the 4H+5H pyramid did not always equal BCD47, and the 1H did not always equal BCD12. The presence of uncharacterized non-target resistance alleles is one explanation for the variance seen among the lines within an introgression. If there are such QTL, they may trace to BCD12. If they trace to BCD47 or Baronesse, they must have small effects, as they remained undetected in a large mapping population involving the same germplasm (Vales *et al*. 2005). An alternative explanation is the unintended

introgression of favorable alleles due to a lack of markers that could identify all three possible alleles at some of the loci that flanked introgression targets. Although there was some variance among the multiple lines within some of the introgressions, the average effects of the BSR resistance allele introgressions at the QTL were always greater.

The effects of these introgressions with the i-BISON corroborates and extends the findings of Castro *et al*. (2003a), who targeted the same QTL alleles and introgressed them into more variable genetic backgrounds. The differences in magnitude of effect of different QTL alleles also corroborate previous results (Castro *et al*. 2003b and c). The 4H QTL allele had the largest effects, followed by 1H and 5H. The results of this experiment confirm multiple phenotypes can be attributed to each QTL allele, as reflected by the high correlations between the four components of resistance. Parlevliet (1979) also reported high correlations between lesion size and infection efficiency. Thus, the data support pleiotropy rather than different and specialized functions attributable to each QTL allele. Nonetheless, the QTL not only vary in effect, but also in their impact on specific components. The 4H QTL allele had the largest effect on all components, as well as a proportionally greater effect on latent period. Therefore, pyramids of multiple resistance QTL alleles where the 4H QTL was present led to lower infection efficiency, lesion size, and pustule density. The i-BISON lines containing three QTL with resistance alleles did not have a significantly longer latent period than those containing only one or two QTL with resistance alleles.

We have verified that MAS is effective for introgressing qualitative and quantitative disease resistance genes into an elite agronomic background. In the case of the single qualitative resistance gene on 7H, similar gains would undoubtedly have been achieved through phenotypic selection and at a reduced cost. In the case of the quantitative resistance allele targets on 1H, 4H, and 5H, it would not have been possible to develop all the single gene and multiple allele combinations without the initial QTL position information and use of markers during the selection process. We have shown that the general barley stripe rust quantitative resistance phenotype "disease severity" can be partitioned into the components of latent period, infection efficiency, lesion size, and pustule density. Our findings concur with previous reports that the four components are highly correlated. Our use of genotype and phenotype information revealed that the QTL with resistance alleles we targeted have pleiotropic effects on all four components, although the QTL alleles varied in the magnitude of their effects. There were linear reductions in infection efficiency, lesion size, and pustule density as more resistance alleles were added to individual genotypes, but resistance pyramiding did not increase latent period. In this study, infection efficiency was the component with the least experimental error, the largest significant differences between resistant and susceptible lines, and was the easiest to measure. Since there is such a high correlation amongst the traits it would be possible to use only infection efficiency as the selection criterion for quantitative resistance. Latent period is a significant component of resistance and it is important to point out that

measurements to determine infection efficiencies will also allow the calculation of latent period.

The introgression of the same resistance alleles at the same QTL into different lines did not always lead to the same resistance: there was more variance among lines within the quantitative resistance allele introgression classes and no variance among lines within the qualitative resistance gene introgression group (all lines were immune). The variance among lines representing the introgression of the same target QTL alleles may be due to the presence of undiscovered resistance factors. Additional evidence for this possibility is the observation that the 0-QTL line was either equal to or more resistant than the susceptible parent. It is also possible that non-target resistance alleles were inadvertently introgressed due to the lack of completely informative (tri-allelic) markers at all loci. During the course of this research, new markers were identified that provided additional and better resolution than the markers that were available for the first round of resistance allele selection. Therefore, the final characterization of the F5 lines provided the best picture of the allele composition of all lines. This information will be essential in selecting parental lines for the next step in this research – development of the QTL-NILs. From the standpoint of measuring allele effects, however, the variance attributable to inadvertent introgressions of known and unknown resistance alleles was always much less than the variance between introgression classes.

This first set of experiments focused on seedling resistance to a single race. Patterns of QTL allele effects in the i-BISON at the seedling stage in response to multiple races and at the adult plant stage in response to field infection are warranted to explore in more depth in the barley/barley stripe rust pathosystem. This first step in the genetic dissection of quantitative resistance to barley stripe rust into component traits raises interesting questions regarding the nature of quantitative resistance genes. The products of the next stage of this research, the QTL- NILs, should ultimately allow for characterization of these genes in terms of their structure and function.

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Table 2.1. The i-BISON lines and their respective introgression assignments. Each graphical genotype represents the QTL present in the line (1H, 4H, 5H, or their combinations) based on genome screening. Black indicates the presence of the resistance allele, white the absence of the allele, and grey the possibility of the presence of the resistance allele (based on the inability to differentiate between two alleles).

		Graphical Genotype		
Line	Introgression	1H	4H	5H
69	1H QTL			
128	1H QTL			
191	1H QTL			
104	4H QTL			
129	4H QTL			
18	4H QTL			
87	5H QTL			
111	5H QTL			
136	5H QTL			
157	5H QTL			
217	5H QTL			
2-20	7H major gene (control)			
2-22	7H major gene (control)			
3-50	7H major gene (control)			
216-4	1H and 4H QTL			
243-4	1H and 4H QTL			
136-2	1H and 4H QTL			
218-1	1H and 5H QTL			
174	1H and 5H QTL			
110-3	1H and 5H QTL			
217-2	4H and 5H QTL			
108	4H and 5H QTL			
22-4	4H and 5H QTL			
95-2	1H, 4H, and 5H QTL			
130	No QTL (control)			

Table 2.2. Mean square (MS) and significance results from analysis of variance examining the effects of genotype, parents, introgressions, lines within introgressions, and the contrast of parents versus introgressions using the pooled error as the error term, on latent period (LP), infection efficiency (IE), lesion size (LS), and pustule density (PD)

		MS			
Source of variation	DF	LP	IE	LS	PD
Reps	2	2.71**	0.87	0.26***	194.02**
Genotypes	24	5.43***	53.84***	0.05**	349.14***
Parents	2	2.69**	199.78***	0.12**	1041.02***
Introgressions	7	10.54***	78.33***	0.09***	468.71***
Parents vs. introgressions contrast	1	2.29*	54.32***	0.08*	37.28
Lines within introgressions	14	3.56	21.11***	0.01	213.25***
Pooled error	48	0.49	1.79	0.02	21.68

* Denotes that *F* value is significant at $P < 0.05$. ** Denotes that *F* value is significant at $P < 0.01$. *** Denotes that *F* value is significant at $P < 0.0001$.

Table 2.3. Comparison of the i-BISON lines to the controls: Baronesse (Bar), 0-QTL (0), BCD12 (B12), and BCD47 (B47) (including which QTL are present in the controls) for latent period, infection efficiency, lesion size, and pustule density By running all possible pair-wise t-tests on LSmeans when the overall F-test for introgressions was significant and focusing only on pre-planned comparisons.

			1H	4H	5H	1H+4H	1H+5H	4H+5H	1H+4H+5H
Latent Period	Bar	(0)		*				*	*
	0	(0)		*				*	*
	B12	(1H)		*				*	*
	B47	(4H+5H)			*				*
Infection Efficiency	Bar	(0)	*	*	*	*	*	*	*
	0	(0)		*		*		*	*
	B12	(1H)			*				
	B47	(4H+5H)			*				
Lesion Size	Bar	(0)	*	*	*	*	*	*	*
	0	(0)	*	*	*	*	*	*	*
	B12	(1H)							
	B47	(4H+5H)							
Pustule Density	Bar	(0)	*	*	*	*	*	*	*
	0	(0)		*		*	*	*	*
	B12	(1H)			*				
	B47	(4H+5H)	*		*				

* Denotes a significant difference at $P < 0.05$ level.

Table 2.4. Results from the analysis of correlation of latent period, infection efficiency, lesion size, and pustule density.

	Infection Efficiency	Lesion Size	Pustule Density
Latent Period	-0.78 *	-0.83 *	-0.68 *
Infection Efficiency		0.77 *	0.87 *
Lesion Size			0.79 *

* Denotes significance at $P<0.01$ level.

Figure 2.1. The pedigree and derivation of the i-BISON lines indicating generations when phenotypic and genotypic screenings were performed. Black squares indicate resistance allele donors.

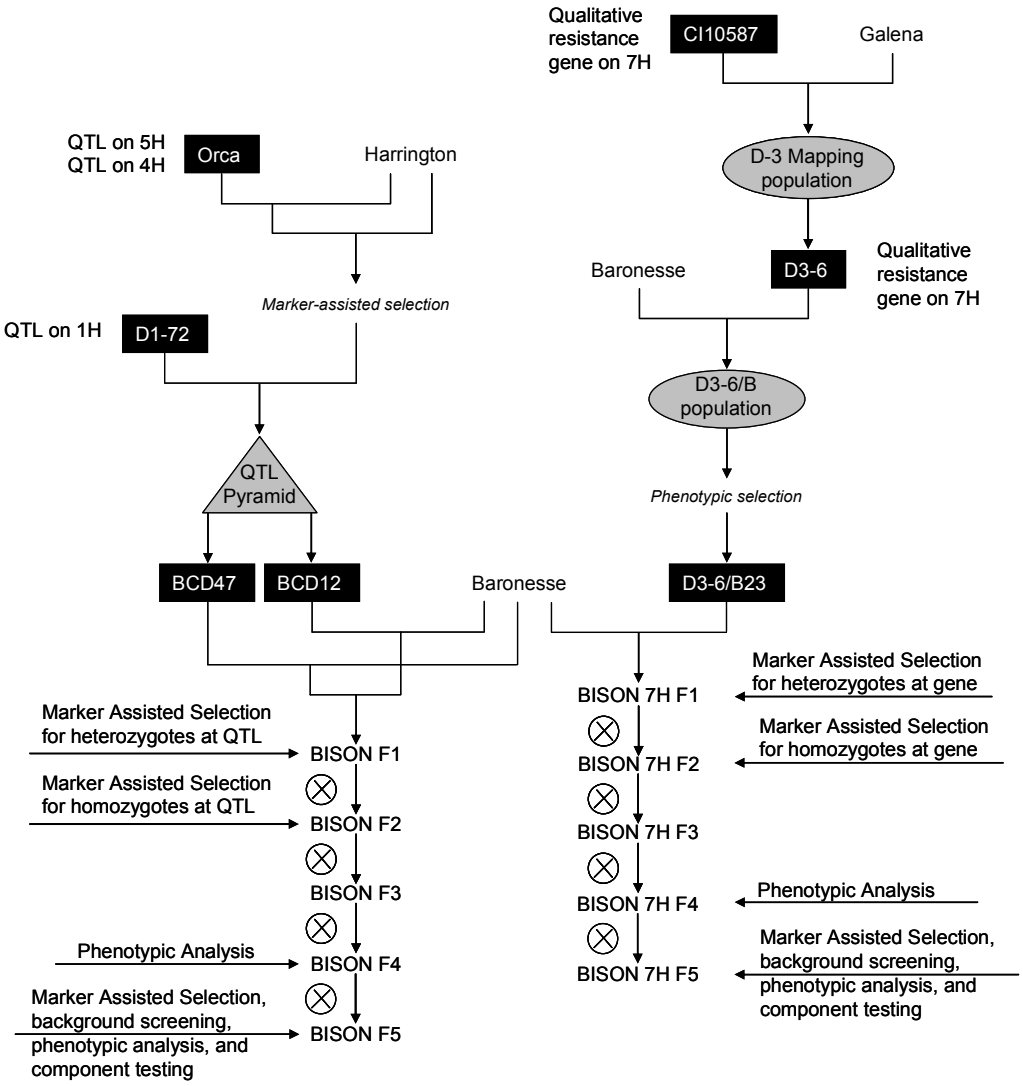


Figure 2.2. Linkage map of the BCD47/Baronesse DH population constructed by Vales *et al*. (2005) showing only markers used for foreground and background screening of the i-BISON lines. The dotted lines indicate regions with distances inferred from Ramsay *et al*. (2000). Markers in bold represent those that flank the QTL target introgression regions and were used for foreground screening. All others were used for background screening.

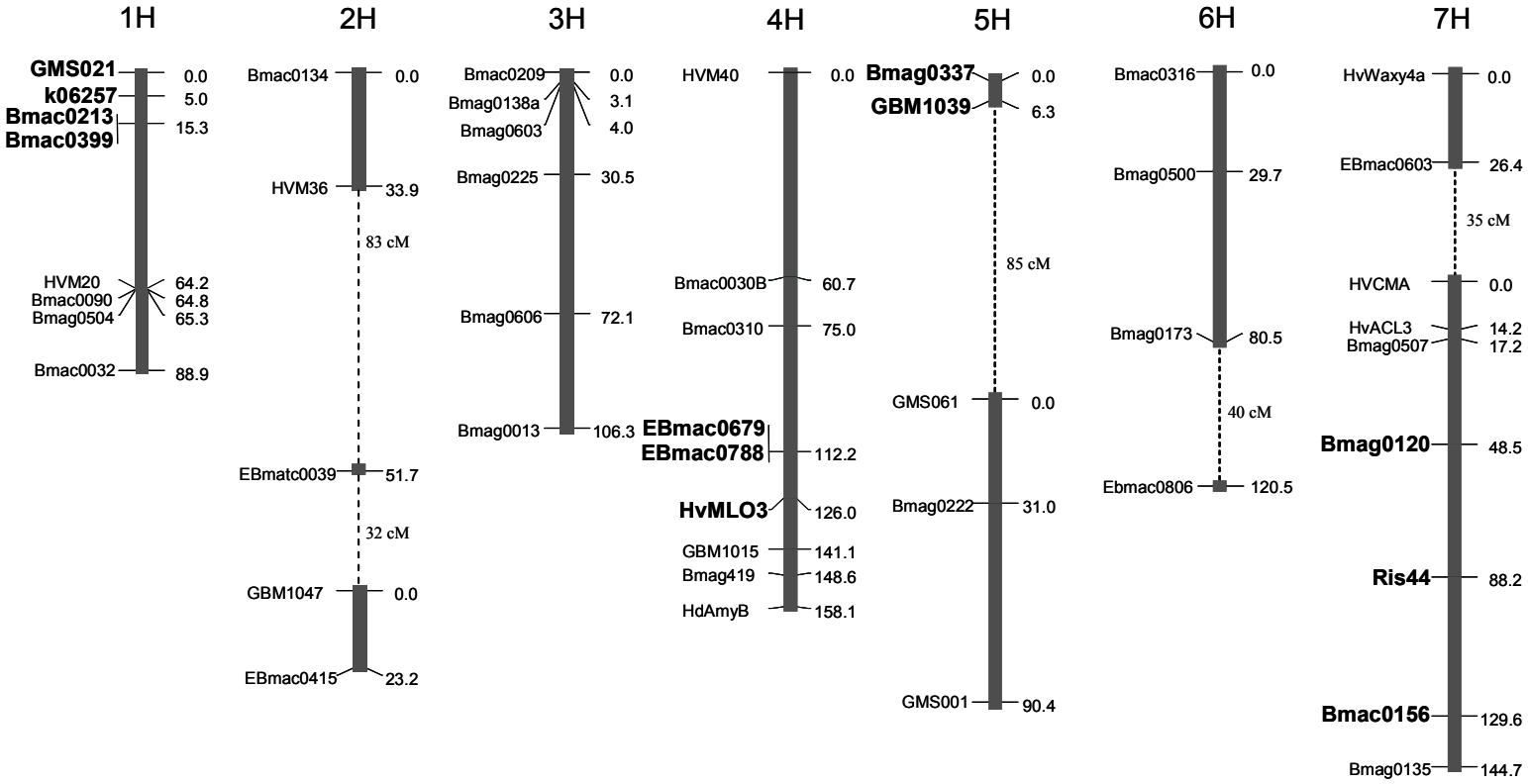


Figure 2.3. The least-squares means of treatments for the five components of resistance. Error bars indicate 95% confidence intervals. Treatments are separated into three groups: white are controls, grey are single QTL target introgressions, and black are combinations of QTL target introgressions.

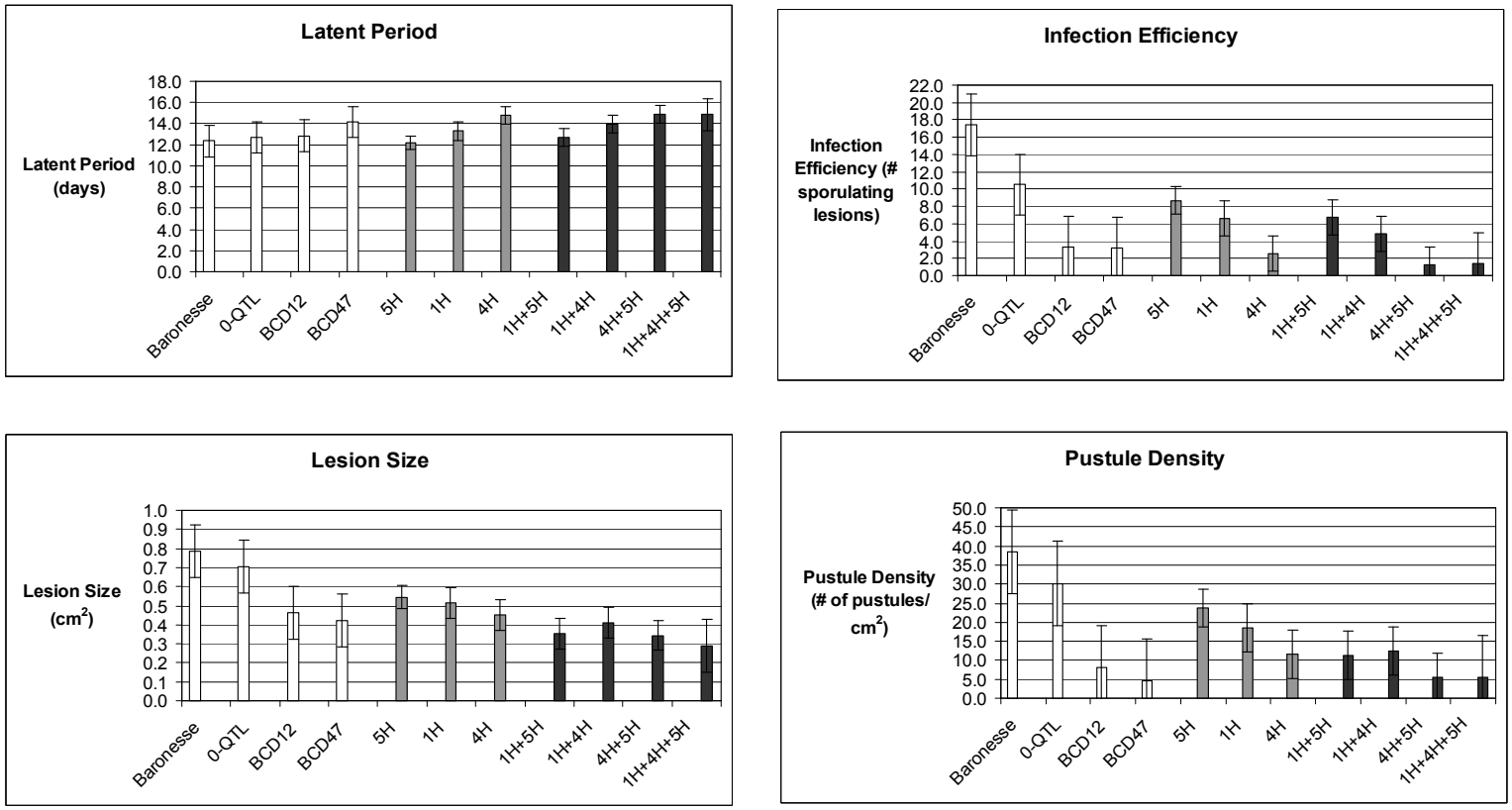
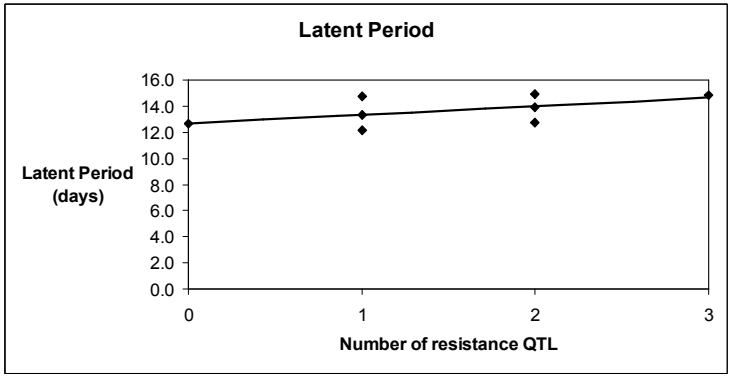
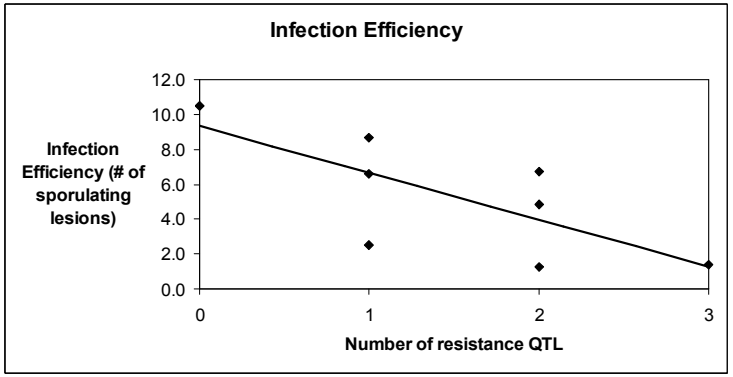
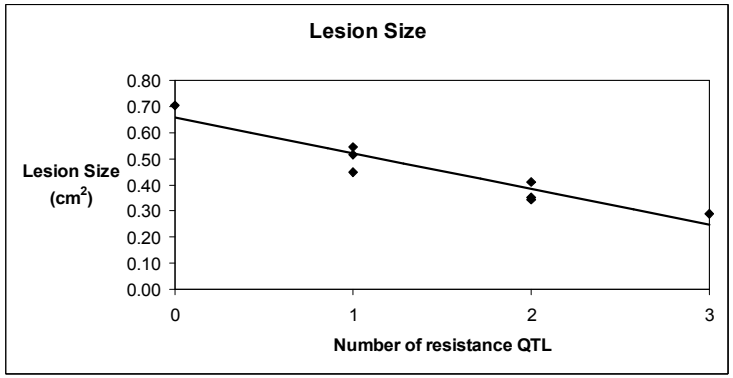


Figure 2.4. Regression of each component of resistance on the number of QTL targets per introgression indicating the effectiveness of pyramiding QTL target introgressions for infection efficiency, lesion size, and pustule density.







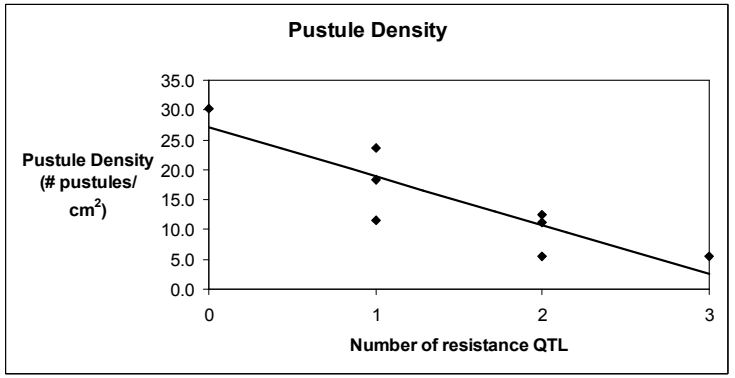
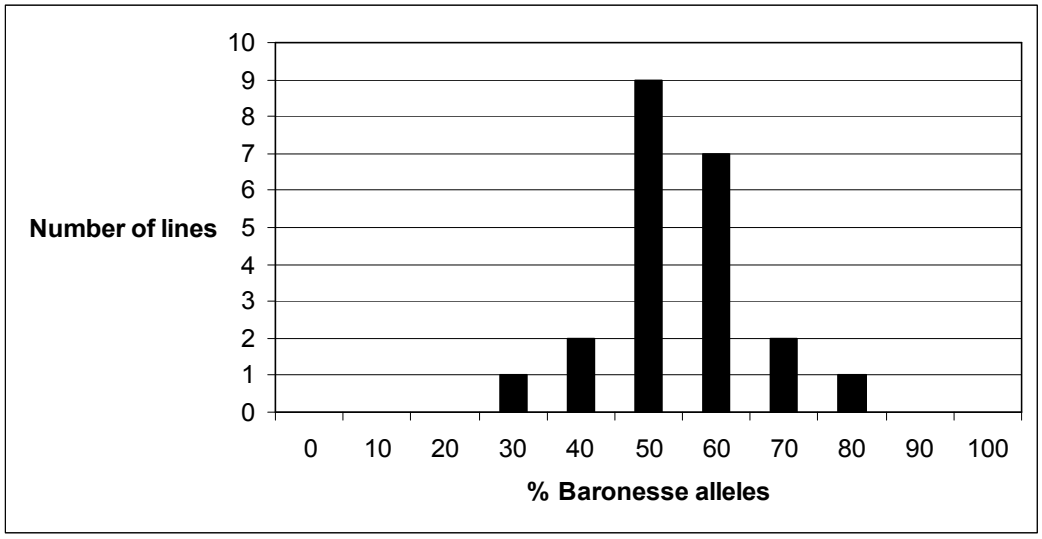


Figure 2.5. Distribution of the percentages of background loci at which Baronesse alleles are fixed for the quantitative resistance introgressions.



BARLEY STRIPE RUST RESISTANCE QTL ALLELES ARE EFFECTIVE ACROSS GROWTH STAGES, RACES, AND ENVIRONMENTS

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Abstract

Quantitative resistance (QR) to disease is usually more durable than qualitative resistance, but its genetic basis is not well understood. We used the barley/barley stripe rust (BSR) pathosystem as a model to characterize the QR phenotype and associated genomic regions. Midway through the development of near-isogenic lines representing individual QTL alleles, and combinations of QTL alleles, in a homogeneous genetic background, we developed a set of QTL introgression lines. These intermediate barley near-isogenic (i-BISON) lines represent BSR resistance QTL alleles in one-, two-, and three-way combinations in a susceptible background. We measured the response to inoculation with the pathogen, as either infection type or percent disease severity, on the i-BISON lines at the seedling and adult plant stages, in controlled and field environments, with varying races of the pathogen, and combinations thereof. The i-BISON QTL allele introgression effects were consistent across controlled and field testing environments and across Toluca Valley, Mexico and Washington, USA locations. BSR resistance QTL alleles have consistent effects across varying races but show interactions with growth stage, mainly due to magnitude of response. The results do not conclusively demonstrate a benefit to pyramiding multiple resistance alleles. However, potential durability of such constructs may justify their development.

Introduction

There is extensive literature on the advantages and disadvantages of qualitative vs. quantitative resistance to plant diseases (Kameswara Rao *et al*., 2002; Michelmore, 2003; Niks and Rubiales, 2002). Although theory predicts, (Jiménez-Gasco *et al*., 2004; Johnson, 1981; Vera Cruz *et al*., 2000) and experience has shown (Sandoval-Islas *et al*., 1998) that quantitative resistance (QR) has a higher probability of durability that qualitative resistance, effective breeding for quantitative resistance requires information on the number, location, and effects of the determinant genes. Quantitative trait locus (QTL) mapping provides this information and establishes the basis for marker assisted selection (MAS) (Borevitz and Chory, 2004). Key advantages to QR-MAS are the ability to select for resistance alleles in the absence of the disease (Bariana *et al*., 2001) and the option to pyramid multiple resistance genes (Kameswara Rao *et al*., 2002).

In the case of resistance to barley stripe rust (BSR) (incited by *Puccinia striiformis* Westend. f. sp. *hordei*) in barley (*Hordeum vulgare* ssp. *vulgare*) we have mapped QR alleles (Castro *et al*., 2002; Castro *et al*., 2003a; Toojinda *et al*., 2000; Vales *et al*., 2005) and used MAS to introgress and pyramid these alleles (Castro *et al*., 2003b; Castro *et al*., 2003c; Richardson *et al*., 2006). This has led to the development of BSR-resistant germplasm (Castro *et al*., 2003a; Castro *et al*., 2003b; Toojinda *et al*., 2000) and allowed for the characterization of the components of QR in near-isogenic genetic stocks (Richardson *et al*., 2006).

The availability of the near-isogenic stocks allows us to address important questions in breeding for QR to BSR including (i) race-specificity; (ii) effects of resistance alleles at the adult vs. seedling stage, and (iii) the use of controlled vs. field environments.

Materials and methods

Germplasm

The development of a set of BSR QTL allele introgression lines was described in detail by Richardson *et al*. (2006). Briefly, the intermediate barley isogenic lines (i-BISON) were developed by marker assisted introgression of BSR resistance QTL alleles into a BSR- susceptible background, the cultivar 'Baronesse'. 'Baronesse' is a two-rowed, spring growth habit, feed barley grown extensively in the Pacific Northwest of the USA. Based on repeated tests in Mexico, 'Baronesse' is susceptible to BSR, although under less disease pressure in the Pacific Northwest it is not as susceptible as other varieties (Vales *et al*., 2005). The resistance QTL allele donors were 'BCD47' and 'BCD12'. These are two-rowed, spring growth habit doubled-haploid (DH) experimental lines developed via marker-assisted selection (MAS) for BSR resistance QTL alleles. BCD47 contains resistance QTL alleles on chromosomes 4H and 5H, and BCD12 on 1H (Castro *et al*., 2003a). The i-BISON lines were derived from the cross of BCD47/ 'Baronesse', F1//BCD12/ 'Baronesse', F1 (Figure 3.1). Resistance alleles at QTL

on three chromosomes (designated as targets 1H, 4H, and 5H) were tagged for introgression. As described in detail by Richardson *et al*. (2006), there were different numbers of lines representing each QTL allele, or allele combination, introgression. There were four lines for the 1H QTL, three for the 4H, 14 for the 5H, 7 for the 7H, three for the 1H+4H, 7 for the 1H+5H, 6 for the 4H+5H, and one each for the 1H+4H+5H and 0-QTL.

Genotyping

Nine Simple Sequence Repeat (SSR) markers (Figure 3.2) were used for foreground screening and MAS. All SSRs were described by Ramsay *et al*. (2000) except for GMS021, which was first described by Struss and Plieske (1998) and k06267 (an EST from the Research Institute for Bioresources, Okayama University, Japan). Genotyping was performed with ABI PRISM DNA sequencers equipped with Genescan and Genotyper software (PE Biosystems, Foster City, CA) or on 6% polyacrylamide gels (C.B.S Scientific Co., Del Mar, CA) (Wang *et al*., 2003). Additional detail on genotyping protocols and primer sequences is provided in Richardson *et al*. (2006).

As an example of the MAS strategy, a line selected at the F1 generation for the 1H QTL target introgression was heterozygous for the 1H QTL and heterozygous (or homozygous for the susceptible allele) at the 4H and 5H QTL. The same SSRs were used to screen lines in the F2 generation to identify and select homozygotes for the resistance alleles at one, two, or three of the target QTL

introgressions and homozygotes for the resistance allele at the major gene. The lines identified at the F2 were only selected if, besides being homozygous for the resistance allele at the target QTL, they were also homozygous for the susceptible allele at the other target QTL.

Disease Phenotyping

Washington Controlled Environment Seedling (WCS)

The standard procedures for seedling testing under controlled greenhouse conditions described by Chen and Line (1992) were used. Briefly, for each line, 5-7 seeds were planted in a 5 cm square plastic pot. The seedlings were grown in a greenhouse at a diurnal temperature cycle of 10-25^{O}C. The five races - PSH-1, PSH-13, PSH-14, PSH-31, and PSH-54 have been described (Chen *et al*., 1995; Line and Chen, 1999; Chen, 2004). The virulence patterns on differential cultivars of these five races were compared to that of race BSTR 97, used in the Montana experiments, to confirm the differences between the Washington and Montana races (Table 3.1). A mixture of spores with talc powder (Sigma) at a ratio about 1:20 was dusted onto plants at the two-leaf stage. Seedlings were uniformly inoculated with urediniospores of a specific test race, placed in a dew chamber at 10^{O}C for 24 h, and then placed in a growth chamber at a diurnal temperature cycle that gradually changed from 4^{O}C at 2 am to 20^{O}C at 2 pm. Metal halide lights supplemented natural daylight and extended the photoperiod cycle to 16 h light per

24 h period. Approximately 20 to 22 days after inoculation, infection types (IT) were recorded according to the 0-9 scale described by Line and Qayoum (1991).

Washington Controlled Environment Adult (WCA)

The conditions for growing plants before inoculation were the same as above and the conditions for growing the plants after inoculation were described by Chen and Line (1995). Briefly, three seeds of each test genotype were planted in a 15.2 cm square plastic pot with three replicates. Races PSH-31 and PSH-54 were used. Plants at Feekes stages 10-10.5 (Large, 1954) were inoculated with the spore-talc mixture and transferred to a dew chamber at 10^{O}C for 24 h. Plants were then transferred to a greenhouse with the temperature and light conditions described for the seedling tests. Infection type data were recorded for each plant 20-22 days after inoculation.

Washington Field Adult (WFA)

The full procedures for field evaluation of stripe rust resistance were described by Chen and Line (1995). Briefly, for each line, approximately 5g seed was hand planted in a single 61 cm long row with 30.5 cm between row spacing and three replicates. Stripe rust developed in response to natural infection. Both infection type (0 - 9, as described for the controlled environment tests) and disease severity (percentage of leaf areas infected on a plot basis) data were recorded at Feekes stage 11.1.

Montana Controlled Environment Seedling (MCS)

Three replicates of twelve seeds per line were sown in 10x10 cm pots and grown in a greenhouse with a 14 h light per 24 h period augmented by sodium halide lights. Temperatures were 15/20^{O}C±1 (night/day). Plants were inoculated when the third leaf started to emerge using inoculum that traces to an isolate collected in 1997 from a field near Bozeman, Montana (BSTR 97). One mg of rehydrated lyophilized spores were mixed with 9mg of talcum and lightly dusted onto the leaf surface. Inoculated plants were then placed in a dew chamber at 10-12^{O}C for 24 h. Plants were subsequently returned to the greenhouse. Plants were examined daily to determine the first onset of disease symptoms in the form of chlorotic spots (incipient lesions). When pustules had fully developed, typically after 14 days, infection type was rated on a scale of 0-9.

Montana Controlled Environment Adult (MCA)

Six plants representing each genotype were retained after the seedling inoculation and re-inoculated, as described previously, at Feekes stages 8 and 10.5. Infection types (0-9) were assigned 14 days after inoculation.

Mexico Field Adult (MFA)

The set of i-BISON lines and the parental lines were grown in a two-replicate alpha-lattice design with two planting dates at the ICARDA/CIMMYT barley

breeding program facilities in the Toluca Valley of Mexico (hereafter referred to as "TVM"). Disease severity was assessed visually, as the percentage of diseased foliage per plot. Each plot consisted of two 1m rows. Spreader rows surrounded the entire nursery and consisted of a mixture of eight susceptible varieties. Spreaders were inoculated twice with applications of spores suspended in oil. Spores were collected locally and the race composition of the inoculum was not determined. Disease severity readings were conducted at Feekes stage 10.5. Stripe rust epidemics were very consistent and intense, based on the high disease severity observed in susceptible checks and in spreader rows.

Statistical analyses

Combined analyses of variance (ANOVAs) were performed on subsets of the total data using the SAS general linear model (GLM) procedure (SAS Institute, Cary, NC). The first combined ANOVA involved the IT data from the controlled environment tests at Montana and Washington (Table 3.2). Sources of variation were partitioned into races (PSH 31, PSH 54, and BSTR 97), growth stages (seedling and adult), genotypes, and the interactions of these terms. The genotypes term was further partitioned into QTL resistance allele introgressions, parental lines, and an introgression vs. parent contrast. *F*-tests were performed using the pooled error term as the denominator. In order to test the additivity of pyramiding multiple resistance alleles, we performed regression of the infection type LSmeans on the number of QTL resistance alleles.

A second combined ANOVA was based on the IT data from the Washington controlled environment adult (WCA) and Washington field adult (WFA) tests. The average IT's in response to inoculation with PSH 31 and 54 were used for WCA. The sources of variation were partitioned as environments (controlled and field), genotypes (further partitioned into QTL resistance allele introgressions, the parental lines, and an introgression vs. parent contrast), and the interaction of environment and genotype. *F*-tests were performed using the pooled error term as the denominator. The additivity of resistance allele pyramiding was tested using regression.

A third combined ANOVA was based on the percent disease severity data from the WFA and Mexico field adult (MFA) tests. The sources of variation were partitioned as location (Washington and Mexico), genotypes (further partitioned into QTL resistance allele introgressions, the parental lines, and an introgression vs. parent contrast), and the interaction of location and genotype. *F*-tests were performed using the pooled error term as the denominator. The additivity of resistance allele pyramiding was tested using regression.

Results

Stripe rust resistance QTL alleles have consistent effects across races but show interactions with growth stage

When four of the six data sets (sets 1, 2, 4, and 5 as listed in Table 3.2) were used in a combined ANOVA (Table 3.3), neither the three-way interaction (stage*genotype*race) nor the two-way stage*race interaction were significant. There was a highly significant growth stage*genotype interaction. Further partitioning of this source of variation revealed no significant parental line*growth stage interaction and a highly significant introgression*stage interaction. As shown in Figure 3.3, this interaction was due primarily to changes in magnitude of response: all introgressions had higher infection types at the seedling stage than at the adult plant stage. Considering the main effect of growth stage, the average seedling infection type was 6.0 and the average adult plant infection type was 3.8. These values are significantly different at $P < 0.0001$.

However, as is apparent in Figures 3.3 and 3.4, there were notable differences in the magnitudes of response in some of the two-way comparisons of introgressions at the two stages. For example, at the seedling stage, the 5H and 1H + 5H introgressions did not have significantly different infection types (7.0 and 7.2, respectively), but at the adult stage, the introgression with two resistance alleles had a significantly lower ($P < 0.0001$) infection type (3.9) than the single resistance allele introgression (6.2). Similarly, the 1H and 4H + 5H introgressions had seedling infection types of 6.1 and 6.4 , respectively, but at the adult stage the infection types were significantly different ($P < 0.0001$). Again, the introgression line with two resistance alleles had a significantly lower infection type than the line with only one (4.5 and 2.1, respectively).

In order to determine if the introgression of QTL resistance alleles into a stripe rust susceptible background improved resistance at the seedling and adult stages, each introgression class was compared to Baronesse. As shown in Table 3.4, the 5H allele introgression did not confer resistance at either plant stage, the 1H+5H introgression only conferred resistance at the adult stage, and the 0-QTL allele introgression was not significantly different from 'Baronesse' at either growth stage. Increasing the number of QTL resistance alleles led to more resistant types at the adult plant stage, but not at the seedling stage (Figure 3.5).

The genotype*race interaction was significant at $P = 0.07$ (Table 3.3). Further partitioning of this source of variation revealed no significant parental line*race interaction and a $P = 0.06$ significance level for the introgression*race interaction. Given the importance of race specificity in the context of quantitative resistance (Johnson, 1981; Niks and Rubiales, 2002), we believe that these interaction data merit closer examination. We accordingly performed an ANOVA on the combined MCS and WCS data sets; this time including races PSH 1, PSH 13, and PSH 14, which had not been included in the overall combined ANOVA (Table 3.3) due to the inclusion of only the races which were used at both growth stages. In this ANOVA of seedling data involving six races, the introgression*race interaction effect was not significant (data not shown). There was, however, a significant difference amongst infection types: BSTR 97 had the lowest and PSH 54 the highest infection type (Figure 3.6a). The combined ANOVA based on the MCA and WCA data revealed that neither the

introgression*race nor race effects were significant (data not shown). As shown in Figure 3.6b, the trend was for the lowest infection type with BSTR 97 and the highest with PSH 54.

Considering the significant race main effect in the combined analysis (Table 3.3), the only significant difference in infection type was that between BSTR 97 (4.4) and PSH-54 (5.3). A notable exception was the 4H introgression, which, averaged over growth stages, showed a significantly higher infection type in response to inoculation with PSH-31 than in response to BSTR 97 (P=0.0002) or PSH- 54 (P=0.008).

Due to the highly significant stage*genotype interaction we present infection type data for each of the QTL resistance allele introgression classes at each growth stage, averaged over races. At the seedling stage, Baronesse and the 0-QTL introgression line were not significantly different and had the highest infection types (Figure 3.4a). BCD12 and BCD47, the resistance allele donor parents, did not have significantly different infection types (4.5 and 4.3) and both were significantly lower than Baronesse (6.7). The 5H and 1H+5H i-BISON had infection types (7.0 and 7.2) that were not significantly different from Baronesse. All other resistance alleles, and allele combinations, introgressed into Baronesse conferred infection types equal to the resistant parents. No introgression lines surpassed the level of resistance observed in BCD12 and BCD47. The effects of resistance allele introgressions at the adult stage were similar to those at the seedling stage (Figure 3.4b). Baronesse and the 0-QTL introgression were not

significantly different and had the highest infection types (Figure 3.4b). BCD12 and BCD47 were not significantly different from each other and had significantly lower infection types than Baronesse (2.2 and 2.4, respectively). The 5H, 1H, and 1H+5H QTL allele introgressions had infection types (6.2, 4.5, and 3.9, respectively) that were not significantly different from Baronesse. All other resistance alleles and allele combinations introgressed into 'Baronesse' conferred infection types equal to the resistant parents but no resistance allele introgression line surpassed the level of resistance in BCD12 and BCD47.

The magnitudes and ranks of resistance QTL allele effects are consistent across controlled environment and field tests

In the combined ANOVA of the infection type data from WCA and WFA (data sets 2 and 3 in Table 3.2) there was no significant environment*genotype interaction, confirming that all genotypes, at the adult stage, had comparable infection types in controlled environment and field tests (Table 3.5). The environment main effect was also non-significant, indicating that, averaged over genotypes, infection types were comparable in controlled environment and field tests. The genotype main effect was highly significant, and when it was partitioned, both the parent and introgression components were highly significant. The lack of significance of the parent vs. introgression contrast indicates that there was not a significant difference between the average of the introgressions and the average of the parents. Baronesse and the 0-QTL introgression were not

significantly different and had the highest infection types (Figure 3.7). BCD12 and BCD47, the resistant parents, were not significantly different from each other and had significantly lower infection types Baronesse (1.4 and 2.4, respectively). The 5H and 1H+5H resistance allele introgressions did not lead to infection types significantly different from the 0-QTL allele introgression and Baronesse. All other resistance alleles, and allele combinations, introgressed into Baronesse conferred infection types equal to the resistant parents but no introgression line surpassed the level of resistance of BCD12 and BCD47. Considering all allele introgressions, pyramiding multiple QTL resistance alleles led to significantly lower infection types (P=0.008) (Figure 3.8).

The magnitudes and ranks of resistance QTL allele effects are consistent across Washington and Mexico field tests

In the combined ANOVA of the percent disease severity data from the two field data sets (WFA and MFA), there was no significant location*genotype interaction, confirming that adult plants of all genotypes had comparable ranks and magnitudes of adult plant disease severity in the Washington and Mexico tests (Table 3.6). The location main effect was significant, with Mexico having a higher disease severity than Washington (averaged across genotypes). The genotype main effect was highly significant, and when it was partitioned, both the parent and introgression components were highly significant. The lack of significance of the parent vs. introgression contrast indicates that there was not a significant

difference between the average of the introgressions and the average of the parents. Baronesse and the 0-QTL allele introgression lines were not significantly different and had the highest disease severities (Figure 3.9). BCD12 and BCD47 were not significantly different from each other and had significantly lower disease severities than Baronesse (6.5% and 1.6%, respectively). The i-BISON representing 5H, 4H+5H, and 1H+5H resistance allele introgressions had disease severities (29.1%, 19.1%, and 18.0%, respectively) that were not significantly different from 'Baronesse'. All other resistance alleles and allele combinations introgressed into Baronesse conferred levels of resistance, as measured by disease severity, equal to the resistant parents. No BISON lines surpassed the level of resistance of BCD12 and BCD47, but considering al introgression lines, pyramiding multiple QTL resistance alleles led to significantly lower disease severities (P=0.03) (Figure 3.10).

Discussion

Key considerations in breeding for resistance to barley stripe rust, as with many plant diseases, are (i) the growth stage at which disease resistance is assessed, (ii) whether plant material is assessed in controlled environment or field tests, (iii) the race specificity of resistance genes, and (iv) the prospects for MAS as a tool for disease resistance breeding. These considerations are to some extent confounded and require trade-offs. For example, a principal advantage of

controlled environments is the capacity to assess large numbers of seedlings inoculated with multiple races of known composition. However, adult plant resistance in response to field inoculum is usually the measure of agronomic worth, and in the case of the cereal rusts, there is evidence that adult plant resistance has a higher likelihood of durability than seedling resistance (Atienza *et al*., 2004; Sandoval-Islas *et al*., 1998). These considerations will be addressed in turn, based on the results of the experiments described in this report.

We found that all genotypes had higher seedling than adult infection types. The higher seedling infection type may be attributable to the deliberate selection for high seedling infection type and low adult plant disease severity in the ICARDA/CIMMYT breeding program (Sandoval-Islas *et al*., 1998). This program was the original source of the resistance alleles in BCD47 and BCD12 (Hayes *et al*., 2001). If a program chose to capitalize on the advantages controlled environment seedling screens offer in terms of control of race source and population size, these high seedling infection types could be problematic, especially in view of the genotype*stage interaction. For example, both theoretical and empirical studies support the value of resistance gene pyramiding (Kameswara Rao *et al*., 2002) and yet, as detailed in the Results section, at the seedling stage the 5H and 1H + 5H introgressions did not have significantly different infection types whereas at the adult stage, the introgression with two resistance alleles had a significantly lower infection type than the single resistance allele introgression. A similar pattern was observed for the 1H and 4H+5H introgressions. If genotyping

costs could be justified, a combination of phenotypic screening at the seedling stage coupled with marker confirmation of the presence of target alleles would be effective. Otherwise, phenotypic confirmation of adult plant resistance at some point in the breeding program would be necessary.

The literature provides mixed reports on the consistency of controlled environment seedling versus field screening for disease, supporting the notion that seedling phenotyping may only approximate adult plant phenotyping. Castro *et al.* (2002), using antecedents of the resistance donors used for the current investigations, reported that 50% of the QTL were in common to seedling and adult data sets and that the largest effect QTL were those that were in common to the two growth stages. Similarly, Prioul *et al.* (2004) assessed recombinant inbred lines (RILs) for quantitative resistance to *Mycosphaerella pinodes* in pea (*Pisum sativum* L.), at the seedling and adult plant stages. Six QTL were identified affecting resistance at the seedling stage and ten QTL at the adult plant stage, with four QTL common to both stages. We concur with these authors that in our model as well, additional research is necessary to determine if stage-specific QTL are a consequence of developmental gene expression and/or interactions with environmental signals. In contrast, using the downy mildew/ pearl millet system, Jones *et al*. (2002) mapped two QTL identified under field conditions in India that were mapped to the same location in glasshouse screens carried out in both India and the UK.

Of course, measurement of some phenotypes may require specific phenotyping procedures. For example, in a preceding report involving the same germplasm, we measured four individual components of quantitative resistance (latent period, infection efficiency, lesion size, and pustule density) that necessitated the use of a controlled environment (Richardson *et al.*, 2006). Likewise, since adult plant epidemics are usually the cause of significant yield loss, we have used the high disease pressure environment of Toluca, Mexico for resistance gene mapping and validation (Castro *et al*., 2003a; Castro *et al*., 2003c; Toojinda *et al*., 2000; Vales *et al*., 2005).

The QTL allele* race interaction issue is a complicated one. In principle, Parlevliet (1979) defined qualitative and quantitative resistance in the cereal rusts in terms of race-specificity, or the lack thereof. Strictly speaking, quantitative resistance is race-nonspecific with no gene-for-gene interaction between the host and pathogen. The advent of QR dissection technologies, however, has revealed race specific QTL for a number of host: parasite models including potato: *Phytophthora infestans* (Leonards -Schippers *et al*. (1994), barley: *Puccinia hordei* (Atienza *et al*., 2004) and both rice and barley: *Magnaporthe oryzae* (Chen *et al*., 2003). In the current experiments, we detected nearly-significant QTL x race interactions, which could justify further experiments involving more races and replications.

It is often necessary and advisable in disease resistance breeding to use multiple field environments for resistance phenotype validation. For example, in

the case of BSR, epidemics in the Pacific Northwest of the USA are often of lower intensity than in the Toluca Valley of Mexico. Therefore, we have availed ourselves of the latter location for rigorous disease assessments, although cost considerations favor the former location. We determined that there was no significant genotype*location interaction for the resistance QTL targeted in this study: the QTL effects for each line in Washington were consistent with those in Mexico. The results of Vales *et al*. (2005) confirm this finding. Using a large (n=409) population of doubled haploid lines for barley stripe rust severity in the Toluca Valley, Mexico and in Washington state, USA, under field conditions at the adult plant stage, of the eight QTL detected in Mexico and the five detected in Washington, five were coincident between the two locations. Despite dramatic climatologic and environmental variance between the two testing locations, the ranks and magnitudes of the QTL effects were consistent.

Our data confirm the value of the targeted BSR resistance alleles and the utility of marker assisted quantitative resistance gene mapping and introgression. They also reveal the necessity of validating the value of resistance alleles as these are introgressed into susceptible backgrounds. The data do not conclusively support a benefit to pyramiding multiple resistance alleles in single genotypes. However, the potential durability of pyramids, as compared to simpler genetic constructs, may justify their construction.

Acknowledgements

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Table 3.1: Comparison of patterns of virulence of nine differential barley cultivars inoculated with six races of *Puccinia striiformis* f. sp. *hordei*: barley stripe rust (BSTR) 97 (97), *Puccinia striiformis hordei* (PSH) 31 (31), PSH 54 (54), PSH 1 (1), PSH 13 (13), and PSH 14 (14).

	Races					
Differential	97	31	54	1	13	14
Abed Binder 12	S	S	S	R	R	S
Asterix	S	S	R	R	R	S
Bigo	R	S	R	R	S	R
Emir	S	S	R	R	S	S
Heils Franken	R	S	R	S	S	S
I5	R	R	R	R	R	R
Mazurka	R	S	R	R	S	R
Trumph	R	S	S	R	S	S
Varunda	R	S	R	R	S	S

S=susceptible, R=resistant

Table 3.2: Six data sets used for analyses of barley stripe rust infection (IT=infection type and %=percent disease severity).

Location	Environment	Plant Stage	Abbreviation	Race makeup	Data
Washington	Controlled	Seedling	WCS	PSH-1, PSH-13, PSH-14, PSH-31, and PSH-54	IT
Washington	Controlled	Adult	WCA	PSH-31 and PSH-54	IT
Washington	Field	Adult	WFA	Local field race composition	IT and %
Montana	Controlled	Seedling	MCS	BSTR 97	IT
Montana	Controlled	Adult	MCA	BSTR 97	IT
Mexico	Field	Adult	MFA	Local field race composition	%

Table 3.3: Combined ANOVA of barley stripe rust infection type data considering the effects of growth stage, stripe rust race, genotypes (further partitioned into parents and resistance QTL introgressions), and their interactions. The data sets included in this ANOVA were Washington Controlled Seedling, Washington Controlled Adult, Montana Controlled Seedling, and Montana Controlled Adult.

Source	DF	MS	*P*-value
Rep(Stage)	4	0.84	0.8909
Stage	1	160.11	< 0.0001
Race	2	9.79	0.0414
Genotypes	10	58.33	< 0.0001
Introgressions	7	66.80	< 0.0001
Parents	2	54.90	< 0.0001
Introgressions vs. Parents contrast	1	0.57	0.6667
Genotype*Race	20	4.63	0.0704
Introgression*Race	14	5.09	0.0596
Parent*Race	4	2.42	0.5299
Stage*Genotype	10	12.31	< 0.0001
Stage*Introgression	7	17.53	< 0.0001
Stage*Parent	2	0.13	0.9595
Stage*Race	2	5.54	0.1639
Stage*Genotype*Race	22	2.31	0.7752
Pooled Error	412	3.05	

Table 3.4: Significance of least square means for infection type of barley stripe rust at the seedling and adult growth stages when comparing resistance QTL introgressions to the susceptible parent ('Baronesse'). The data sets included in this analysis were Washington Controlled Seedling, Washington Controlled Adult, Montana Controlled Seedling, and Montana Controlled Adult.

	Infection type and *P*-value			
	Seedling		Adult	
'Baronesse'	8.62		6.70	
1H	6.07	0.0107	4.51	0.0109
4H	4.24	<0.0001	2.31	<0.0001
5H	7.03	0.0840	6.21	0.5398
1H+4H	5.22	0.0010	2.59	<0.0001
1H+5H	7.17	0.1267	3.92	0.0010
4H+5H	6.40	0.0214	2.08	<0.0001
1H+4H+5H	4.96	0.0038	0.96	<0.0001
0-QTL	7.96	0.5966	7.56	0.4234

Table 3.5: Combined ANOVA of stripe rust infection type data considering the effects of barley genotype and controlled environment versus field data. The data sets included in this ANOVA were Washington Controlled Adult and Washington Field Adult.

Source	DF	MS	*P*-value
Rep(Environment)	4	3.70	0.7152
Environment	1	0.31	0.8331
Genotypes	10	63.55**	<0.0001
Introgressions	7	75.18**	<0.0001
Parents	2	44.18*	0.0021
Introgressions vs. Parents contrast	1	4.62	0.4171
Environment*Genotype	10	7.65	0.3682
Environment*Introgression	7	10.52	0.1665
Environment*Parent	2	1.31	0.8292
Pooled Error	226	6.99	

Table 3.6: Combined ANOVA of stripe rust percent disease severity data considering the effects of barley genotype and Mexico versus Washington data. The data sets included in this ANOVA were Washington Field Adult and Mexico Field Adult.

Source	DF	Mean Square	*P*- value
Rep(location)	3	1.94	0.4199
Location	1	27.15	0.0004
Genotype	10	11.03	<0.0001
Introgression	7	12.23	<0.0001
Parent	2	11.52	0.0044
Introgression vs. parent contrast	1	0.54	0.6803
Location*Genotype	10	1.60	0.6442
Location*Introgression	7	1.63	0.5905
Location*Parent	2	2.13	0.3550
Error	151	2.05	

Figure 3.1: The pedigree and derivation of the intermediate- barley near-isogenic (i-BISON) lines for stripe rust resistance QTL alleles indicating generations when phenotypic and genotypic screenings were performed. Black squares indicate resistance allele donors.

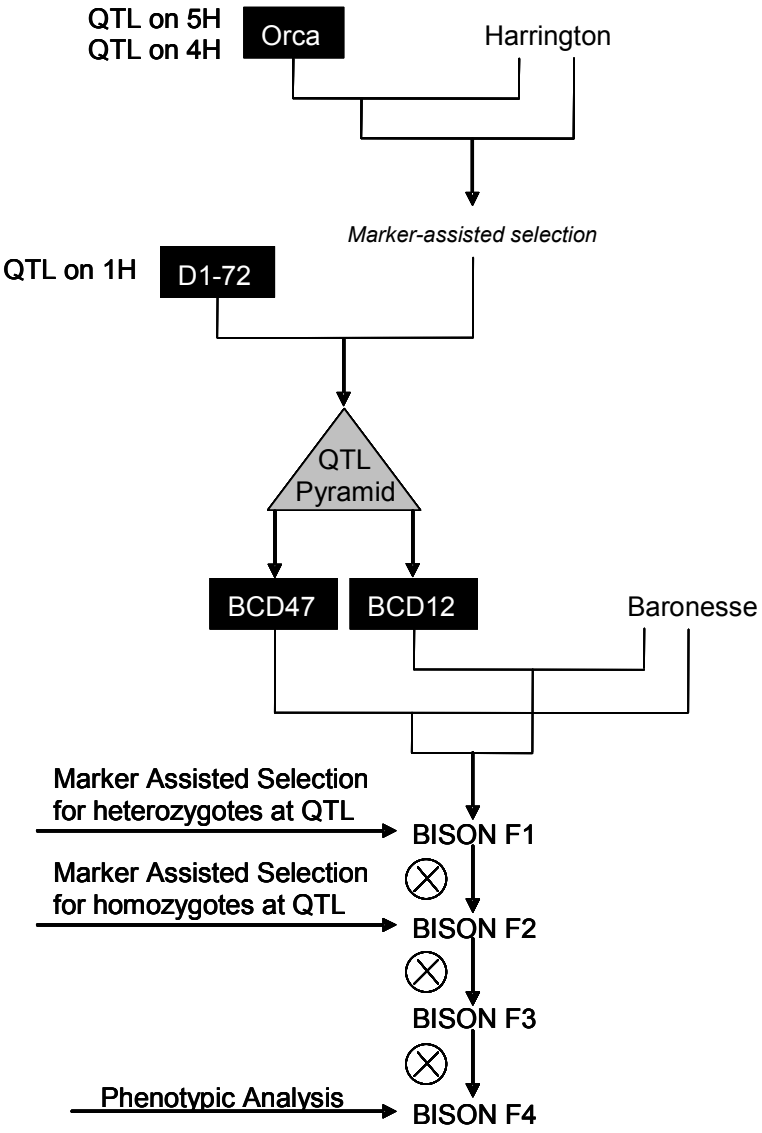


Figure 3.2: Chromosomes 1H, 4H, and 5H from the linkage map of the BCD47/'Baronesse' DH population constructed by Vales *et al*. (2005) showing only markers used for foreground screening of the intermediate-barley near-isogenic (i-BISON) lines for stripe rust resistance QTL alleles. The dotted lines indicate regions with distances inferred from Ramsay *et al*. (2000).

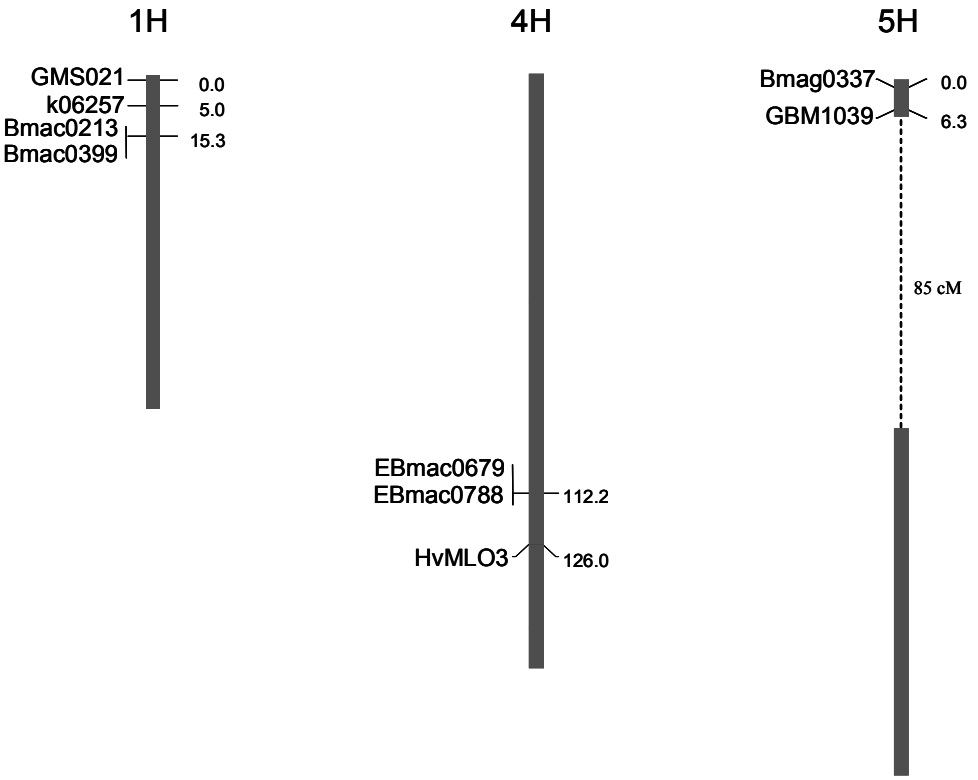


Figure 3.3: Least square means for infection type, averaged over barley stripe rust races, for seven QTL resistance allele introgression combinations, and the 0-QTL control, at the seedling and adult plant stages. The data sets included in this analysis were Washington Controlled Seedling, Washington Controlled Adult, Montana Controlled Seedling, and Montana Controlled Adult.

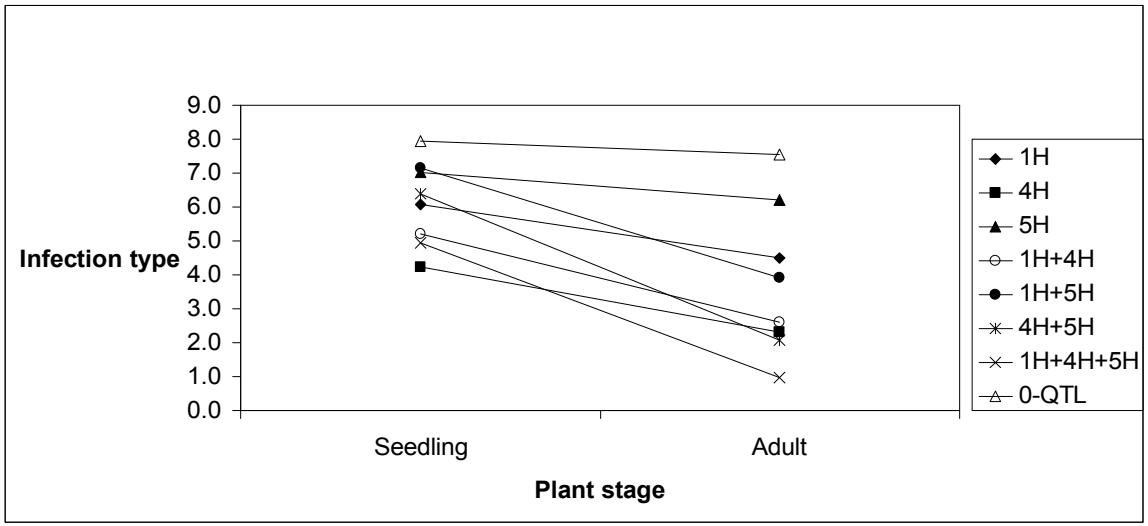
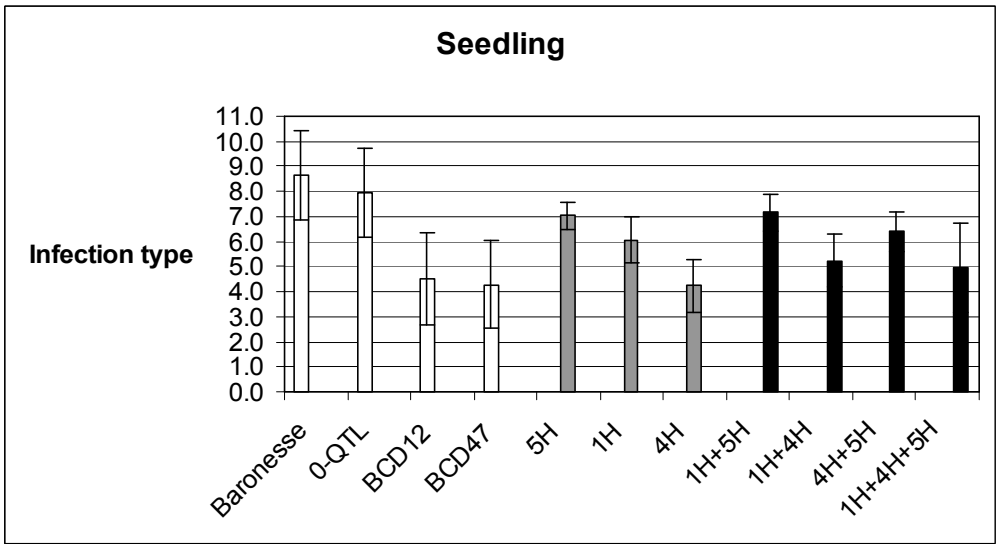


Figure 3.4: Least-square means for infection type in barley genotypes after inoculation with three different stripe rust races at the (a) seedling and (b) adult growth stages. White bars are parents and the 0-QTL control; grey are single QTL allele introgressions, and black are combinations of QTL allele introgressions. Error bars indicate 95% confidence intervals. The data sets included in this analysis were Washington Controlled Seedling, Washington Controlled Adult, Montana Controlled Seedling, and Montana Controlled Adult.

(a)



(b)

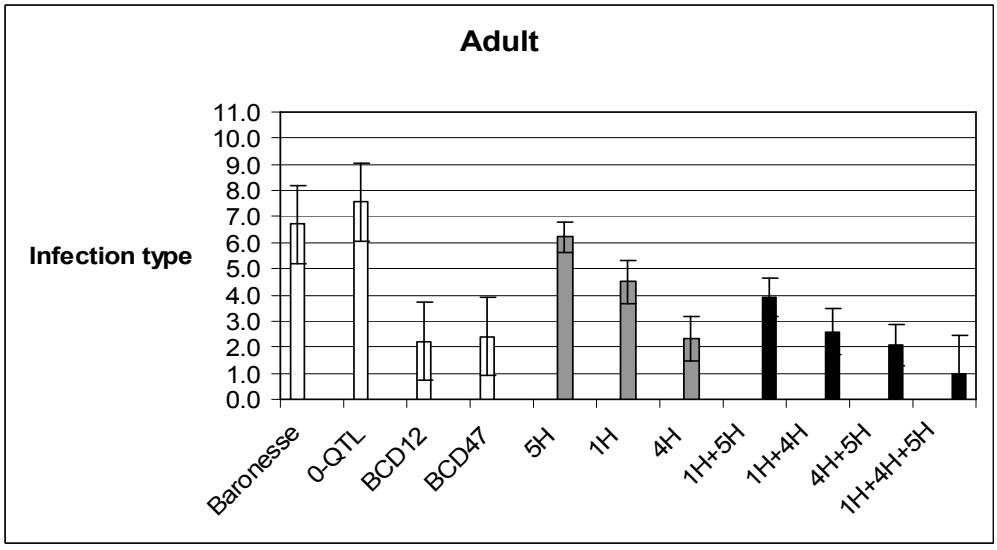
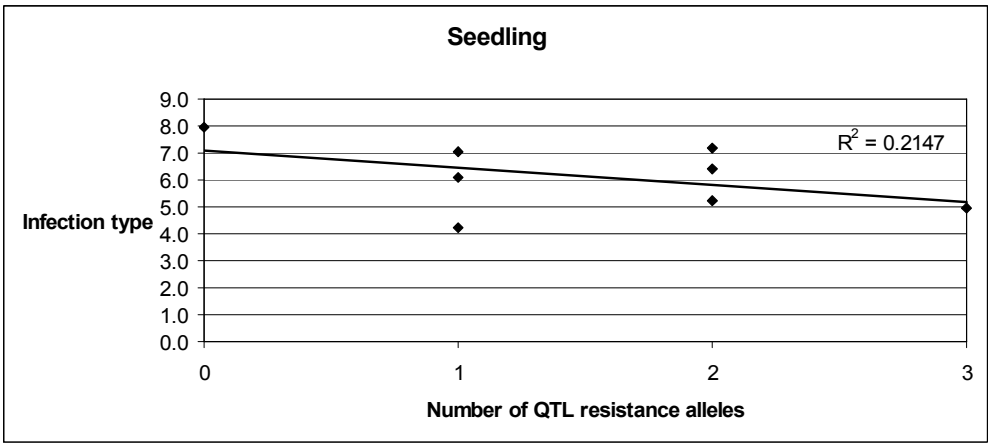


Figure 3.5: Regression of infection type of barley stripe rust on the number of QTL targets per introgression at the (a) seedling and (b) adult plant stages. The data sets included in this analysis were Washington Controlled Seedling, Washington Controlled Adult, Montana Controlled Seedling, and Montana Controlled Adult.

(a)



(b)

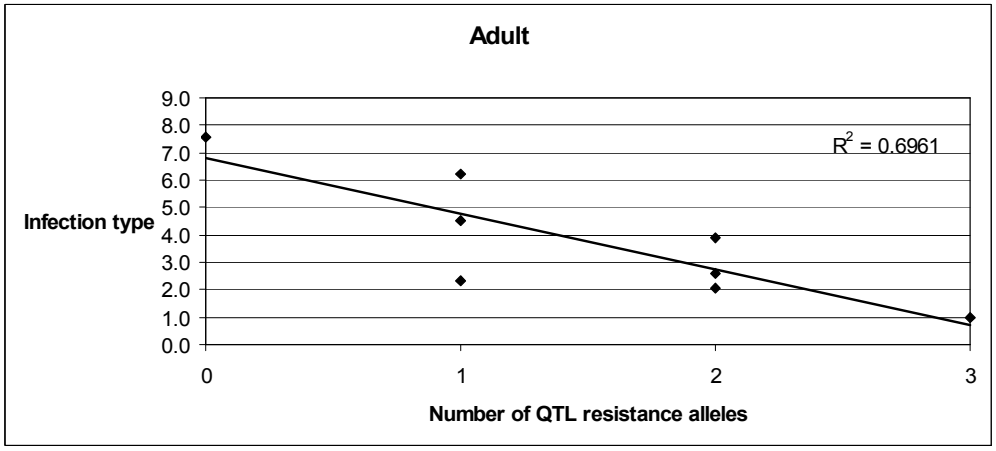
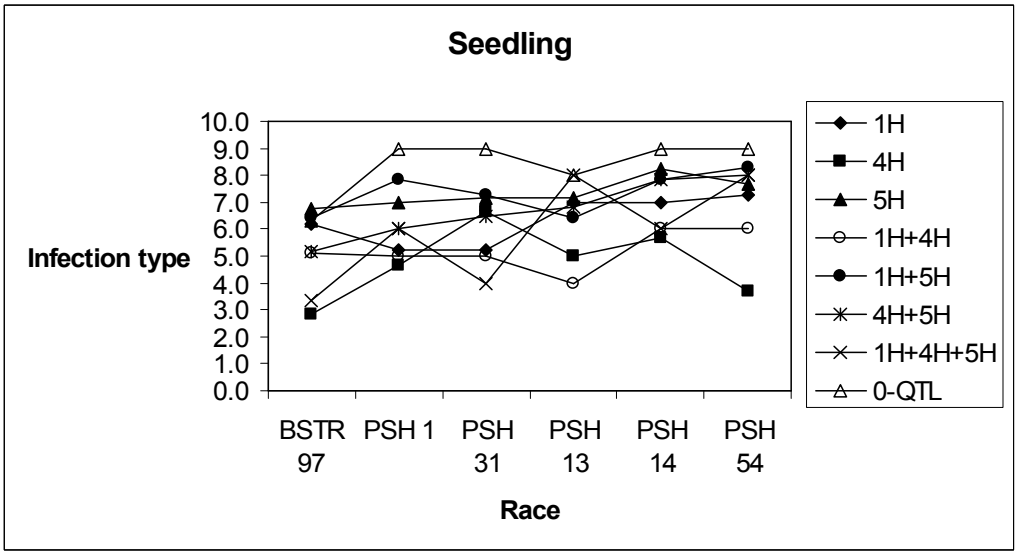


Figure 3.6: Least- square means for infection type of barley stripe rust, averaged over growth stages, for seven QTL resistance allele introgression combinations, and the 0-QTL control, after inoculation with three different isolates at the (a) seedling and (b) adult stages. The data sets included in this analysis were Washington Controlled Seedling, Washington Controlled Adult, Montana Controlled Seedling, and Montana Controlled Adult.

(a)



(b)

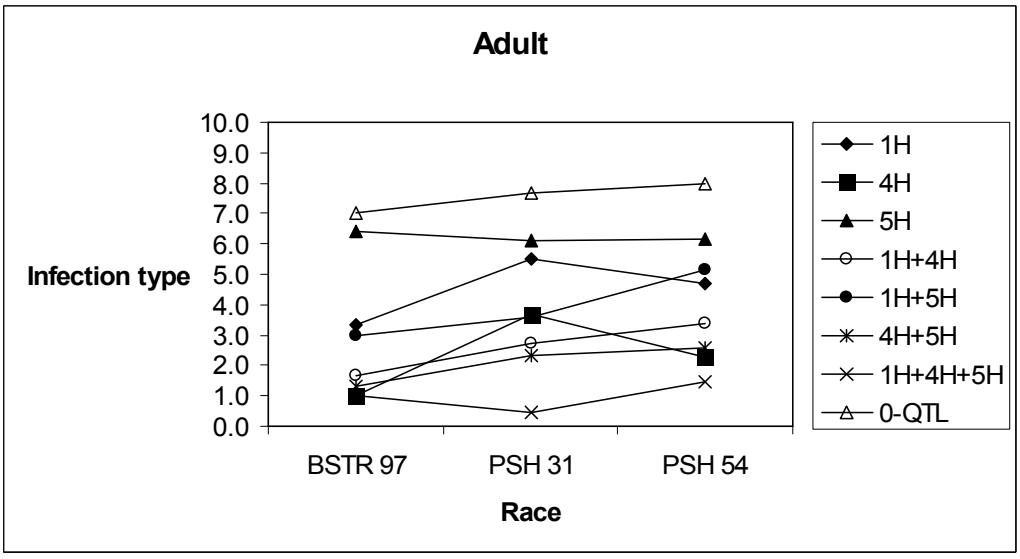


Figure 3.7: Least-squares means for infection type of stripe rust in barley genotypes averaged across controlled and field adult plant testing environments. White bars are parents and the 0-QTL control; grey are single QTL allele introgressions, and black are combinations of QTL allele introgressions. Error bars indicate 95% confidence intervals. The data sets included in this analysis were Washington Controlled Adult and Washington Field Adult.

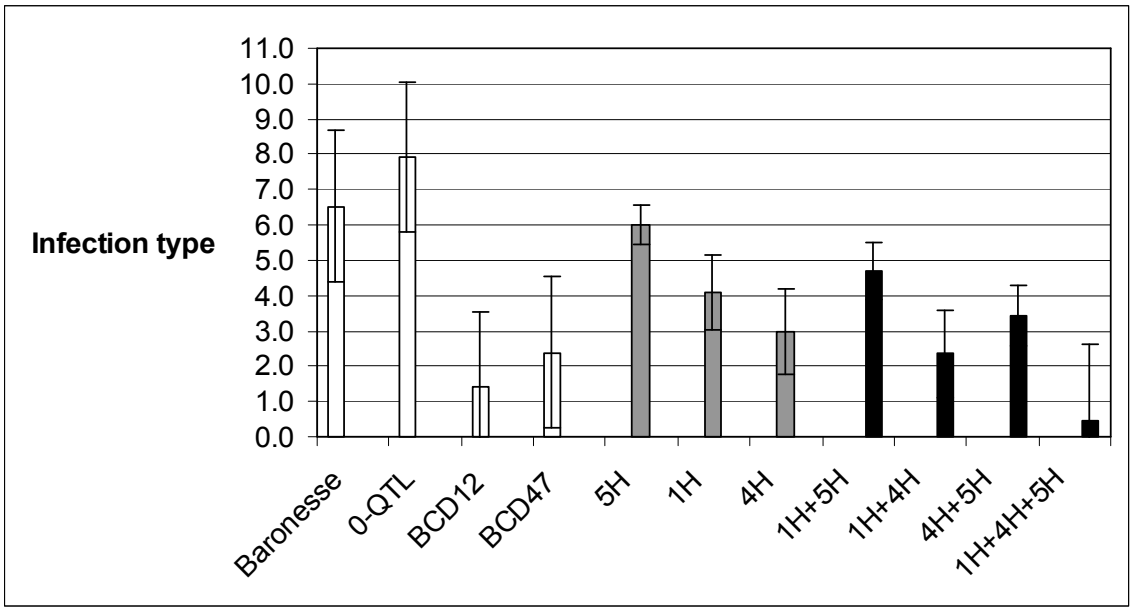


Figure 3.8: Regression of infection type of barley stripe rust on the number of QTL resistance alleles averaged across controlled environment and field tests. The data sets included in this analysis were Washington Controlled Adult and Washington Field Adult.

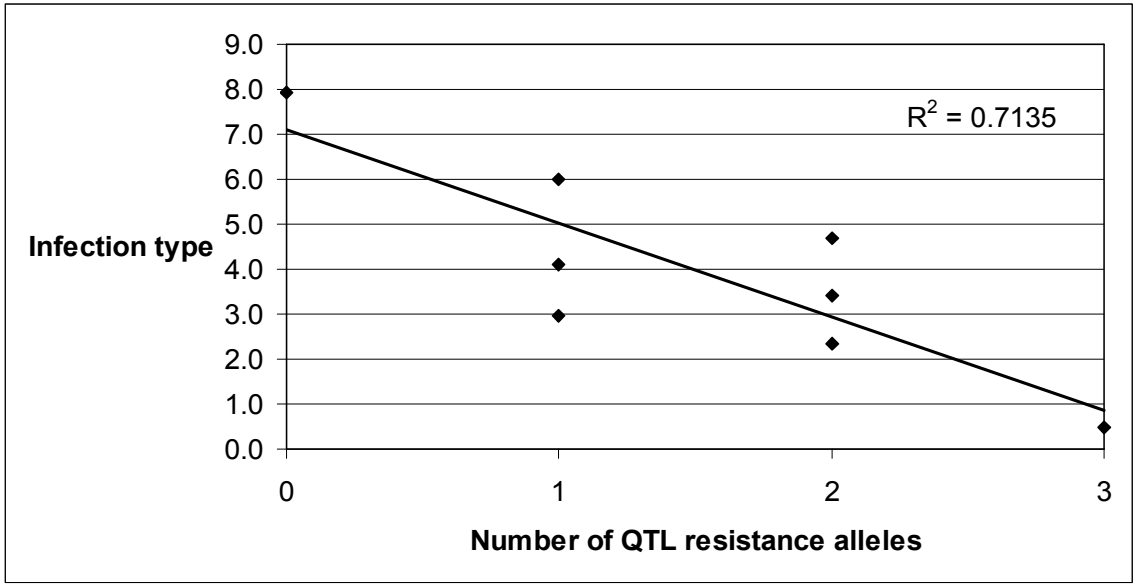


Figure 3.9: Least-square means for percent disease severity of stripe rust in barley genotypes averaged across Mexico and Washington adult plant field tests. White bars are parents and the 0-QTL control; grey are single QTL allele introgressions, and black are combinations of QTL allele introgressions. Error bars indicate 95% confidence intervals. The data sets included in this analysis were Mexico Field Adult and Washington Field Adult.

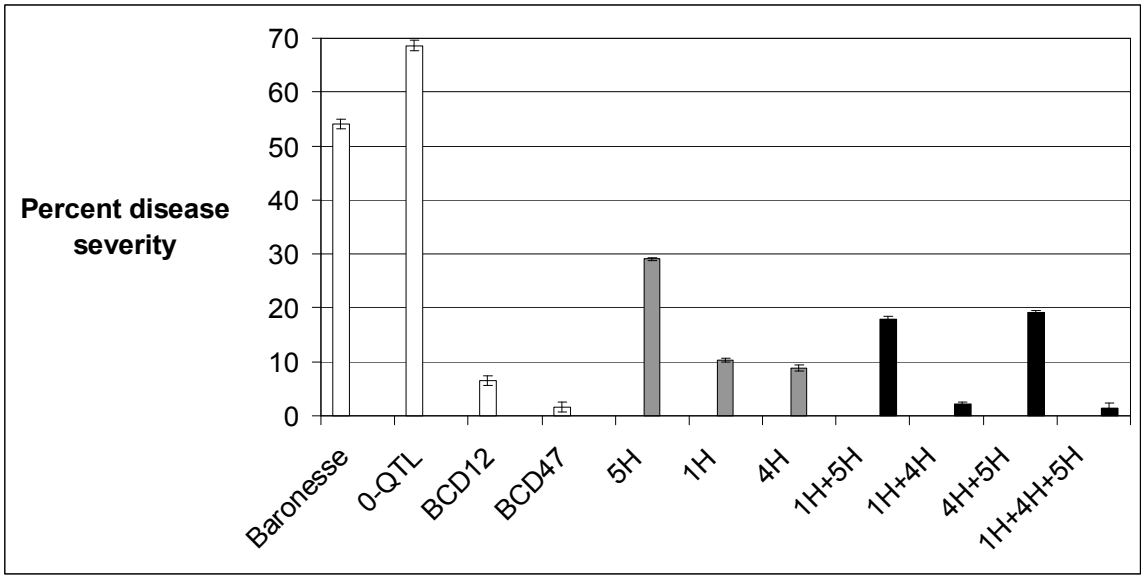
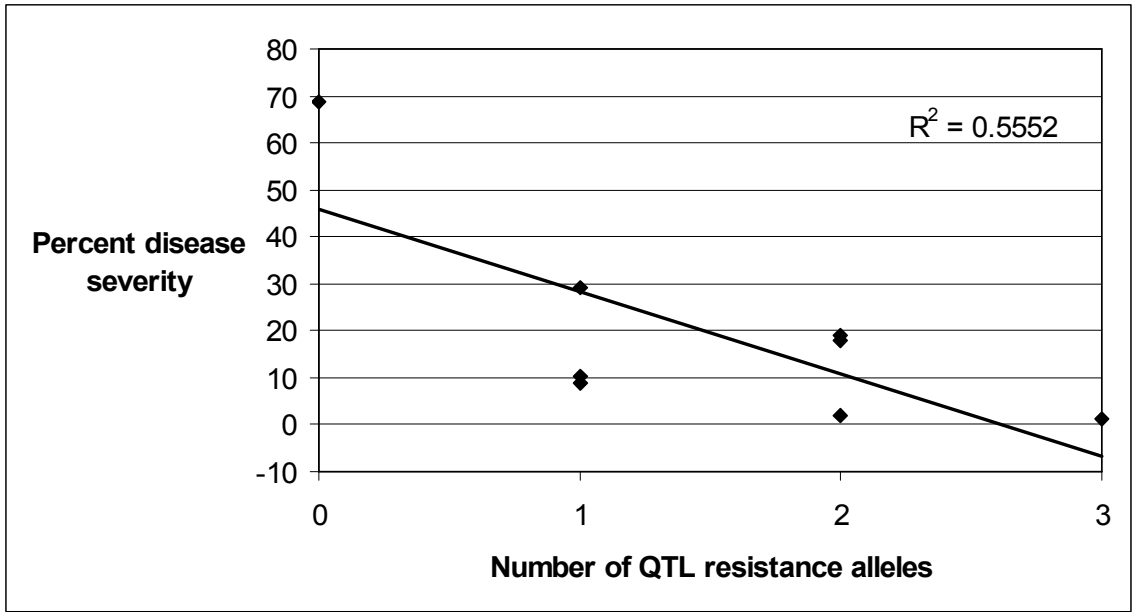


Figure 3.10: Regression of percent disease severity of barley stripe rust on the number of QTL resistance alleles averaged across Mexico and Washington field adult test indicating the effectiveness of pyramiding QTL target introgressions. The data sets included in this analysis were Mexico Field Adult and Washington Field Adult.



GENERAL CONCLUSIONS

Quantitative resistance (QR) to plant diseases has been a long sought after trait in plant breeding. The broad spectrum resistance and, therefore, potential durability of QR most often outweigh the ease of introgression of qualitative, gene-for gene sources of resistance. Marker assisted selection (MAS) for QR is certainly more complex, yet information from QTL discovery and linkage mapping efforts can be used to design schemes for resistance breeding. We used the barley/barley stripe rust pathosystem as a model for the characterization of the QR phenotype and associated genomic regions. The results of this research demonstrate that MAS is effective for introgressing resistance QTL alleles with consistent effects.

The BISON lines were developed primarily for determining the individual effects and interactions of barley stripe rust (BSR) resistance QTL alleles when introgressed into a susceptible variety grown extensively in the Pacific Northwest of the USA. Midway through the preparation of these BISON lines, we developed a set of QTL introgression lines representing disease resistance QTL alleles in one-, two-, and three-way combinations in a susceptible background: the intermediate barley near-isogenic (i-BISON) lines. In this research, (i) four components of disease resistance: latent period, infection efficiency, lesion size, and pustule density and (ii) infection type or percent disease severity at the seedling and adult plant stages, in controlled and field environments, with varying races of the

pathogen, and combinations thereof were measured on the i-BISON lines. The goals were to determine (i) if the disease components are QTL-specific, (ii) if pyramiding resistance alleles at multiple QTL leads to higher levels of resistance, (iii) if there is resistance QTL allele race-specificity; (iv) the effects of resistance alleles at the adult vs. seedling stage, and (v) the effects of resistance alleles under controlled versus field environments.

This research was prompted by the fact that while quantitative disease resistance is highly valued for its higher probability of durability, the genetic basis and underlying mechanisms are not as well understood as with qualitative resistance.

Key results of these experiments

Pyramiding and dissecting disease resistance QTL to barley stripe rust

- When comparing introgressions with the same QTL allele architecture as the resistance donor parents, e.g. 4H+5H vs. BCD47 and 1H vs. BCD12, there were no significant differences for latent period, infection efficiency, lesion size, and pustule density.
- The 4H introgression had the largest effect on the four components of resistance. The order of magnitude of effects for latent period, infection efficiency, and pustule density was 4H > 1H > 5H. For lesion size, there was no significant difference in the effects of 1H, 4H, and 5H.

- The four components of resistance were highly correlated.
- Increasing the number of QTL resistance alleles in single genotypes led to more resistant infection efficiency, lesion size, and pustule density.

Barley stripe rust resistance QTL alleles are effective across growth stages, races, and environments

Stripe rust resistance QTL alleles have consistent effects across races but show interactions with growth stage

- There were notable differences in the magnitudes of response (crossover interactions) in some of the two-way comparisons of introgressions at the two stages resulting in a significant introgression*stage interaction.
- Increasing the number of QTL resistance alleles led to more resistant types at the adult plant stage, but not at the seedling stage.
- Further examination of the genotype*race interaction ($P = 0.07$) revealed no significant adult*race or seedling*race interactions.
- No introgression line surpassed the level of resistance observed in the resistance QTL allele donors, BCD12 and BCD47, at either the seedling or adult plant stage.

The magnitudes and ranks of resistance QTL allele effects are consistent across controlled environment and field tests

- The non-significant genotype*environment interaction effect indicated that all genotypes had comparable infection types in controlled environment and field tests.

- Considering all allele introgressions, pyramiding multiple QTL resistance alleles led to significantly lower infection types.

The magnitudes and ranks of resistance QTL allele effects are consistent across Washington and Mexico field tests

- There was no significant location*genotype interaction, confirming that adult plants of all genotypes had comparable ranks and magnitudes of adult plant disease severity in the Washington and Mexico tests.
- The location main effect was significant, with Mexico having a higher disease severity than Washington (averaged across genotypes).
- Considering al introgression lines, pyramiding multiple QTL resistance alleles led to significantly lower disease severities.

Recommendations for future research

The first set of experiments focused on seedling resistance to a single race. The second set of experiments focused on seedling and adult resistance in different environments and with different races. The final products of this research, the BISON lines, should ultimately allow for characterization of quantitative resistance genes in terms of their structure and function through additional experiments such as:

- Measuring the four components of disease resistance: latent period, infection efficiency, lesion size, and pustule density, on the final BISON lines at both the seedling and adult plant stage to determine (i) if the disease components are

QTL-specific and (ii) if pyramiding resistance alleles at multiple QTL leads to higher levels of resistance.

- Developing additional sets of BISON lines in different genotypes to determine the effect of background genotype on BSR resistance QTL alleles.
- Analyzing gene expression differences, through microarray analysis, in the quantitatively resistant BISON 4H and the qualitatively resistant BISON 7H when inoculated with the pathogen at both the seedling and adult plant stage and with varying races.
- Further dissecting the resistance QTL allele on chromosome 4H, the most important QTL target, by analyzing the progeny of a BISON 4H x Baronesse cross with defined breakpoints throughout the 4H region.

We have shown how developing NILs for the barley/ barley stripe rust pathosystem has improved our understanding of the importance of resistance QTL alleles, the nature of quantitative disease resistance, and how utilizing molecular breeding tools can lead to the development of durable and consistently resistant cultivars.

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APPENDIX

A DETAILED DESCRIPTION OF THE DEVELOPMENT OF THE BARLEY NEAR-ISOGENIC (BISON) LINES

Overview

The BISON (barley near-isogenic) lines are genetic stocks with individual, or combinations of, barley stripe rust resistance QTL alleles introgressed into a Baronesse background. The target QTL alleles on chromosomes 4H and 5H come from BCD47 and the target QTL allele on chromosome 1H comes from BCD12. Lines with a designation 1H, 4H, or 5H only have a single QTL allele introgressed, from the corresponding chromosome. Lines with combinations (e.g. 1H+4H) have multiple resistant QTL alleles introgressed. The lines with the 7H designation have a qualitative (major) gene for barley stripe rust resistance introgressed. This resistance gene was originally discovered in CI10587; the donor for development of the BISON was D3-6/B23. The "control" line (0-QTL) was selected from the same population as the other BISON lines, but was selected for the susceptibility alleles (tracing to Baronesse) at each QTL region (1H, 4H, and 5H).

Germplasm development

Two initial crosses were made for the generation of the BISON population: BCD12/Baronesse and BCD47/Baronesse. The single cross F1s were then crossed: BCD12/Baronesse // BCD47/Baronesse. The resulting progeny were designated as the F1 generation of the barley near-isogenic (BISON) lines. A

second population was generated to introgress the qualitative source of BSR resistance on chromosome 7H into the susceptible Baronesse background. The source of the major gene on chromosome 7H, D3-6/B23, was crossed with Baronesse and the resulting progeny were designated as the F1 generation of the BISON 7H population.

Marker assisted selection (MAS) in the F1 generation

247 F1 BISON lines were screened with twelve simple sequence repeat (SSR) molecular markers: GMS21, Bmac0399, Bmac0213, and Bmac0032 spanning the 1H QTL region; EBmac0701, EBmac0635, EBmac0788, and HvMlo3 spanning the 4H QTL region; and Bmac0096, Bmag323, Bmag0337, and EBmac0684 spanning the 5H QTL region. Subsequent mapping efforts revised the map position of EBmac0684 to chromosome 2H, so it was removed from the screening. 126 lines were selected for further analysis for the 1H QTL region, 92 for the 4H QTL region, and 115 for the 5H QTL region. The lines were selfed and seed was collected. Four F1 BISON 7H lines were screened with one SSR, Bmag0120, to confirm heterozygosity. The lines were selfed and seed was collected.

MAS in the F2 generation

The BISON F2 lines were screened with eleven SSRs: GMS21, Bmac0399, Bmac0213, and Bmac0032 spanning the 1H QTL region; EBmac0701,

EBmac0635, EBmac0788, and HvMlo3 spanning the 4H QTL region; and Bmac0096, Bmag323, and Bmag0337 spanning the 5H QTL region. For the 1H QTL region, four lines (BISON 69, 128, 160, and 191) were selected because they were homozygous for the BCD12 allele for only the 1H QTL. All four lines were advanced to the F5 generation through single seed decent. For the 4H QTL region, four lines (BISON 18, 104, 107, and 129) were selected because they were homozygous for the BCD47 allele for only the 4H QTL. BISON 107 was not advanced to further generations because of poor seed development. BISON 18, 104, and 129 were advanced to the F5 generation through single seed decent. For the 5H QTL region, 19 lines (BISON 5, 9, 10, 19, 29, 40, 25, 60, 87, 96, 100, 110, 111, 136, 157, 168, 170, 194, and 217) were selected because they were homozygous for the BCD47 allele for only the 5H QTL. BISON 194 was not advanced to further generations because of poor seed development. All remaining lines were advanced to the F5 generation through single seed decent. BISON 130, the "control" line, was selected from the F2 BISON population for the susceptibility alleles (tracing to Baronesse), instead of the resistance alleles, at each QTL region (1H, 4H, and 5H). This line was advanced to the F5 generation through single seed decent.

In order to increase the chances of finding lines homozygous for the QTL resistance alleles at multiple target introgressions, four seeds from each of 24 selected F2 BISON lines (3, 22, 29, 32, 45, 50, 53, 60, 63, 95, 110, 113, 129, 136, 138, 157, 208, 210, 212, 216, 217, 218, 231, and 243) were planted and seedlings

were screened with three SSRs: Bmac0213, HvMlo3, and Bmag0337. These three SSRs represented the closest marker available to the QTL peaks on chromosomes 1H, 4H, and 5H, respectively. The 24 lines were selected because at the F2 generation they were either homozygous for multiple QTL resistance alleles or heterozygous. Of the resulting 96 lines (24 x 4), 17 were selected and are hereinafter referred to as BISON P.T.C. (putative triple crown) lines. For the 1H+4H QTL target, BISON P.T.C. 136-2, 216-4, and 243-4 were selected because they were homozygous for the BCD47 allele for the 4H QTL and for the BCD12 allele for the 1H QTL. All three lines were advanced to the F5 generation through single seed decent.

For the 1H+5H and 4H+5H QTL targets, both BISON and BISON P.T.C. lines were selected. For the 1H+5H QTL target, eight lines (BISON P.T.C. 32-1, 32-3, 50-1, 53-1, 110-3, 210-2, 218-1, and BISON 174) were selected because they were homozygous for the BCD12 allele for the 1H QTL and for the BCD47 allele for the 5H QTL. BISON P.T.C. 32-1 was not advanced to further generations because of poor seed development. BISON P.T.C. 32-3, 50-1, 53-1, 110-3, 210-2, 218-1, and BISON 174 were advanced to the F5 generation through single seed decent. For the 4H+5H QTL regions, seven lines (BISON P.T.C. 3-4, 22-4, 110-2, 113-4, 208-3, 217-2, and BISON 108) were selected because they were homozygous for the BCD47 allele for the 4H QTL and for the BCD47 allele for the 5H QTL. BISON P.T.C. 110-2 was not advanced to further generations because of poor seed development. BISON P.T.C. 3-4, 22-4, 113-4, 208-3, 217-2,

and BISON 108 were advanced to the F5 generation through single seed decent. For the most challenging target - the 1H+4H+5H QTL allele pyramid - one line (BISON P.T.C. 95-2) was selected because it was homozygous for the BCD12 allele at the 1H QTL, and for the BCD47 alleles at both the 4H and 5H QTL. This line was advanced to the F5 generation through single seed decent.

50 F2 seeds from each of the four BISON 7H lines were planted and all 200 lines were genotyped with two SSRs and one STS: Bmag0120, Bmac0156, and Ris44, respectively. Ten lines (BISON 7H 1-31, 1-37, 2-20, 2-22, 2-36, 3-38, 3-39, 3-40, 3-50, and 4-10) were selected because they were homozygous for the D3-6/B23 allele for the three loci screened. BISON 7H 1-31, 2-36, and 3-39 were not advanced to further generations because of poor seed development. All remaining lines were advanced to the F5 generation.

Crossing F5 selections to Baronesse

All lines that were advanced to the F5 generation were crossed to Baronesse. In all crosses, Baronesse served as the male parent. These are referred to as backcross (BC1) lines because Baronesse was the principal allele donor in the original four-way cross. BC1 seed was harvested and three seeds per BC1 were planted and genotyped to confirm heterozygosity at QTL target introgression sites of interest. These selected BC1 lines were backcrossed a second time, again with Baronesse serving as the male parent. BC2 seed from all crosses of each line was bulked. From the bulk for each line, up to 10 BC2 seeds were planted. A total of

152 BC2 seedlings representing 18 lines for the eight QTL target introgressions (three lines for 1H, one for 4H, five for 5H, three for 7H, one for 1H+4H, two for 1H+5H, two for 4H+5H, and one for 1H+4H+5H), were genotyped to confirm heterozygosity and to remove lines homozygous for the Baronesse allele at QTL target introgression sites of interest. Five BC2 seedlings representing one line for the 0-QTL target introgression were genotyped to identify and select lines homozygous for the Baronesse allele at QTL target introgression sites of interest.

While the genotyping was being conducted, phenotypic data for all lines were generated in order to select one representative line per introgression. In Washington, USA infection type (IT) was measured in a controlled environment at the seedling and adult plant stages. IT and percent disease severity were measured on adult plants in the field in Washington, USA. IT was measured in a controlled environment at the seedling and adult plant stages in Montana, USA. Percent disease severity was measured on adult plants in the field in the Toluca Valley, Mexico. For example, of the three lines for the 1H QTL target, (BISON 69, 128, and 191) BISON 69 was selected. BISON 18 was selected for the 4H QTL, BISON 111 for the 5H QTL, BISON 7H 2-22 for the 7H major gene, BISON P.T.C. 136-2 for the 1H+4H QTL, BISON P.T.C. 110-3 for the 1H+5H QTL, BISON P.T.C. 22-4 for the 4H+5H QTL, BISON P.T.C. 95-2 for the 1H+4H+5H QTL, and BISON 130 for the 0-QTL. Of the ten (or fewer) BC2 seedlings, per introgression, genotyped, four were selected for 1H, two for 4H, four for 5H, two for 7H, one for 1H+4H, two for 1H+5H, and two for 4H+5H. Of the seven BC2

seedlings genotyped for the 1H+4H+5H QTL none were heterozygous for the resistance allele at all three of the QTL target introgression sites of interest. All remaining BC2 seeds from BISON 95-2 were planted and the 29 seedlings were screened in hopes of finding at least one heterozygous for the resistance allele at all three of the QTL target introgressions. Five of those screened were selected for the 1H+4H+5H QTL introgression. One BC2 seedling for the 0-QTL target introgression (BISON 130-3) was selected as the final representative line. These 23 seedlings were selfed and BC2S1 seed was collected.

For each of the single QTL allele introgressions (1H, 4H, 5H, and 7H) 20 BC2S1 seeds were planted from each of the selected lines. For each of the combination QTL allele introgressions (1H+4H, 1H+5H, 4H+5H, and 1H+4H+5H) 80 BC2S1 seeds were planted from each of the selected lines. All BC2S1 seedlings were genotyped with three SSRs: Bmac0399 (1H), EBmac0679 (4H), and Bmag0337 (5H), to select lines homozygous for the resistance allele at the target QTL and for the susceptible allele at the other QTL. For example, a line representing the 1H QTL target was selected if it was homozygous for the BCD12 allele at Bmac0399 and homozygous for the Baronesse allele at EBmac0679 and Bmag0337. Eleven lines were selected for the 1H QTL target introgression, eight for the 4H QTL, 10 for the 5H QTL, six for the 7H major gene, five for the 1H+4H QTL, seven for the 1H+5H QTL, and eight for the 4H+5H QTL. One BC2S1 seedling for the 1H+4H+5H QTL target introgression (BISON P.T.C. 95-

2-5-10) was selected as the final representative line. All selected plants were selfed and BC2S2 seed was collected.

Three BC2S2 seeds from each selected line were planted and seedlings were genotyped with the same three foreground SSRs previously mentioned as well as with 42 additional background SSR, STS, and EST molecular markers. The selected line for the 0-QTL introgression (BISON 130-3) and the 1H+4H+5H QTL introgression (BISON P.T.C. 95-2-5-10) were also included in the background molecular screening. Final representative lines were selected based on the percentage of Baronesse background. The selected lines were 69-9-11-1 for 1H, 18-7-4-2 for 4H, 111-5-20-3 for 5H, 2-22-4-7-1 for 7H, 136-2-7-58-1 for 1H+4H, 110-3-2-71-1 for 1H+5H, 22-4-7-44-3 for 4H+5H, BISON P.T.C. 95-2-5-10 for 1H+4H+5H, and BISON 130-3 for 0-QTL.

Utilization of BISON lines

Our ultimate goal was to develop a set of near-isogenic lines (NILs) representing resistance alleles at individual QTL and combinations of QTL, yet we were able to utilize intermediate-stage germplasm to answer some important questions about the nature of quantitative resistance to barley stripe rust. As an intermediary step between the available QTL allele introgression lines in variable genetic backgrounds and our final goal, we developed a set of QTL resistance allele introgression lines in a more homogenous genetic background. 25 F5 generation BISON, BISON P.T.C., and BISON 7H lines representing eight QTL

target introgressions (1H, 4H, 5H, 1H+4H, 1H+5H, 4H+5H, 1H+4H+5H, and 0-QTL) and one major gene introgression (7H) were selected and are hereinafter referred to as the i-BISON lines. We measured four components of disease resistance on the i-BISON lines: latent period, infection efficiency, lesion size, and pustule density. Our goals were to (i) determine if the disease components are QTL-specific, and (ii) if pyramiding resistance alleles at multiple QTL leads to higher levels of resistance. Our results showed that the greatest differences between the target QTL introgressions and the susceptible controls were for the latter three traits. On average, however, the QTL introgressions also had longer latent periods than the susceptible parent (Baronesse). There were significant differences in the magnitudes of effects of different QTL alleles. The 4H QTL allele had the largest effect, followed by the alleles on 1H and 5H. Pyramiding multiple QTL alleles led to higher levels of resistance in terms of all components of quantitative resistance except latent period.

In a second experiment we used the i-BISON lines to study the effectiveness of QTL effects across growth stages, races, and environments. We measured the response to inoculation with the pathogen, as either infection type or percent disease severity, on the i-BISON lines at the seedling and adult plant stages, in controlled and field testing environments, with varying races of the pathogen, and combinations there of. Our goals were to determine if the magnitudes and ranks of QTL effects are consistent (i) across plant stages, (ii) in response to varying races, and (iii) across controlled and field testing

environments. Through our results we determined that the i-BISON QTL allele introgression effects are consistent across controlled and field testing environments and across the Mexico and Washington locations. Pyramiding multiple resistance alleles in a single genotype is effective at the adult plant stage, yet the data do not conclusively support this benefit at the seedling stage. However, the potential durability of pyramids, as compared to simpler genetic constructs, may justify their construction. Also, the data reveal the necessity of validating the value of resistance alleles as these are introgressed into susceptible backgrounds.

Nine final BISON lines are available for further experiments. Each final line represents resistance alleles at individual QTL and combinations of QTL introgressed into at least an 85% Baronesse background. For the single and double resistance allele introgression BC2S3 seed is available for each of the seven final lines hereinafter referred to as BISON 1H, BISON 4H, BISON 5H, BISON 7H, BISON 1H+4H, BISON 1H+5H, and BISON 4H+5H. BC2S2 seed is available for the BISON 1H+4H+5H line and BC2S1 seed is available for the BISON 0-QTL line. A third experiment will follow the format of the first experiment, but use the final BISON lines. These final lines should ultimately allow for characterization of these genes in terms of their structure and function.