

AN ABSTRACT OF THE THESIS OF

Ariel Julio Castro Tabó for the degree of Doctor of Philosophy in Crop Science
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Patrick M. Hayes

Genetic resistance is the most appropriate way to control diseases in crop plants. Resistance can be described as qualitative (involving a gene-for-gene system) or quantitative (with continuous distribution of disease levels). The relationships between different types of resistance remain obscure, as does the genetics of quantitative traits. The use of quantitative trait locus (QTL) analysis tools for dissecting such traits has raised concerns regarding bias in QTL estimation and lack of QTL validation. We report data for barley (*Hordeum vulgare* L. subsp. *vulgare*) and barley stripe rust (BSR, caused by *Puccinia striiformis* f.sp. *hordei*) that contribute to understand the relationships between different types of disease

resistance, and of the utility of QTL analysis tools for studying and manipulating the genetic determinants of complex traits.

BSR is an important disease of barley. Using QTL mapping and logistic regression we mapped BSR seedling resistance QTL on chromosomes 5(1H) and 6(6H) in the cultivar Shyri that showed complementary gene action. We also mapped one qualitative BSR resistance gene on chromosome 1(7H) in the accession CI10587 (BSTR1). We developed a pyramid of previously mapped BSR resistance QTL alleles on chromosomes 4(4H) (QTL4), 5(1H) (QTL5) and 7(5H) (QTL7), and pyramids of BSTR1 with QTL4 and QTL5 (two populations), and of BSTR1 with QTL5 (one population). These populations provided independent estimates of QTL effects and interactions.

The results validated QTL effect estimates for the three QTL in the first resistance gene pyramid and for QTL4 and QTL5 in the other three pyramids. These studies confirmed the additivity of the QTL effects and the limited importance of QTL x QTL interactions. The original QTL estimates were biased. QTL estimates obtained from the first resistance gene pyramid provided better estimates of QTL effects. These studies also validated the coincidental seedling resistance effects on QTL4 and QTL5 as well as the statistical procedure used for their estimation. The presence of a resistance allele from CI10587 precluded the detection of significant QTL effects, but in the absence of the qualitative resistance gene, they lead to resistance. Our results suggest that QTL analysis can help to understand and

manipulate genes underlying quantitative traits of biological and economical importance.

Stripe Rust Resistance Pyramids in Barley

by

Ariel Julio Castro Tabó

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Dr. Patrick M. Hayes initiated, advised, and supervised all aspects of the project. He substantially contributed in formulating hypotheses, and discussing and revising manuscripts. Dr. Sergio Sandoval-Islas conducted the disease evaluations in Toluca, Mexico. Drs. Xianming Chen, Ronald Line, and Mareike Johnson conducted the seedling testing in controlled conditions. Dr. Hugo Vivar developed the original resistance sources, collaborated in the development of the populations and coordinated the testing in Mexico. Dr. Flavio Capettini also helped to coordinate the testing in Mexico. Dr. Theerayut Toojinda developed the Shyri x Galena map. Dr. Christopher C. Mundt helped in the analysis of epidemiological data and contributed to the preparation of manuscripts. Dr. Steven J. Knapp helped in the statistical analysis and contributed to the preparation of manuscripts. Ing. Agr. Carlos Rossi mapped the resistance gene in the CI10587 x Galena population. Dr. Andris Kleinhofs and Dr. David Kudrna were responsible for the RFLP assays.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
COINCIDENT QTL THAT DETERMINE SEEDLING AND ADULT PLANT RESISTANCE TO STRIPE RUST IN BARLEY	9
ABSTRACT	10
INTRODUCTION	12
MATERIALS AND METHODS	17
RESULTS	21
DISCUSSION	32
REFERENCES	38
MOLECULAR MARKER ASSISTED PYRAMIDING OF QUANTITATIVE TRAIT LOCUS (QTL) ALLELES VALIDATES QTL AND LEADS TO HIGH LEVELS OF PLANT DISEASE RESISTANCE	44
ABSTRACT	45
RESULTS AND DISCUSSION	46
NOTES AND REFERENCES	59
MOLECULAR MARKER ASSISTED (MMAS) PYRAMIDING OF QUANTITATIVE TRAIT LOCUS (QTL) ALLELES DETERMINING RESISTANCE TO BARLEY STRIPE RUST: EFFECTS ON SEEDLING RESISTANCE	63
ABSTRACT	64
INTRODUCTION	66

TABLE OF CONTENTS (CONTINUED)

	Page
MATERIALS AND METHODS.....	70
RESULTS	75
DISCUSSION	83
REFERENCES.....	89
 MAPPING AND PYRAMIDING OF QUALITATIVE AND QUANTITATIVE RESISTANCE TO STRIPE RUST IN BARLEY	 94
ABSTRACT.....	95
INTRODUCTION	97
MATERIALS AND METHODS.....	101
RESULTS	109
DISCUSSION	120
REFERENCES.....	124
CONCLUSIONS.....	129
BIBLIOGRAPHY	132

LIST OF FIGURES

Figure	Page
2.1. Phenotypic distribution of infection type in the Shyri x Galena doubled haploid mapping population when inoculated at the seedling stage with three North American races of <i>Puccinia striiformis</i> f.sp. <i>hordei</i>	22
2.2. MultiQTL LOD scores of BSR seedling reaction to inoculation with three isolates, based on individual and joint datasets, on chromosomes 5(1H) and 6(6H) in the Shyri x Galena population.....	27
2.3. Linkage maps of chromosomes 5(1H) and 6(6H) of the Shyri x Galena population based on Toojinda et al. (2000), showing seedling resistance QTL.....	28
2.4. Least squares means of the probability of occurrence of the disease phenotype in individuals with resistance alleles on QTL5, on QTL6, on both QTL5 and QTL6, and with no resistance alleles for each stripe rust race and for all three races.....	31
3.1. Development of the MAS derived QR allele pyramid population.....	50
3.2. Alignment of the original source populations for the target regions on chromosomes 4H, 1H and 5H showing the markers used for genotyping the QR allele pyramid population.....	54
3.3. Average disease progress curves for doubled haploid lines with different combinations of resistance QTL alleles in two of the six experiments in which this phenotype was measured.....	56
4.1. Phenotypic distribution of infection type in the MAS derived pyramid doubled haploid population when inoculated at the seedling stage with four different isolates of <i>Puccinia striiformis</i> f.sp. <i>hordei</i>	76

LIST OF FIGURES (CONTINUED)

Figure	Page
4.2. Least squares means of the probability of occurrence of the susceptible phenotype in individuals with resistance alleles on QTL4, on QTL5, on both QTL4 and QTL5, and with no resistance alleles for each stripe rust isolate and for all four isolates (left vertical axis).....	82
5.1. Development of the quantitative/qualitative disease resistance populations.	104
5.2. Alignment of the chromosome 1(7H) maps of the CI10587 x Galena, AJ, BU and OP populations	110
5.3. Alignment of the chromosomes 4(4H), 5(1H) and 7(5H) maps of the AJ, BU and OP populations, showing the expected and actual locations of stripe rust resistance QTL	117
5.4. Least squares means of disease severity in DH lines of the OP population classified according to the presence or absence of the resistance alleles at BSTR1, QTL5 and QTL4C QTL regions	118
5.5. Least squares means of disease severity in DH lines of the AJ and BU populations classified according to the presence or absence of the resistance alleles at BSTR1, QTL5 and QTL4B QTL regions.....	119

LIST OF TABLES

Table	Page
2.1. Numbers of resistant and susceptible doubled haploid lines in the Shyri x Galena population when inoculated at the seedling stage with three North American races of <i>Puccinia striiformis</i> f.sp. <i>hordei</i>	23
2.2. Numbers of resistant and susceptible doubled haploid lines in the Shyri x Galena population when inoculated at the seedling stage with three North American races of <i>Puccinia striiformis</i> f.sp. <i>hordei</i> , classified according to reaction to each of the three races.....	24
2.3. SIM and sCIM scores of the significant peaks from the MQTL combined analysis of the three races and LOD scores of the same QTL detected using the multi-trait procedure of MultiQTL	26
2.4. Numbers of resistant (R) and susceptible (S) doubled haploid lines in the Shyri x Galena population, inoculated at the seedling stage with three North American races of <i>Puccinia striiformis</i> f.sp. <i>hordei</i> , classified according to their reaction to each one of the races and the alleles present in the QTL regions on chromosomes 5(1H) and 6(6H).	26
2.5. Likelihood ratios for tests of significance of the QTL main and interactions effects from separate analyses for each stripe rust, and QTL and race main and interactions effects for the combined analysis across stripe rust races (<i>p</i> -values were calculated using asymptotic chi-square distributions).....	30
3.1. Least squares means of disease severity and area under the disease progress curve (AUDPC), and least squares estimates of the infection rate in individuals according to the presence or absence of the resistance alleles on the QTL regions under study on chromosomes 1H, 4H and 5H....	55
3.2. Comparison of the amount of genotypic variance of disease severity (σ_G^2) explained by the QTL effects in the original reports and in the MAS derived QR pyramid population.....	57

LIST OF TABLES (CONTINUED)

Table	Page
4.1. Numbers of resistant and susceptible MAS-derived doubled haploid lines when inoculated at the seedling stage with different isolates of <i>Puccinia striiformis</i> f.sp. <i>hordei</i> , classified according to reaction to each isolate	77
4.2. Numbers of resistant (R) and susceptible (S) MAS-derived doubled haploid lines inoculated at the seedling stage with different isolates of <i>Puccinia striiformis</i> f.sp. <i>hordei</i> , classified according to their reaction to each one of the races and the alleles present in the QTL regions on chromosomes 4(4H) and 5(1H).....	79
4.3. Likelihood ratios for tests of significance of QTL main and interaction effects from separate analyses for each of four stripe rust isolates, and QTL and isolate main and interaction effects for the combined analysis of all isolates.....	80
4.4. Comparison of least squares means for the probability of occurrence of the susceptible phenotype in individuals with different allele configurations at QTL4 and QTL5, in source QTL mapping populations, and in MAS-derived DH lines.	86
5.1. Summary of barley stripe rust severity QTL, corresponding LOD scores and significance level.....	112
5.2. ANOVA table of the candidate gene analysis for stripe rust resistance..	115

STRIPE RUST RESISTANCE PYRAMIDS IN BARLEY

INTRODUCTION

Barley stripe rust (BSR), caused by *Puccinia striiformis* f.sp. *hordei*, is an important disease of barley (*Hordeum vulgare* L. subsp. *vulgare*) in the Pacific Northwest of the United States. A highly virulent race of this pathogen was first observed in the Western Hemisphere in Colombia in 1975. This race was probably introduced from Europe (Dubin and Stubbs, 1985). The disease spread southward, reaching Argentina in 1982, and northward, reaching Mexico in 1987 (Calhoun et al., 1988). In the United States it was first observed in Texas in 1991 (Marshall and Sutton, 1995). By 1995, the disease was reported in every state of the western U.S. Commercial-scale epidemics have occurred annually in California and Oregon since 1995. Originally described as race 24, barley stripe rust in North America is now known to be a very heterogeneous population (Dubin and Stubbs, 1985; Roelfs and Huerta-Espino, 1994; Chen et al., 1995; Marshall and Sutton, 1995). Wheat stripe rust (*Puccinia striiformis* f.sp. *tritici*) is the most important wheat disease in

the Pacific Northwest, but both f.sp. are highly specialized: cross-infection is not of epidemiological importance.

Genetic resistance is the most economical and environmentally appropriate strategy for disease control in plants. It is a major objective in most crop breeding programs and its durability is a key consideration. In order to develop varieties with durable resistance, a comprehensive knowledge of the specifics of the host-pathogen system of interest is required. Disease resistance classification is a controversial subject, with extensive literature on the merits of different types of resistance. Resistance phenotypes are a continuum, ranging from the hypersensitive response to a modest reduction in the rate of epidemic development. Throughout the continuum, there are different combinations of locus number, allele effects, stage of expression, and race specificity (Browning et al., 1977). All these aspects need to be defined on a case-by-case basis before making broad generalizations about a specific source of resistance.

Qualitative resistance refers to a disease response that can be easily described in terms of resistant vs. susceptible. It is frequently described in terms of a gene-for-gene system (Flor, 1946), in which the presence of an avirulence gene in the pathogen is required for the resistance gene in the host to be effective. Quantitative resistance is used to describe a disease response that defies easy rating (i.e. resistant vs. susceptible). This type of resistance can be described in terms of a scale, such as percent severity on a plot basis, and generally allows some symptom development

under intense epidemic conditions. Qualitative resistance is usually measured at the seedling stage while quantitative resistance is measured at adult plant stage.

Vanderplank (1963, 1968, 1978) stated that these classifications represent genes with distinctly different mechanisms and race specificities. Others consider that all resistance genes are similar, but are expressed differently in different combinations and in different genetic backgrounds (Nelson, 1978). There are suggestions that quantitative resistance could follow a gene-for-gene system (Parlevliet and Zadoks, 1977; Parlevliet, 1981) and that failure to fit quantitative resistance to such models is due to inadequate experimental technique (Ellingboe, 1975).

The characterization of plant resistance at the molecular level has provided information indicating that gene-for-gene relationships are recognition processes that turn on multiple genes in a resistance pathway (Bourett and Howard, 1990; Choi and Dean, 1997; Hamer et al., 1998). At the same time quantitative trait loci (QTL) analysis procedures have facilitated the dissection of quantitative disease resistance, locating relatively few determinants of complex resistance phenotypes (Michelmore, 1995; Young, 1996; Kover and Caicedo, 2001). The relationships between the two types of resistance, and the relationships of disease resistance with plant morphological and phenological traits, are areas requiring additional study.

One of the theoretical advantages of quantitative disease resistance is its potentially higher probability of durability (Parlevliet, 1989). A gene-for-gene qualitative resistance system will not remain effective if the pathogen acquires the

corresponding virulence (Johnson, 1992). The risks involved in using gene-for-gene resistance are well known (Johnson, 1981; Parlevliet, 1983; Vanderplank, 1963, 1968) and there is evidence that pathogen virulence can evolve more quickly than plant breeders can incorporate single resistance genes into new varieties (Parlevliet, 1977). The problems arising from the use of race-specific resistance genes have led to the development of novel genetic strategies for disease control, including mixtures (Wolfe, 1985), multilines (Allan et al. 1993; Browning and Frey, 1969), resistance gene pyramids (Schafer and Roelfs, 1985; Mundt, 1990) and partial resistance (Parlevliet, 1975).

The first studies on genetic resistance to BSR were conducted in India in the 1960's (Chen and Line, 1999). Later, Lehman et al. (1975) described four qualitative resistance genes, *Yr1* – *Yr4*. Of these genes, only *Yr4* has been mapped, and it is on the short arm of chromosome 5(1H) (von Wettstein-Knowles, 1992).

Quantitative resistance should be more effective and durable against barley stripe rust than qualitative resistance. Adult plant resistance to wheat stripe rust is non race-specific, and sensitive to high temperature (Qayoum and Line, 1985), while seedling resistance is race-specific and expressed in all stages of plant growth (Chen and Line, 1995). In barley, Sandoval-Islas et al. (1998) reported very low correlation between these two types of resistance.

The ICARDA/CIMMYT program in Mexico has developed barley germplasm that allows limited symptom development when exposed to the spectrum of virulence

encountered in field tests in South America, Mexico, and the U.S. According to Sandoval-Islas et al. (1998), this resistance can be considered quantitative and durable. With the objective of breeding for durable resistance to stripe rust in barley, Oregon State University (OSU) initiated a collaborative effort (reviewed by Hayes et al, 2001) to map and introgress those resistance sources into germplasm adapted to the Pacific Northwest. The focus of the collaborative work has been on adult plant field resistance, based on the experience and success of the ICARDA/CIMMYT barley program and perspectives on durable resistance obtained in the Pacific Northwest with the wheat/wheat stripe rust model (Milus and Line, 1986a, 1986b). However, it is also of interest and importance to determine the genetics of seedling resistance.

The use of molecular markers and quantitative trait locus (QTL) analysis procedures has provided tools for relating complex traits, such as quantitative resistance, to specific regions of the genome (Doerge, 2002). These developments led to tremendous enthusiasm in the plant breeding community, where these tools promised to increase the efficiency and rate of selection response. However, the initial enthusiasm for QTL analysis and the potential gains to be achieved by marker-assisted selection (MAS) has been tempered by concerns regarding bias in QTL estimation (Beavis, 1998), lack of studies validating QTL alleles in different genetic backgrounds and few examples of successful MAS (Dekkers and Hospital, 2002). The small population sizes typically used for QTL detection lead to

overestimation of QTL effects and underestimation of QTL number and interaction (Melchinger et al., 1998).

In order to correctly assess the potential of QTL analysis there are several issues that need to be addressed. One of those issues is the number of genes that are necessary to recover the target phenotype. Bernardo (2001), using simulation studies, suggested that if the number of genes is high, simultaneous selection for all the genes affecting the trait could be detrimental in terms of selection response. However if the number of genes is small, and the heritability of the trait low, then the incorporation of genotypic information could increase the selection efficiency. Other issues that need to be addressed are the effect that a change in the genetic background can have on QTL allele expression, the relative importance of QTL additive main effects compared with QTL x QTL interaction, and the importance of QTL x environment interaction.

QTL mapping tools have been applied by the OSU barley project to map and introgress the BSR resistance in germplasm selected by ICARDA/CIMMYT. BSR resistance QTL have been mapped in multiple populations (Chen et al., 1994; Toojinda et al., 2000), and introgressed in different genetic backgrounds using MAS (Toojinda et al., 1998). Hayes et al. (1996) mapped seedling resistance QTL alleles in Calicuchima-sib to chromosomes 4(4H) and 6(6H). According to Castro et al. (2002b) both QTL mapped in the same regions as the adult plant resistance QTL, and both had complementary gene effects, meaning that resistance alleles at

two QTL are necessary in order to achieve resistance at the seedling stage. The growth stage and race specificities of other QTL mapped in this project were not known.

The different locations of the detected BSR resistance QTL opened another possibility: they could be combined in the same genetic background. Since pyramiding resistance genes with different race-specificities is a way of increasing the likelihood of resistance durability, pyramiding QTL alleles should also accomplish the same objective. That reasoning can also be extended to the combination of quantitative and qualitative resistance in the same genetic background. Furthermore, the pyramids of QTL alleles provide an independent validation of QTL effects detected in mapping populations and allow for estimation of QTL interactions. With this information at hand, it should be possible to assess the validity of the use of QTL analysis tools for the study and manipulation of the genes determining quantitative and qualitative resistance to BSR.

Using combinations of BSR resistance QTL alleles pyramided in the same genetic background, and a series of combinations of BSR resistance QTL alleles and a qualitative BSR resistance gene, and generating phenotypic data covering different growth stages and pathogen isolates, the objectives of this work were, accordingly, to: (i) complete the information regarding stage specificity of previously mapped adult plant disease resistance QTL alleles; (ii) validate QTL allele effect estimation in a different genetic background; (iii) study QTL x QTL and QTL x environment

interactions; and (iv) study the relationships between quantitative and qualitative resistance genes.

**COINCIDENT QTL THAT DETERMINE SEEDLING AND ADULT PLANT
RESISTANCE TO STRIPE RUST IN BARLEY**

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ABSTRACT

Barley stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) is an important disease of barley (*Hordeum vulgare* L. subsp. *vulgare*). This disease reached the Americas in 1975. It is now endemic from the Andean region of South America to the Western North America. We are systematically mapping quantitative resistance genes present in ICARDA/CIMMYT germplasm and introgressing these genes into barley germplasm adapted to Western North America. Resistance to stripe rust in the Triticeae can be race- and growth stage-specific. In this study, we mapped genes conferring resistance at the seedling stage, after inoculation with defined isolates (PSH-1, PSH-13, PSH-14), in a doubled haploid population in which adult plant resistance genes had previously been mapped. The disease reaction data for each of three isolates fit a 3:1 (susceptible: resistant) ratio, indicating that two genes are required for resistance. QTL effects and significance were estimated using QTL mapping procedures and logistic regression analysis, taking into account the binomial distribution of the trait. Two resistance QTL – one on chromosome 5 (5H) and one on chromosome 6 (6H) – were detected and in all cases Shyri contributed the resistance alleles. No QTL x race interaction was detected. The two seedling resistance QTL map to the same regions of the genome as two of the four adult plant resistance QTL. These data lay the foundation for more detailed analyses

directed at unraveling the genetics of qualitative and quantitative disease resistance mechanisms.

INTRODUCTION

Barley stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) is an important disease of barley (*Hordeum vulgare* L. subsp. *vulgare*). This disease has caused serious yield losses throughout the world (Dubin and Stubbs, 1985). In the Americas, barley stripe rust (BSR) was first observed in Colombia in 1975 and Dubin and Stubbs (1985) postulated that the disease was introduced from Europe. The disease spread southward, reaching Argentina in 1982, and northward, reaching Mexico in 1987 (Calhoun et al., 1988). In the United States, BSR was first observed in Texas in 1991 (Marshall and Sutton, 1995). By 1995, the disease was reported throughout the western U.S. Commercial-scale epidemics have occurred annually in California and Oregon since 1995. At least one million of the approximately 3.2 million acres of barley in the western U.S. could be considered at risk to BSR.

The population of BSR in the Americas was first described as race 24 (Dubin and Stubbs, 1985), which was first reported in Europe in the early 1960's. Considerable variation has since been reported in pathogen isolates collected in the U.S. (Chen et al., 1995; Marshall and Sutton, 1995; Roelfs and Huerta-Espino, 1994). Chen et al. (1995), based on seedling resistance of barley genotypes to races of *P. striiformis* f.sp. *hordei*, selected a series of differentials. Genotypes were identified which

were resistant to all North American isolates. Chen et al. (1995, 1998), and Chen and Line (1999) reported that most of the seedling resistance genes were recessive.

Genetic resistance is the most successful, efficient and economical means to control rusts in cereals and it should be used for the control of BSR. Since most of the cultivars currently grown in the United States are susceptible to BSR, development of resistant cultivars is a priority in the Pacific Northwest, where the disease can be a principal production constraint. Breeders face the question: "what type of resistance to use: quantitative or qualitative?".

Qualitative resistance can be described in terms of resistant vs. susceptible reaction and it is frequently interpreted in terms of a gene-for-gene system (Flor, 1946). Its use in breeding resistant varieties can be straightforward. Quantitative resistance is a disease response that defies easy rating (i.e. resistant vs. susceptible). This type of resistance can be described in terms of a scale, such as percent severity on a plot basis. A quantitatively resistant genotype allows some symptom development under intense epidemic conditions. In the case of stripe rust, the wheat/wheat stripe rust model has been much more extensively studied than the barley/barley stripe rust model, and in the former, quantitative resistance is defined as resistance which is non race-specific and expressed only at the adult plant stage (Milus and Line, 1986a, 1986b). The use of quantitative resistance in a breeding program requires extensive field-testing and this type of resistance is generally more difficult to

breed for than qualitative resistance. The interest in quantitative resistance is due to its probable durability.

In the case of BSR, the germplasm developed by the ICARDA/CIMMYT program in Mexico allows limited symptom development when exposed to the spectrum of virulence encountered in field tests in South America, Mexico, and the U.S. Sandoval-Islas et al (1998) determined the resistance of 500 accessions from this breeding program at the seedling and adult plant stages. Eight-six percent of the accessions showed a susceptible reaction when inoculated at the seedling stage with a Mexican isolate of *P. striiformis* f. sp. *hordei* corresponding to race 24. Seventy six percent of the lines had low disease severities (10% or less) at the adult plant stage. The fact that ICARDA/CIMMYT germplasm has remained resistant to BSR over a 15-year period may be grounds for describing it as having durable resistance. Accordingly, one approach to develop resistant varieties for the Pacific Northwest of the U.S. would be to introgress quantitative resistance genes from the unadapted ICARDA/CIMMYT germplasm.

In order to accomplish this resistance gene identification and introgression as quickly and efficiently as possible, we initiated a collaborative effort to use molecular markers for resistance QTL mapping and marker-assisted selection (reviewed by Hayes et al., 2001). We mapped QTL for BSR resistance to barley chromosomes 4(4H) and 7(5H) in one accession (Chen et al., 1994) and chromosomes 2(2H), 3(3H), 5(1H) and 6(6H) in another (Toojinda et al. (2000).

We hypothesized that these accessions have different BSR resistance QTL alleles and proceeded to develop a complex population pyramiding the resistance QTL alleles on chromosome 4(4H) and 7(5H) -sib with the resistance QTL alleles on chromosome 5(1H) (Castro et al., 2000). Experimental results have confirmed the QTL effects in the new genetic background (Castro et al., 2002b). Thomas et al. (1995) reported BSR resistance QTL alleles in the variety “Blenheim” in the same region as reported by Toojinda et al. (2000), and Chen et al. (1994). The chromosome 5(1H) QTL maps to the same region as *Rps4* (previously called *Yr4*), a resistance gene present in several European barley cultivars (Von Wettstein-Knowles, 1992).

The focus of the OSU/ICARDA/CIMMYT mapping and introgression experiments has been on adult plant field resistance, based on the experience and success of the ICARDA/CIMMYT barley program and perspectives on durable resistance obtained in the Pacific Northwest with the wheat/wheat stripe rust model (Milus and Line, 1986a, 1986b). It is also of interest to determine the race specificity and growth stage specificity of resistance, although these specificities are often confounded, in the sense that race specificity is typically defined based on the reaction of seedlings to inoculation with defined isolates under controlled environment conditions.

The availability of “immortal” doubled haploid mapping populations allows for mapping determinants of multiple phenotypes, including resistance at different

growth stages and the reaction to different isolates. Hayes et al. (1996) mapped seedling and adult plant resistance QTL in the same ICARDA/CIMMYT accession and reported that seedling and adult plant resistance QTL coincided on chromosome 4(4H). Shyri, the variety studied by Toojinda et al. (2000) showed a highly resistant reaction to three isolates, when inoculated at the seedling stage. These three isolates – PSH-1, PSH-13, and PSH-14 – represent races showing a range of virulence (Chen et al., 1995). Accordingly, the objectives of this study were to determine (i) the number and location of BSR seedling resistance genes in Shyri and (ii) the linkage relationships of these seedling resistance genes with the adult plant resistance QTL reported by Toojinda et al. (2000).

MATERIALS AND METHODS

PLANT MATERIALS AND EVALUATIONS OF DISEASE RESISTANCE

Ninety-four F₁-derived doubled haploid (DH) lines were produced from the cross of Shyri x Galena as described by Toojinda et al. (2000), using the *Hordeum bulbosum* technique (Chen and Hayes, 1989). Shyri is a two-rowed feed barley developed by ICARDA/CIMMYT (Mexico) and released by INIAP (Ecuador). Galena is a proprietary two-rowed malting barley belonging to the Coors Brewing Company, Inc.

Adult-plant disease severity assessments, linkage mapping, and QTL analysis procedures for adult plant resistance were described by Toojinda et al. (2000). For the current study, the parents and the doubled haploid population were assayed for resistance to BSR, at the seedling stage, following the procedures described by Chen and Line (1992). Isolates corresponding to races PSH-1, PSH-13 and PSH-14 of *P.striiformis* f. sp. *hordei* (Chen et al., 1995) were used to inoculate the seedlings. Infection types were recorded 20 days after inoculation using a 0-9 scale (0: complete resistance; 9: complete susceptibility) as described by Line et al. (1974).

DATA ANALYSES

Chi-square tests were used to determine the goodness of fit of the phenotypic segregation ratios. For the chi-square tests, DH lines with 0 to 5 scores were considered as resistant and DH lines with of 6 to 9 scores were considered as susceptible (Line et al., 1974; Line and Qayoum, 1991; Chen and Line, 1999).

For mapping the genes conferring resistance at the seedling stage, we used the linkage map and data reported by Toojinda et al. (2000). QTL were mapped using the interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures of MQTL (Tinker and Mather, 1995), regression procedures, and the single and multi-trait options implemented in MultiQTL 1.55 (<http://esti.haifa.ac.il/~popthor/MultiQtl/MultiQtl.htm>).

Intermediate values may represent misclassifications of disease reaction; accordingly, we repeated our analyses treating these intermediate values as missing observations. Because the results were the same as those obtained with the full dataset, we report results only from the latter. For the MQTL analysis each dataset was analyzed with 1000 permutations, a 5 cM walk speed, and a Type I error rate of 5%. For sCIM, 18 background markers with approximately even spacing were specified, with a maximum of three background markers per linkage group. For the MultiQTL analysis each dataset was analyzed with 1000 permutations in order to establish the significance of the QTL and a bootstrap simulation (with 1000 samples) was used for the assignment of each significant QTL to a defined marker

interval. Datasets corresponding to each race were analyzed individually, and then jointly in order to test for QTL x race interaction.

Genome regions affecting resistance to stripe rust revealed by the QTL scans were used in performing a QTL analysis analogous to candidate gene analysis, where the genotypes of the flanking markers are used as independent variables. Recombinant genotypes were not included in the analysis. Therefore, the independent variables had two levels each, with each level corresponding to a parental genotype. The treatment design was a 2 x n factorial, where n is the number of genome regions detected. The difference between parental marker class means estimates the additive effect of the QTL flanked by the markers. Double crossovers between the QTL and marker loci downwardly bias estimates of the effects. Thus, differences between parental marker genotype means are conservative estimates of the effects of QTL residing in the n chromosomal regions.

Because the response (dependent) variable was binomial (1 = resistant and 0 = susceptible) and the response probability distribution was binomial, the analysis was performed using a generalized linear model (Nelder and Wedderburn, 1972; McCullagh and Nelder, 1989) with a logit link function, $g(\mu) = \log[\mu/(1 - \mu)]$, and binomial errors, where μ is the expected value of $y = r/n$ (the probability of resistance to stripe rust), r is the number of resistant lines, n is the total number of lines, and $r = 1, 2, \dots, n..$ The probability distribution and variance of y are

$$f(y) = \binom{n}{r} \mu^r (1 - \mu)^{n-r}$$

and $[\mu(1 - \mu)]/n$, respectively. Statistical analyses were performed using the SAS (2001) GENMOD procedure. Parameters and test statistics were estimated using a Type III analysis (analogous to partial sums of squares analyses of general linear models). We performed separate analyses of the effects for each stripe rust race and a combined analysis across stripe rust races. The former analyses entailed estimating the least square means for each QTL and their interactions, the additive effects, the additive by additive interaction effects, and likelihood ratio statistics for tests of significance of the effects (p -values were calculated using asymptotic chi-square distributions). The latter analysis entailed estimating the least square means and test statistics for the effect of stripe rust race (R) and interaction effects between R and QTL, in addition to the main and interaction effects across races. The probability of resistance to stripe rust was estimated for genotypes by $e^p/(1 + e^p)$, where p is the least square mean for the individual QTL and QTL x QTL interaction.

RESULTS

SEEDLING RESISTANCE/SUSCEPTIBILITY PHENOTYPE DATA

When the Shyri x Galena DH population was inoculated, under greenhouse conditions, with races PSH-1, PSH-13, and PSH-14 of *P. striiformis* f. sp. *hordei*, the reactions to each isolate (Figure 2.1) fit a 3:1 (susceptible: resistant) ratio (Table 2.1). In a doubled haploid population, segregation of alleles at a single locus gives a 1:1 phenotypic ratio and dihybrid segregation with independent assortment gives a 1:1:1:1 ratio. Therefore, a 3:1 ratio can be interpreted as evidence that the resistant phenotype is conferred only when the two resistance genes are present. Examples of this type of digenic resistance have been described for stem and leaf rust of wheat (Knott and Anderson, 1956; Singh and McIntosh, 1984).

Although the number of DH lines in each of the phenotypic classes is almost the same for each of the three races (Table 2.1), the DH lines in each group were not always the same (Table 2.2). This could be due to misclassification of the resistance phenotype, or, as elaborated upon in the *Discussion*, to linked, race-specific resistance genes.

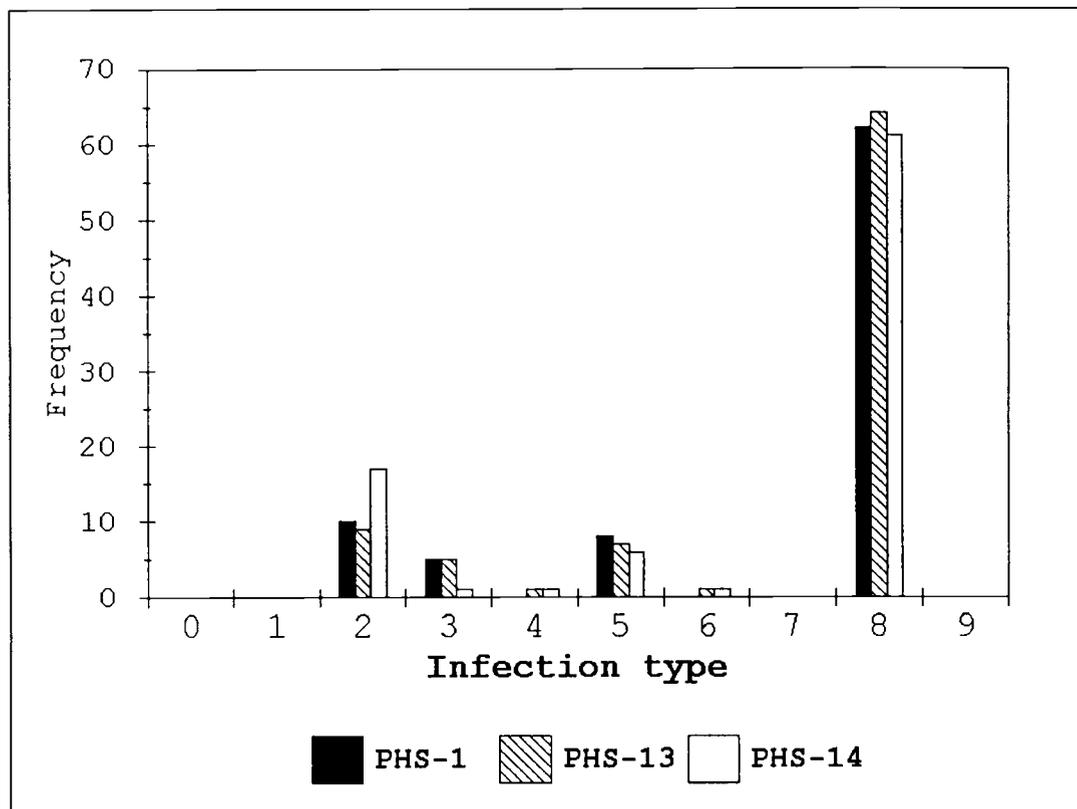


Figure 2.1. Phenotypic distribution of infection type in the Shyri x Galena doubled haploid mapping population when inoculated at the seedling stage with three North American races of *Puccinia striiformis* f.sp. *hordei*.

Table 2.1. Numbers of resistant and susceptible doubled haploid lines in the Shyri x Galena population when inoculated at the seedling stage with three North American races of *Puccinia striiformis* f.sp. *hordei*. The hypothesized ratios and corresponding Chi-square test are shown.

Race	No. of doubled haploid lines		Hypothesized phenotypic ratio	P
	Susceptible	Resistant	S:R	
PSH-1	62	23	3:1	0.661
PSH-13	65	22	3:1	0.951
PSH-14	62	25	3:1	0.421

Table 2.2. Numbers of resistant and susceptible doubled haploid lines in the Shyri x Galena population when inoculated at the seedling stage with three North American races of *Puccinia striiformis* f.sp. *hordei*, classified according to reaction to each of the three races (resistant: score ≤ 5 ; susceptible score ≥ 6).

Race			
PHS-1	PHS-13	PHS-14	No. of DH lines
R	R	R	13
R	R	S	1
R	S	R	2
R	S	S	6
S	R	R	4
S	R	S	2
S	S	R	4
S	S	S	52

MAPPING RESISTANCE GENES

In order to locate the genes responsible for the seedling resistance we used QTL analysis tools, recognizing that the phenotypic values were not normally distributed. Our expectation was that the QTL analysis tools would provide us with estimates of the numbers of genes determining resistance and approximate locations of these genes.

For each one of the three races, two large-effect QTL were detected. These QTL were detected on chromosomes 5(1H) and 6(6H) with all QTL analysis procedures. These QTL will be referred to as QTL5 and QTL6 in the remainder of this manuscript (Table 2.3). No significant QTL x race interaction was detected.

Using MultiQTL, two significant QTL were detected at the previously mentioned positions on chromosomes 5(1H) and 6(6H). LOD values were lower for QTL6. The multitrait analysis performed with MultiQTL also detected the same two QTL determining resistance to all three races (Table 2.3). In both cases the alleles associated with the higher level of resistance came from Shyri (Table 2.4).

The locations of the seedling resistance QTL on the Shyri x Galena map are presented in Figure 2.2, based on the QTL peaks detected with both analysis procedures. The confidence intervals are based on the bootstrap simulations from MultiQTL. The QTL on chromosome 5(1H) (QTL5) is located in a confidence interval spanning 23.3 cM (*LM637/8-3* to *Bmac213*), and the QTL on chromosome 6(6H) (QTL6) is located in a confidence interval spanning 37.0 cM (*MWG652A* to *Linka*). Both QTL are coincident in their location with the two most important adult plant resistance QTL reported by Toojinda et al. (2000) (Figure 2.3). Because of the presence of crossovers in the QTL5 and QTL6 regions nine DH lines (seven for QTL5 and two for QTL6) were not included in the subsequent analyses.

Table 2.3. SIM and sCIM scores of the significant peaks from the MQTL combined analysis of the three races and LOD scores of the same QTL detected using the multi-trait procedure of MultiQTL.

	MQTL scores			MultiQTL	
	SIM	5% threshold.	sCIM	LOD score	p.value
QTL5	89.1	29.2	91.2	11.68	<0.001
QTL6	23.9	29.2	23.7	3.19	0.030

Table 2.4. Numbers of resistant (R) and susceptible (S) doubled haploid lines in the Shyri x Galena population, inoculated at the seedling stage with three North American races of *Puccinia striiformis* f.sp. *hordei*, classified according to their reaction to each one of the races and the alleles present in the QTL regions on chromosomes 5(1H) and 6(6H).

Alleles in		PSH-1		PSH-13		PSH-14	
QTL5	QTL6	R.	S	R	S	R	S
Shyri	Shyri	11	6	11	3	11	3
Shyri	Galena	4	8	4	8	4	8
Galena	Shyri	2	14	2	14	4	12
Galena	Galena	2	20	0	22	0	22

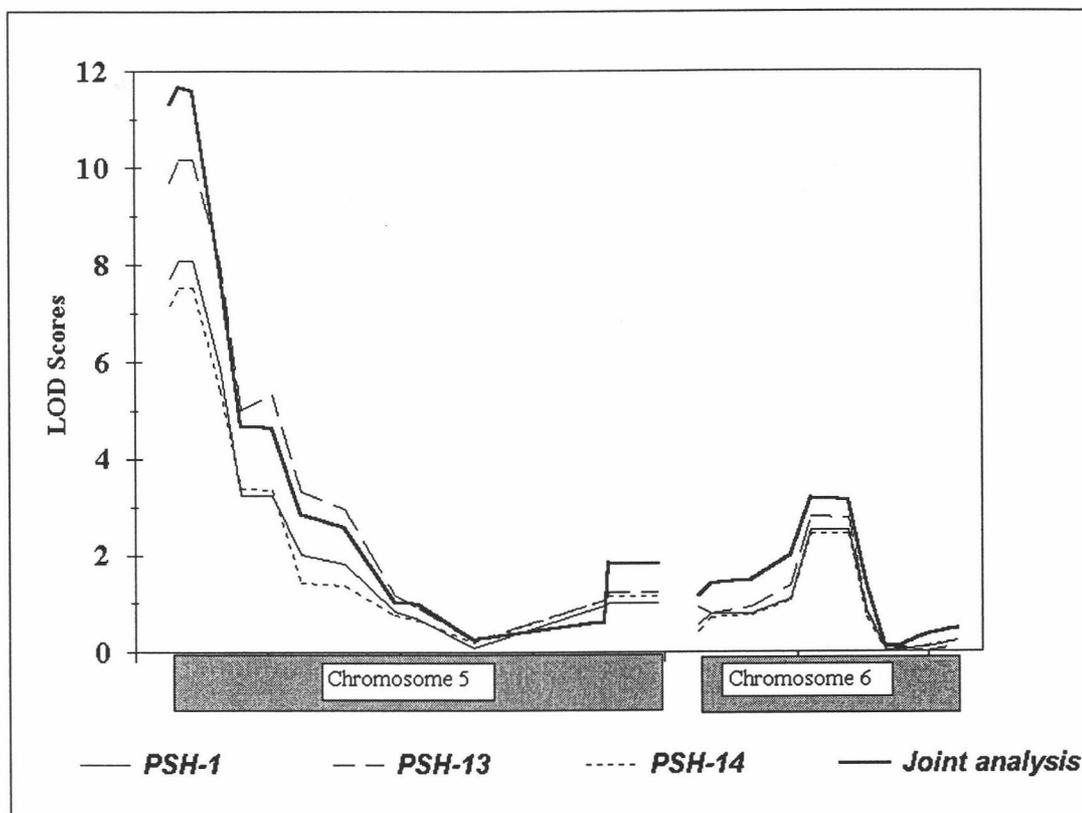


Figure 2.2. MultiQTL LOD scores of BSR seedling reaction to inoculation with three isolates, based on individual and joint datasets, on chromosomes 5(1H) and 6(6H) in the Shyri x Galena population.

Chromosome 5(1H)

Chromosome 6(6H)

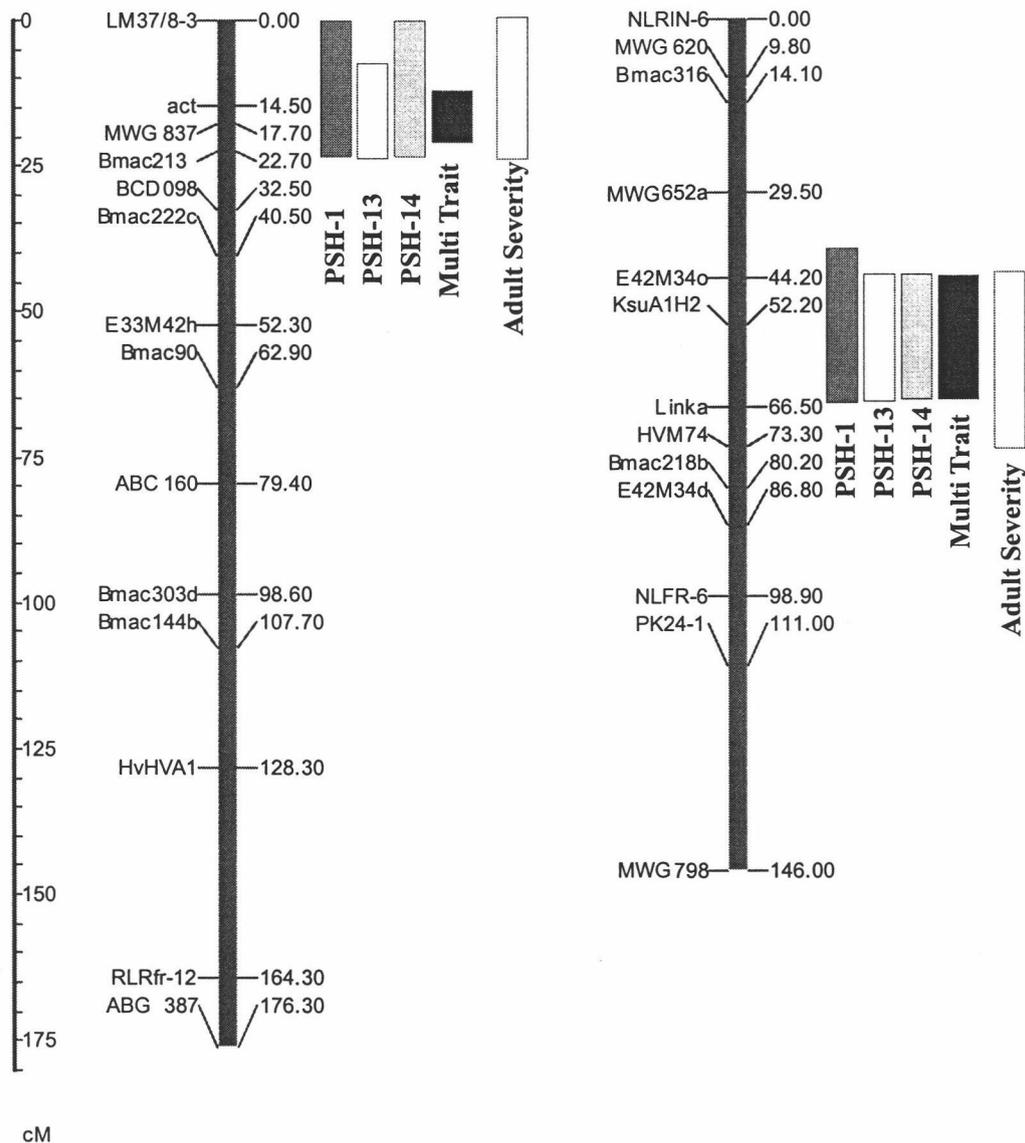


Figure 2.3. Linkage maps of chromosomes 5(1H) and 6(6H) of the Shyri x Galena population based on Toojinda et al.(2000), showing seedling resistance QTL to each of the three isolates (PHS-1, PHS-13, PHS-14), the combined seedling reaction data (multi trait) and the adult-plant resistance QTL reported by Toojinda et al. (2000). Marker locus names are on the left side of each linkage group and distances (Kosambi cM) are shown for each marker interval.

The likelihood ratio statistics were significant for QTL5 and QTL6 (except for QTL6, race PHS-1) when races were analyzed individually, and in the joint analysis of all three races (Table 2.5). No significant QTL by race interaction was detected in the joint analysis. No QTL5 x QTL6 interaction was detected in any of the analyses. As shown in Figure 2.4, the probability of resistance was between 70 and 80% when both resistance genes are present. The probability of resistance was lower than 40% for all other combinations of alleles at the two QTL regions. This distribution of allele values may account for the observed 3:1 phenotypic ratio.

Table 2.5. Likelihood ratios for tests of significance of the QTL main and interactions effects from separate analyses for each stripe rust, and QTL and race main and interactions effects for the combined analysis across stripe rust races (p -values were calculated using asymptotic chi-square distributions).

	Effect	χ^2 statistic	$P < \chi^2$
PHS-1	QTL5	11.66	0.0006
	QTL6	1.85	0.1741
	QTL5*QTL6	0.71	0.3997
PHS-13	QTL5	21.65	0.0001
	QTL6	7.66	0.0057
	QTL5*QTL6	0.68	0.4081
PHS-14	QTL5	17.64	0.0001
	QTL6	13.03	0.0003
	QTL5*QTL6	1.39	0.2383
Joint analysis	QTL5	50.67	0.0001
	QTL6	20.05	0.0001
	QTL5*QTL6	1.67	0.4334
	Race	1.12	0.2906
	Race*QTL5	2.84	0.2413
	Race*QTL6	4.35	0.1136
	Race*QTL5*QTL6	2.75	0.2530

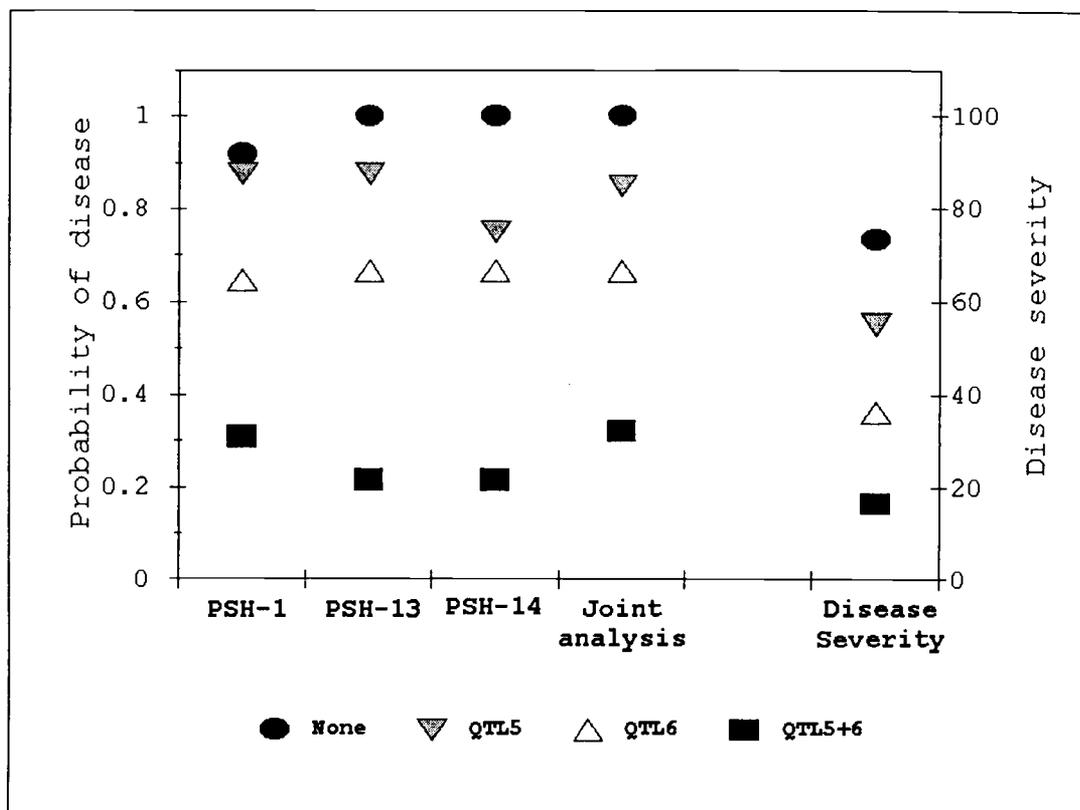


Figure 2.4. Least squares means of the probability of occurrence of the disease phenotype in individuals with resistance alleles on QTL5, on QTL6, on both QTL5 and QTL6, and with no resistance alleles for each stripe rust race and for all three races (left vertical axis). Least squares means of adult plant disease severity for the same individuals in four environments (right vertical axis) (Marquez-Cedillo et al., 2001).

DISCUSSION

At the level of resolution afforded by QTL analysis, we can conclude that determinants of resistance to the three isolates at the seedling stage, and determinants of adult plant resistance, map to the same regions of the genome. QTL coincidence can be due to linkage or pleiotropy, and QTL confidence intervals span large physical distances, according to the physical map of Künzel et al. (2000). Accordingly, we do not know if the same genes, or if linked genes, are involved in seedling and adult plant resistance. The QTL5 is located in a region of intermediate recombination frequency, while QTL6 is located in the border between high and low recombination frequency zones (Künzel et al., 2000; Hayes et al., 2000). The QTL5 region comprises a relatively small physical part of the chromosome. The QTL6 region however, with the present level of resolution, covers approximately half of the corresponding chromosome.

Resistance genes determining responses to the same and/or different pathogens are known to cluster in plants (Michelmore, 1995; Kanazin et al., 1996; Ellis et al., 1998). Multiple quantitative and qualitative resistance genes conferring resistance to different pathogens, and different specificities of the same pathogen, have been mapped to the QTL5 and QTL6 regions (von Wettstein-Knowles, 1992; Thomas et al., 1995; (Steffenson et al., 1996; Hayes et al., 2000; Backes et al., 1995; Spaner et al., 1998; Qi et al., 1998).

In the case of the Shyri x Galena population, the stripe rust resistance phenotype at the adult plant stage, under field conditions, is quantitative while the resistant phenotype at the seedling stage and under controlled environment conditions, is qualitative. Because Shyri has remained resistant to the spectrum of virulence encountered in North and South America over a 15-year period and because this variety allows some symptom development at the adult plant stage when exposed to field inoculum, the variety is considered by the ICARDA/CIMMYT program to have quantitative resistance. Quantitative resistance at the adult plant stage (*sensu* Chen and Line 1995) is considered non race-specific. Seedling resistance is generally thought to reflect gene-for-gene relationships (McIntosh and Wellings, 1986). The three races used in this experiment have shown important differences in their virulence when tested with differential cultivars (Chen et al., 1995). That the same QTL were detected for all three races at the seedling stage and that there was no evidence for QTL x race interaction could be interpreted as evidence for non-race specificity of the seedling resistance QTL. However, this interpretation cannot account for the doubled haploid lines that were resistant to only one or two of the three isolates (Table 2) or the lack of significance of the QTL6 effect for PSH-1, and it does not support the definition of adult plant quantitative resistance, *sensu* Chen and Line (1995) and Sandoval-Islas et al. (1998). Shyri was not among the ICARDA/CIMMYT accessions studied by the latter authors.

An alternative hypothesis, assuming that the coincident QTL for adult and seedling resistance represent effects of the same genes, is that the QTL are race-specific but that the three races, which were used for the seedling tests, did not represent a sufficiently large sample of the pathogen population. This would imply that what the QTL considered to be the determinants of adult plant resistance QTL (Toojinda et al., 2000) may be the effects of race-specific genes. In this scenario, the spectrum of races encountered in the field tests was not sufficiently diverse to reveal race specificity, although the variable magnitude of resistance QTL across environments (Toojinda et al., 2000) could reflect the frequencies of different races in the pathogen population. A third possibility is that each of the QTL regions represents the effects of multiple, linked genes, each of which conditions different race and/or growth stage specificities.

Additional experiments will be necessary to determine the causes of the differences in estimates of the additive effects of QTL5 and QTL 6 at the seedling stage and adult plant stages (Figure 4). The presence of resistance alleles at both QTL increases, more than proportionally, the chances of recovering the resistant phenotype. Even though there is no statistical evidence for QTL x QTL interaction, from a disease management point of view, having resistances alleles at two QTL loci is 30% more likely to give a disease resistance phenotype than having resistance alleles at only one of the two loci. Resistance alleles at the two QTL are necessary but not sufficient, for the resistance phenotype. These seedling resistance

genes may show incomplete penetrance. Similar results were reported (in this case including significant epistasis) for seedling resistance QTL on chromosomes 4(4H) and 6(6H) in the Calicuchima/Bowman population (Castro et al., 2002a).

Two possible explanations are (i) the two QTL control different components of the resistance pathway and (ii) the expression is a function of the inoculum load. The degree of disease resistance is determined by various epidemiological components such as number of infections, rate of lesion expansion, pathogen fructification, length of latent or incubation period, spore deposition, and infectious period, and number of propagules necessary to establish infection (Berger, 1977).

If the seedling and adult plant stripe rust resistance QTL in Shyri represent the effects of the same genes, and each of these genes determines a different component of resistance, it is possible that a single component could slow the rate of epidemic progression under field conditions but not under greenhouse inoculation. There is some evidence for the “cascade” effect of multiple resistance genes in the seedling resistance data in that QTL5 is necessary, but not sufficient for resistance: considering only the lines which have the Shyri allele at QTL5, the ratio of susceptible: resistant lines is 1:1, and the probability of resistance in the joint analysis was 33%. In contrast, considering only the lines that have the Shyri allele at QTL 6, the ratio of susceptible: resistant lines is 3:1 and the probability of resistance in the joint analysis was 17% (Figure 4).

Regarding inoculum load, Luke et al. (1972) reported "threshold-related" late resistance to crown rust in oats, which was inoculum load dependent. In our data the differential response observed between greenhouse and field evaluation could be related to differences in inoculum load. Under field conditions, the expectation is that inoculum load was typical for an environment favorable for disease development. Under controlled environment conditions, the inoculum load per plant may be higher. Under such conditions of high inoculum load, incomplete resistance mechanisms, which are effective under field conditions, may be overwhelmed. Van Silfhout (1993) suggests that a different classification of seedling reaction must be used, one that considers "intermediate" types (infection scores of 4-6), in order to evaluate these kinds of gene effects. In our case, intermediate infection types were observed on six DH lines when the population was inoculated with races PSH-1, PSH-13, and PSH-14. The lines with intermediate infection types were not the same in each of the three groups.

Finally, our data have implications for BSR resistance breeding. The regions of the genome where adult plant resistance QTL alleles in Shyri were identified could be selected for, phenotypically, at the seedling stage under controlled environment conditions. This could reduce the time required to develop resistant varieties because multiple generations could be advanced under controlled environment conditions in the same time a single generation is evaluated under field conditions. However, under such conditions, only genotypes with Shyri alleles at resistance

QTL5 and QTL6 would be selected. Under field conditions, the presence of the resistance allele on QTL5 has higher probability of conferring the resistance phenotype at the adult plant stage than at the seedling stage. For example, we have introgressed the chromosome 5(1H) QTL allele from Shyri into a new genetic background and the selection "BCD12" has a level of adult plant resistance comparable to Shyri (Castro et al., 2002). At the seedling stage, however, Shyri is highly resistant while BCD12 shows an intermediate reaction (infection type 5) (Chen, unpublished data). Accordingly, germplasm, such as that described by Sandoval-Islas et al (1998) which is susceptible at the seedling stage and resistant at the adult stage may have fewer resistance alleles than germplasm such as Shyri, which may have accumulated multiple resistance alleles through the recurrent selection process. In this regard, molecular marker information could help in determining the number of resistance genes and in constructing multiple resistance genes pyramids in single genotypes.

Experiments are underway to determine the effects, interactions, mechanisms, and specificities of each of all mapped barley stripe rust resistance genes, both QTL and Mendelian, in a common genetic background. These experiments should prove useful in unraveling the complexities of stripe rust resistance in barley and they will hopefully prove useful as a model for developing varieties with durable resistance and for integrating molecular and epidemiological approaches to understanding plant disease resistance.

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**MOLECULAR MARKER ASSISTED PYRAMIDING OF QUANTITATIVE
TRAIT LOCUS (QTL) ALLELES VALIDATES QTL AND LEADS TO
HIGH LEVELS OF PLANT DISEASE RESISTANCE.**

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ABSTRACT

Understanding the genetics of quantitative traits has been a challenge. The use of molecular and quantitative trait loci (QTL) analysis tools initially lent support to the idea that relatively few genetic factors were the principal determinants of complex traits. However, QTL analyses have been questioned by concerns regarding bias in QTL estimation and reproducibility of QTL effects in different genetic backgrounds. Using pyramids of quantitative disease resistance QTL developed in barley, we provide independent estimates of: QTL effects, influence of genetic background on QTL effects, QTL x QTL interaction, and QTL x environment interaction. Our results validate QTL effect estimates, showing that a small number of QTL explained 94% of the total genetic variation in trait expression in a new genetic background. Original QTL estimates were quantitatively biased, but that did not preclude the achievement of selection responses. We also confirmed the additive effects of the QTL alleles under study, as well as the consistent effects of QTL alleles across environments. Our results suggest that QTL analysis can indeed help to understand and select for quantitative traits of biological and economical importance.

RESULTS AND DISCUSSION

Classical theory holds that complex (or quantitative) phenotypes are controlled by an infinite number of genes with indistinguishable individual effects, i.e. Fisher's infinitesimal hypothesis (1). Robertson (2) proposed an alternative hypothesis – that the phenotypic manifestation of alleles with low levels of expression, or reduced penetrance, is quantitative variation while alleles at the same locus, or loci, with higher levels of expression and penetrance, are manifested as qualitative variation. The development of abundant and polymorphic molecular markers and quantitative trait locus (QTL) analysis procedures (see review in 3) has provided tools for testing these alternative hypotheses. With complete genome sequences available, new genetic tools can be applied to charting “the land between Mendelian and multifactorial inheritance” as was recently suggested for the case of human diseases with a complex genetic basis (4).

The results of the first generation of QTL analyses in plants lent support to Robertson's hypothesis; relatively few genetic factors were found to be the principal determinants of complex traits, such as grain yield, malting quality, fruit quality, etc (5). In the case of tomato and rice, the genetic dissection of QTL has led to cloning of QTL alleles (6, 7) or at least the assignment of QTL to relatively small physical regions of the genome (8). However, the initial enthusiasm for QTL analysis and the potential gains to be achieved by marker assisted selection (MAS)

has been tempered by concerns regarding bias in QTL estimation (9), a paucity of studies validating QTL alleles in different genetic backgrounds, and few examples of successful MAS (5). The small population sizes typically used for QTL detection lead to overestimation of QTL effect and underestimation of QTL number and interaction (10). Thus, what appeared to be evidence for the Robertsonian hypothesis could in fact be due to a failure of the analysis tools to fully reveal the complexity of quantitative inheritance (11) and if this is the case, the prospects for MAS are not encouraging (12).

Several key issues need to be addressed in order to correctly assess the potential of QTL analysis: (i) how many genes are necessary in order to recover the target phenotype, (ii) what is the expression of QTL alleles in different genetic backgrounds, (iii) what is the relative importance of additive gene action compared with epistasis, (iv) what is the importance of QTL x environment interaction. In this paper we present information that addresses these issues, using the example of quantitative resistance to a plant disease.

Genetic resistance is the most economical and environmentally appropriate strategy for disease control in plants. In the case of qualitative resistance - where genes of large effect clearly interact on a gene-for-gene basis with the pathogen - there is evidence that pathogen virulence can evolve more quickly than plant breeders can incorporate single resistance genes into new varieties (13). Quantitative resistance (QR) shows continuous variation and it is usually incomplete in expression but has

a higher probability of durability (14). Prior to the advent of QTL analysis, estimates of gene number for QR were derived from statistical analyses of phenotypic data collected from segregating crosses and estimates of the number of "effective factors" determining QR ranged from 2 to 10 (15). QTL approaches to estimating the number of genes determining QR have revealed that in some cases a significant proportion of the total variance in QR is attributable to one locus or a few loci. However, small population sizes and model assumptions may cause estimates of gene number to be downward biased for both statistical analyses of phenotypic data (15) and for QTL analyses (16, 17).

We have generated QTL data from a plant disease resistance model that lead us to be cautiously optimistic about the application of QTL analysis to plant genetics and improvement. Barley (*Hordeum vulgare* subsp. *vulgare*) is a diploid crop of genetic and economic importance (18), and can be seriously affected by the disease stripe rust (caused by the fungal pathogen *Puccinia striiformis* f.sp. *hordei*). We initiated a collaborative effort (reviewed in 19) to map and introgress quantitative resistance (QR) to barley stripe rust (BSR) from germplasm that has remained resistant to the spectrum of virulence encountered in Mexico, South America and the U.S (20). We have mapped BSR resistance QTL in multiple populations derived from crosses of resistance sources crossed with susceptible varieties adapted to western North America (21, 22). We have released varieties with QTL resistance alleles (23), and we have introgressed multiple QTL from different genetic backgrounds into single

genotypes using MAS (24). Since QTL from different sources were not coincident, we proposed the development of QTL pyramids as a way to increase the resistance level of new germplasm.

In order to pyramid QR genes, we developed a population of 115 doubled haploid (completely homozygous) (25) lines from a series of matings involving two donors of different QR alleles (Fig. 3.1, 26). The variety Orca is homozygous for BSR resistance QTL alleles on chromosomes 4H and 5H (21), the experimental line D1-72 is homozygous for BSR resistance QTL alleles on chromosome 1H (22) and the variety Harrington has no known QTL resistance alleles. Given this three-parent pedigree, DH lines have one of three possible allele architectures per locus. Each DH line was assayed with PCR primers specific to simple sequence repeat (SSR) loci defining each of the three QTL regions (Fig.3.2). By assaying with multiple multi-allelic SSRs, we could infer the allelic architecture of each DH line for each BSR QTL region. Disease phenotype data were obtained from seven different experiments in which the entire population was tested under severe BSR epidemic conditions (27). Having identified the number and origin of BSR resistance QTL alleles in each line, we proceeded to determine the main effects of each allele and the interactions of alleles in terms of three QR resistance phenotypes: disease severity, the area under the disease progress curve, and the infection rate (28).

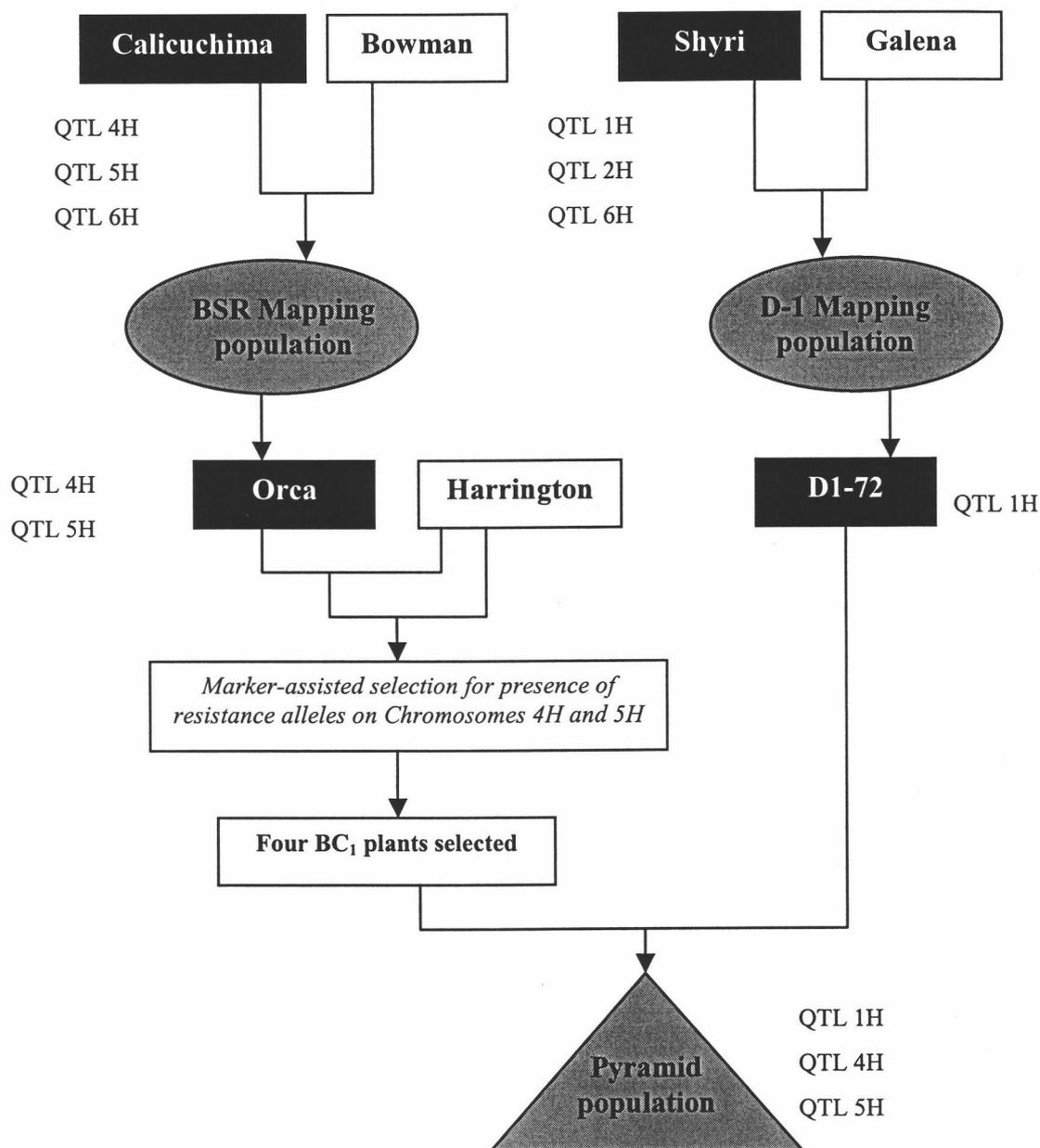


Figure 3.1. Development of the MAS derived QR allele pyramid population. Black boxes represent resistance sources and white boxes represent susceptible parents. All the mapping populations were composed of completely homozygous doubled-haploid lines.

The numbers of lines per allele class (Table 3.1) fit genetic expectations (29), but the number of DH lines in each allele class are not the same. In order to determine the effect of this imbalance in sample size (i.e. more lines representing each of the single QTL resistance allele classes, fewer lines in the two resistance allele classes, and the fewest in the three resistance allele class), we randomly sampled in each class to generate sub-samples with the same number of lines per class ($n = 10$). The results of the analysis of sub-samples were the same – in terms of order and magnitude of differences of phenotypes and in declaration of significant differences – as the analysis based on the full population. Accordingly, we present data from the full population in this report.

The presence of BSR resistance alleles in any of the three QTL regions was significantly associated with lower disease levels, according to the three measures of the QR resistance phenotype (Table 3.1, Figure 3.3). This is evidence that these alleles have an effect on reducing BSR symptoms in a genetic background different from those in which the alleles were originally discovered. The BSR resistance alleles reduced disease in an additive fashion, except for one case of significant QTL X QTL interaction: the QTL resistance allele on chromosome 4H, contributed by the variety Orca, showed an interaction with the QTL resistance allele contributed by D1-72 on chromosome 1H. The presence of both QTL resistance alleles led to greater resistance than the presence of either resistance allele alone, but the reduction in disease conferred by the two resistance alleles did not reduce

disease symptoms as much as would be expected by the sum of the individual effects of the two alleles. The accuracy of the estimates of the combination of resistance alleles on chromosomes 4H and 5H, and of alleles in all three chromosomes is likely constrained by the limited number of DH lines representing the higher order combinations of alleles.

QTL effects were confirmed in each experiment, regardless of epidemic intensity. No QTL x experiment interaction was detected. Fig.3.3 shows results from the experiments in which the greatest disease pressure was observed. Each resistance allele, and combination of alleles, shows a distinct and consistent disease progress curve.

A comparison of QTL effects as estimated in the source mapping populations and in the MAS-derived lines reveals marked changes in magnitude of effect (Table 3.2). This could be due to bias in estimation of QTL effects in the source mapping populations or it could be due to uncharacterized interactions of the resistance QTL alleles with the new genetic background. In the original QTL mapping report (21), the chromosome 4H QTL had a very small effect on reducing disease severity, while the effect of the chromosome 5H allele was much greater. We found the reverse in our MAS-derived lines (Table 3.2). This introgression of QTL alleles into new genetic backgrounds shows that the resistance allele on chromosome 4H has a higher "breeding value" than the one on chromosome 5H. We have developed three other populations including the QTL alleles described in this report, with

resistance alleles at other QTL, and we have found the same relationship between the 4H and 5H alleles.

The QTL alleles that we targeted comprise large genome regions, averaging 20 cM. Estimates of physical (Mb) to genetic (cM) ratios for these regions of the genome range from less than 1.0 to more than 4.4 Mb/cM (32, 33). Given such large physical regions, there is clearly a possibility for linkage drag. This could limit multi-trait gain from selection in the case of repulsion linkages. Resistance genes are known to cluster in certain regions of plant genomes (34, 35), so it is also possible that coupling linkages of multiple resistance alleles could also be introgressed, which could be advantageous from a practical standpoint. If, however, resistance QTL alleles indeed are found in proximity to other resistance genes and if, as Robertson suggested (2), the QTL alleles show low penetrance, it will be challenging to clone the determinants of QR and demonstrate their effects in complementation tests.

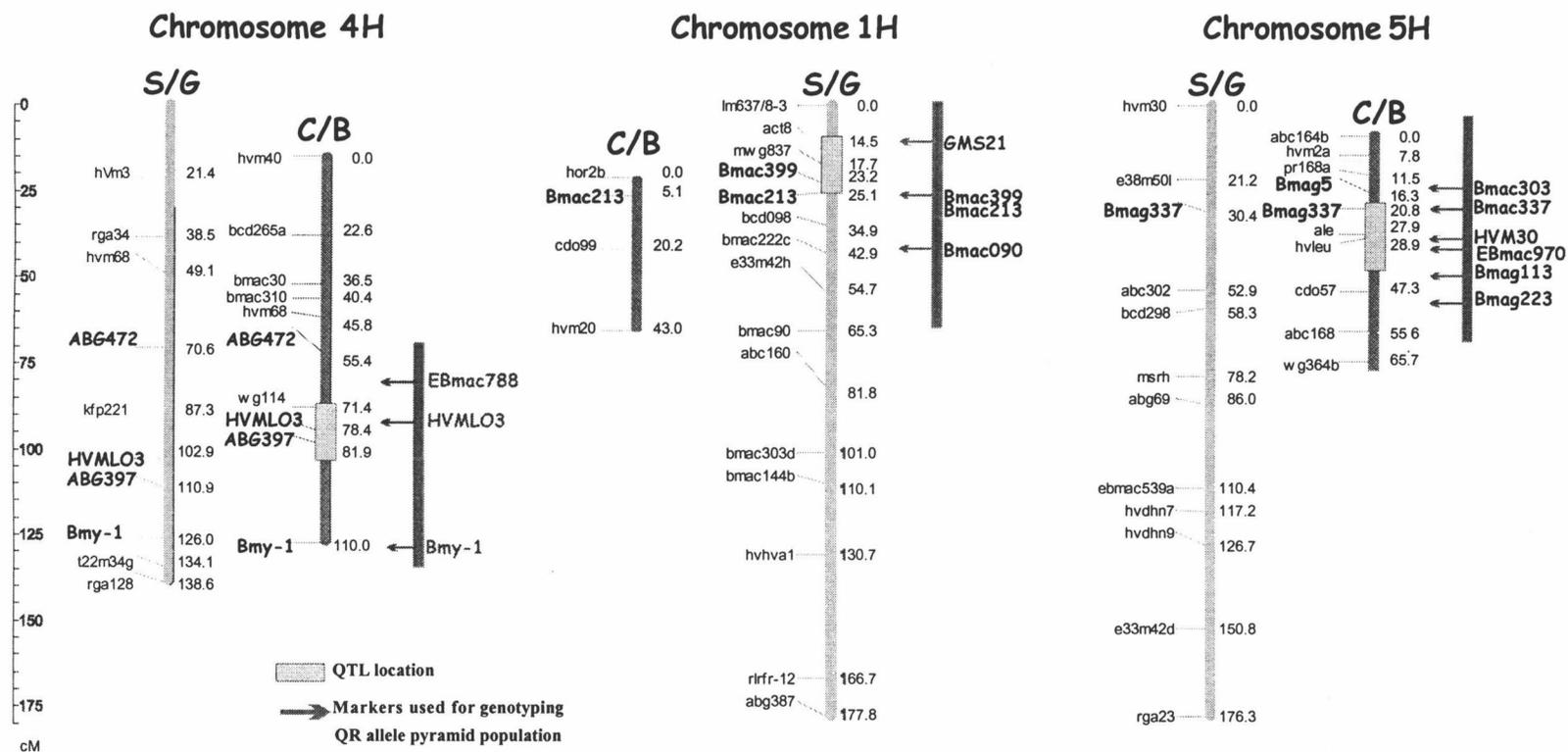


Figure 3.2. Alignment of the QTL mapping populations for the target QR regions on chromosomes 1H, 4H and 5H showing the markers used for genotyping the QR allele pyramid population. “C/B” refers to Calicuchima/Bowman, where QTL on chromosome 4H and 5H were mapped (21). “S/G” refers to Shyri/Galena, where QTL on chromosome 1H was mapped (22). Markers indicated with larger font in the original maps were included to facilitate map alignment. To measure the effect of the presence or absence of the different QTL we performed an analysis analogous to a candidate gene analysis, where the genotypes of the lines (based on the markers shown in the figure) are used as independent variables. Recombinant genotypes were not included in the analysis.

Table 3.1. Least squares means of disease severity and area under the disease progress curve (AUDPC), and least squares estimates of the infection rate in individuals according to the presence or absence of resistance alleles in the QTL regions under study on chromosomes 1H, 4H and 5H. Disease severity was calculated based on data from seven environments, while AUDPC and infection rate were calculated based on data from six environments. Infection rate was determined by adjusting a multiple regression model for all the data, using Gompertz's transformation (30, 31). Values in the same column followed by the same letter are not significantly different ($p < 0.05$) based on pairwise comparisons.

QTL location			Disease Severity ^a	AUDPC ^b	Infection rate ^c
4H (from Orca)	1H (from D1-72)	5H (from Orca)			
-	-	-	50.8 a	850 a	0.0424
+	-	-	25.0 d	438 c	0.0248
-	+	-	31.7 c	587 b	0.0303
-	-	+	40.2 b	648 b	0.0345
+	+	-	15.8 e	241 d	0.0171
+	-	+	7.7 f	160 d	0.0169
-	+	+	21.1 d	341 cd	0.0224
+	+	+	8.9 ef	226 d	0.0092
r ² of the model (%)			46.0	44.6	59.9

^a Measured as maximum % of leaf area infected

^b Measured as the integral of the disease progress curve

^c Calculated as the coefficient of regression after adjusting multiple regression models on transformed data (using Gompertz's transformation: $-\log(-\log(\text{infected area}))$).

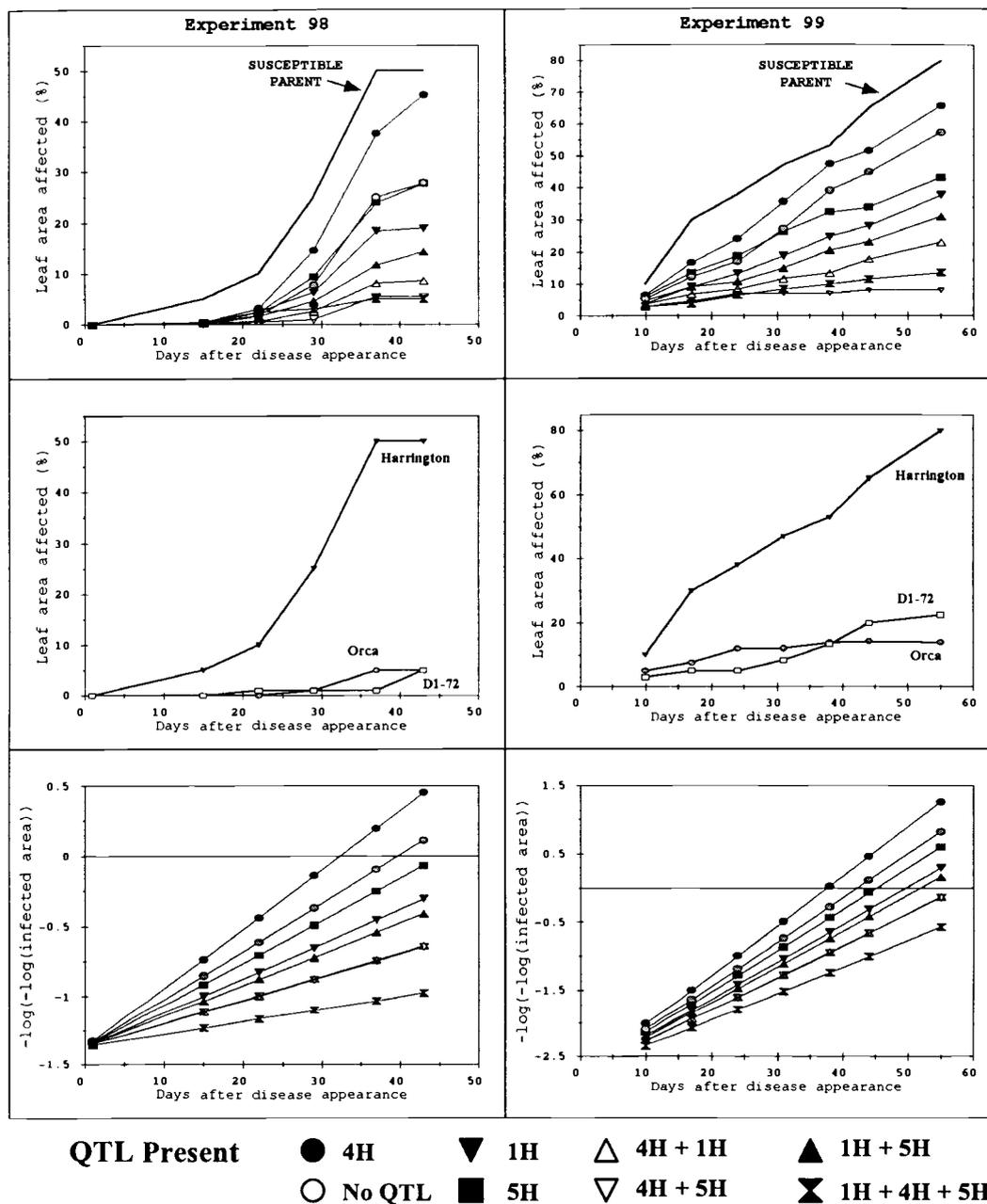


Figure 3.3. Average disease progress curves for doubled haploid lines with different combinations of resistance QTL alleles in two of the six experiments where this phenotype was measured. Figures on the left (98) correspond the third planting date in 1998. Figures on the right (99) correspond to the first planting date in 1999. Figures in first panel show results based on untransformed data; figures in the center show the

Table 3.2. Comparison of the amount of genotypic variance of disease severity (σ_G^2) explained by the QTL effects in the original reports (21, 22), and in the MAS derived QR pyramid population. The heritabilities in the original mapping experiments were 0.77 (Shyri/Galena) and 0.74 (Cali/Bowman). The heritability in the present experiment was 0.56.

QTL location	Original report			Pyramid population	
	Population	% σ_G^2	p-value	% σ_G^2	p-value
Chromosome 1H	Shyri/Galena	0.91	<0.0001	0.22	<0.0001
Chromosome 4H	Cali/Bowman	0.02	0.0089	0.56	<0.0001
Chromosome 5H		0.73	<0.0001	0.16	<0.0001

Our results provide some answers to questions regarding the utility of QTL analysis for understanding the genetics of quantitative traits. We validated the expression of QTL that significantly affect stripe rust infection in a different genetic background. We also showed that, in the population under study, a relatively small number of QTL explained a significant portion of the total genetic variation of the trait (94%), validating the original estimates of the importance of those regions in determining genetic resistance to stripe rust. The estimates of QTL effects in the original mapping populations were different than those in the resistance allele pyramid lines (Table 3.2); this may be due to bias in QTL detection and/or interaction of QTL with genetic background. These biased estimates of allele effect and/or interaction with genetic background did not preclude the achievement of selection responses. We demonstrated that the QTL effects under study were essentially additive, and that the importance of the QTL x environment interaction was low. Thus, our results suggest that QTL analysis can help to identify and manipulate the genes determining an important quantitative trait.

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- (26) The cross was Harrington*2/Orca/2/D1-72. Orca (Calicuchima/Bowman) is a spring feed barley variety developed by Oregon State University that has two BSR resistance QTL, one on chromosome 4H and another on chromosome 5H. D1-72 is an experimental line from the Shyri/Galena mapping population that carries a BSR resistance QTL on chromosome 1H. Harrington, a malting variety developed by the University of Saskatchewan, is the malting quality standard for two-rowed varieties in North America. Backcross-1 generation plants from Harrington*2/Orca were selected for the presence of Orca alleles using RFLP loci flanking the QTL regions on chromosomes 4H and 1H. For more information see: <http://www.css.orst.edu/barley/orbarley/collab.htm>.
- (27) The population was tested in seven experiments during 1996 (1), 1998(3) and 1999(3) at the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) facilities at Toluca, Mexico. Disease severity was measured based on visual assessment of the percentage of infected foliar material in the crop canopy, on a

plot basis. Disease severity readings were taken at the end of the cycle in 1996 and at weekly intervals through grain filling, starting at the beginning of infection, in 1998 and 1999. Stripe rust epidemics achieved in Toluca nurseries were very consistent and intense. Data from multiple growth stages allowed for the calculation of the area under the disease progress curve (AUDPC) and the epidemic infection rate. For the calculation of infection rate we use the Gompertz's transformation ($-\log(-\log(\text{disease severity}))$).

(28) Individual effects were estimated using a QTL analysis analogous to candidate gene analysis, where the genotypes of the flanking markers are used as independent variables. Recombinant genotypes were not included in the analysis. Therefore, the independent variables had two levels each, with one level corresponding to the resistance allele (Orca in chromosomes 4H and 5H, D1-72 in chromosome 1H) and the other level corresponding to the other alleles. The treatment design was a 2 x n factorial, where n is the number of genome regions detected. The difference between parental marker class means estimates the additive effect of the QTL flanked by the markers. Double crossovers between the QTL and marker loci downwardly bias estimates of the effects. Thus, differences between parental marker genotype means are conservative estimates of the effects of QTL residing in the n chromosomal regions. Statistical analyses were performed using the GLM procedure of SAS 7.1 (SAS Inst., 2001).

(29) Individual allele frequencies were tested against expectations using a chi-square test. Orca alleles in the chromosome 4H QTL were significantly higher in frequency than expected. Once observed allele frequencies were considered to calculate the expected genotypic frequencies, the observed genotypic frequencies were not significantly different from the expected ones.

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**MOLECULAR MARKER ASSISTED (MMAS) PYRAMIDING OF
QUANTITATIVE TRAIT LOCUS (QTL) ALLELES DETERMINING
RESISTANCE TO BARLEY STRIPE RUST: EFFECTS ON SEEDLING
RESISTANCE.**

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ABSTRACT

Pyramiding multiple resistance genes in single genotypes may lead to more durable resistance. Quantitative resistance (QR) may be more durable than qualitative resistance. Quantitative trait locus (QTL) analysis tools are used to find determinants of QR. Pyramids of resistance alleles at QTL loci can provide an independent validation of QTL effects and allow for estimation of QTL interactions. We used molecular marker-assisted selection (MAS) to pyramid resistance alleles at two QTL conferring resistance to barley stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) at the seedling stage in a new and agronomically relevant genetic background. The QTL, one on chromosome 4(4H) and the other on chromosome 5(1H), trace to different mapping populations. In the reference mapping populations, QTL alleles showed complementary gene action at the seedling stage, and in both populations seedling and adult plant resistance QTL were coincident. The MAS-derived population of doubled haploid (DH) lines was phenotyped for seedling resistance to four isolates of the pathogen, three representing races PSH-13, PSH-14, and PSH-31, and the fourth an isolate collected in Montana (BSTR-97). Using molecular markers to identify the presence of resistance alleles in the QTL regions, and logistic regression procedures to estimate QTL allele effects, we validated QTL effects and locations in a new genetic background. No QTL x race interaction was detected. In the reference

mapping population, alleles at two QTL (on chromosomes 4(4H) and 6(6H), and on 5(1H) and 6(6H), respectively) were necessary for resistance, whereas in the MAS-derived lines, resistance was achieved with resistance alleles at QTL on chromosomes 4(4H) and 5(1H).

INTRODUCTION

Host pathogen resistance to biotic stresses can be classified as qualitative or quantitative. The former refers to Mendelian genes of large effect that clearly interact on a gene-for-gene basis with the pathogen, whereas the latter describes resistance that shows continuous variation and is usually incomplete in expression. The race specificity of quantitative resistance is still an unresolved question. Qualitative resistance is often measured as the reaction of seedling plants to inoculation, and its use for the development of new varieties can be straightforward. The main problem with qualitative resistance is the lack of durability (Parlevliet, 1977). Quantitative resistance (QR) is more likely to be durable (Parlevliet, 1989). The principal limitation to QR is that it requires extensive field-testing at multiple growth stages.

Pyramiding qualitative resistance genes with different race specificities has been proposed as a way to increase the likelihood of achieving durable resistance with qualitative resistance genes (Schaffer and Roelfs, 1985; Mundt, 1990). Singh et al. (2001) pyramided three bacterial blight resistance genes in rice, providing a wider spectrum of resistance. Tabien et al. (2000) found main effects and non-linear interactions between multiple genes conferring resistance to rice blast (caused by *Pyricularia grisea*) in a rice mapping population. Pyramiding quantitative

resistance alleles in single genotypes may also increase the probability of achieving durable resistance.

Quantitative trait locus (QTL) analysis procedures have facilitated the dissection of QR, revealing that in some cases a significant proportion of the total variance in the expression of the resistance can be attributable to one locus or a few loci (Chen et al., 1994; Michelmore, 1995; Hayes et al., 1996; Young, 1996). However, information on the mechanisms underlying quantitative resistance is still very limited.

The QTL concept represented an important step forward in understanding traits showing quantitative variation (Doerge, 2002). Plant breeders embraced QTL mapping tools in order to increase selection efficiency via marker-assisted selection (MAS). However, QTL analysis has been shown to be subject to serious limitations. These include bias in QTL estimation, lack of studies validating QTL alleles in different genetic backgrounds, and few examples of successful MAS (Dekkers and Hospital, 2002).

In this paper we present QTL and MAS data from the barley: barley stripe rust pathosystem that lead us to be reasonably optimistic about the application of QTL analysis and MAS to the development of disease-resistant genotypes. Stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) is an important disease of barley worldwide. It has been reported in the Americas since 1975, and in the U.S. since 1991 (Dubin and Stubbs, 1985; Marshall and Sutton, 1995). The population of BSR

in the Americas was first described as race 24 (Dubin and Stubbs, 1985), but considerable variation has since been reported in pathogen isolates collected in the U.S. (Chen et al., 1995). Several qualitative and quantitative BSR genes have been reported (Von Wettstein-Knowles, 1992; Chen et al., 1994; Thomas et al., 1995; Hayes et al., 1996; Toojinda et al., 2000; Castro et al., 2002; Castro et al., chapter 2). We have focused our efforts on QR available in ICARDA/CIMMYT germplasm (reviewed by Hayes et al., 2001). BSR QTL from different germplasm accessions mapped on different chromosomes, allowing us to pyramid them in a new genetic background.

The pyramids of QTL alleles provide an independent validation of QTL effects detected in mapping populations and allow for estimation of QTL interactions. Furthermore, MAS is the only way to pyramid QR genes because differential isolates of the pathogen cannot be used to determine the resistance allele architecture of the host, if the resistance is non-race specific.

We developed genotypes combining two BSR resistance QTL alleles from the accession "Calicuchima-sib" on chromosomes 4(4H) and 7(5H) (Chen et al., 1994) (hereafter called QTL4 and QTL7), and a BSR resistance QTL allele from the variety "Shyri" on chromosome 5(1H) (Toojinda et al., 2000) (hereafter called QTL5). We validated the effects of the three QTL, we have found that they act in an additive fashion, and we have demonstrated that genotypes with the most QR

alleles have the highest levels of resistance, under field epidemic conditions at the adult plant stage (Castro et al, chapter 3).

The principal thrust of our QR mapping efforts has been on adult plant resistance under field conditions, but we are also interested in QR QTL effects at the seedling stage. Seedling tests can allow for higher throughput screening and allow for assessment of race specificity of resistance genes. In mapping populations we have found that adult plant QR QTL show complementary gene action at the seedling stage. In the Calicuchima/Bowman population QTL on chromosomes 4(4H) and 6(6H) were required for resistance (Castro et al., 2002), while in the Shyri/Galena population QTL on chromosomes 5(1H) and 6(6H) were required for resistance (Castro et al., chapter 2). The chromosome 6(6H) QTL were coincident in the two populations.

The QR QTL pyramid project was initiated prior to the discovery of the role of the 6(6H) QTL in seedling resistance and accordingly this region of the genome was not targeted for MAS. Thus, we have become interested in determining if seedling resistance could be achieved in the QR QTL pyramids involving QTL4, QTL5 and QTL7. If seedling resistance was conferred by alleles at these QR QTL, we were interested in the nature of the QTL interaction.

MATERIALS AND METHODS

PLANT MATERIAL

A population of 115 doubled haploid (DH) lines (Chen and Hayes, 1989) was developed from the cross Harrington*2/Orca/2/D1-72. Harrington is a two-rowed malting barley variety developed by the University of Saskatchewan. Orca is a two-rowed barley variety obtained from the cross of Calicuchima-sib and Bowman, and has resistance alleles at QTL4 and QTL7 tracing to Calicuchima-sib (Hayes et al., 2000; Castro et al., 2002). D1-72 is a line from the Shyri/Galena population that has a resistance allele at QTL5 tracing to Shyri (Toojinda et al., 2000; Castro et al., Chapter 2). One cycle of marker-assisted selection was performed for resistance alleles at QTL4 and QTL7 in the BC₁ generation (Harrington*2/Orca). Four BC₁ plants with Orca alleles at marker loci flanking QTL4 and QTL7 were crossed with D1-72. DH lines were derived from the F₁ plants of these crosses, following the procedures described by Chen and Hayes (1989).

PHENOTYPING

The parents (Orca, Harrington and D1-72) and the DH progeny were assayed for resistance to BSR, at the seedling stage, in two experiments following the procedures described by Chen and Line (1992) and Hayes et al. (1996). In the first

experiment isolates corresponding to races PSH-13, PSH-14 and PSH-31 of *P. striiformis* f. sp. *hordei*, which represent a range of virulence (Chen et al., 1995), were used. In the second experiment an isolate of *P. striiformis* f.sp. *hordei* collected in Montana in 1997 (hereafter called BSTR-97) was used. Seedling reactions to inoculation with the isolates were measured under controlled environment conditions, following procedures described by Hayes et al. (1996). In both experiments infection types were recorded using a 0-9 scale (0: complete resistance; 9: complete susceptibility) as described by Line et al. (1974).

GENOTYPING

The DH lines were genotyped in the regions defining the BSR QR QTL on chromosomes 4 (4H), 5 (1H), 6(6H), and 7 (5H) using SSR and STS markers. We first screened markers of known map position on Orca, Harrington, and D1-72 for polymorphisms. As our interest was to define the regions of chromosomes 4 (4H) and 7 (5H) that were introgressed from Orca, map positions of markers in these regions were confirmed in the Cali-sib x Bowman mapping population. In chromosome 5 (1H), our interest was in the region tracing to Shyri (via D1-72), and the reference population for confirming map position of polymorphic markers was Shyri x Galena. Neither Harrington nor D1-72 are known to carry resistance alleles at the QTL region on chromosome 6(6H) that was important for seedling resistance in the Cali-sib/Bowman and Shyri/Galena mapping populations (Castro et al.,

2002; Castro et al., chapter 2). As shown in figure 3.2, we used three SSRs (*EBmac788*, *HVMLO3* and *HvAmyB*) for genotyping the chromosome 4(4H) QTL region, four SSRs markers (*GMS21*, *Bmac213*, *Bmac399* and *Bmac90*) for genotyping the chromosome 5(1H) region, and six SSRs (*Bmac303*, *Bmac337*, *HVM30*, *EBmac970*, *Bmac113*, and *Bmac223*) for the chromosome 7(5H) region and one SSR (*Bmag173*) for the chromosome 6 (6H) region. *Bmag173* maps to the seedling resistance QTL peak in both original mapping populations. Based on marker allele genotypes we were able to infer the presence or absence of the corresponding QR loci alleles in each of the lines. The SSR primer sets were developed and mapped by Ramsay et al. (2000), Pillen et al. (2000), Liu et al. (1996) and Becker and Heun (1995). For the SSR genotyping reverse primers were labeled with FAM, TET, NED or HEX fluorescent dye. DNA amplifications were performed using either a Perkin-Elmer 9600 or MJ Research PTC-100 thermal cycler. PCR reactions were carried out in a 10 µl reaction mix containing 37.5 ng of template DNA, 1x PCR buffer, 0.025 units *Taq* DNA polymerase, (Qiagen), 0.2 nM dNTPs and 0.1 picomoles of forward and reverse primers. Information on primer sequences, and PCR amplification conditions for each set of primers, are available at <http://www.scri.sari.ac.uk/ssr> (Ramsay et al. 2000), in Liu et al. (1996) and in Becker and Heun (1995). PCR-amplified fragments from differentially labeled SSR primers and with non-overlapping fragment sizes were simultaneously analyzed in the same gel lane and separated on an ABI Prism 377 DNA Sequencer at the Oregon State University (Central Service Lab) or on an ABI Prism 3700

DNA Sequencer at OMIC, Inc., Portland, OR. Gene Scan® and Genotyper® Software (Applied Biosystems, Perkin Elmer, Foster City, CA) were used for automated data collection and to determine the allele sizes in base pairs, based on the internal standard.

DATA ANALYSES

DH lines with infection type scores of 0 to 5 were considered resistant and DH lines with scores of 6 to 9 were considered susceptible (Line et al., 1974; Line and Qayoum, 1991; Chen and Line, 1999). The population structure precluded the use of QTL analysis tools based on interval mapping. Accordingly, we used a QTL analysis analogous to candidate gene analysis, where the genotypes of the flanking markers are used as independent variables. Recombinant genotypes were not included in the analysis. Therefore, the independent variables had two levels each, with one level corresponding to the resistance allele (tracing to Orca in chromosomes 4(4H) and 7(5H), and tracing to D1-72 in chromosome 5(1H)) and the other level corresponding to the alternative alleles at these QTL. The treatment design was a 2 x n factorial, where n is the number of genome regions targeted. The difference between parental marker class means estimates the additive effect of the QTL. Double crossovers between the QTL and marker loci would downward bias estimates of QTL effects. Thus, differences between parental marker genotype means are conservative estimates of the effects of QTL residing in the chromosomal regions under study. Because the response (dependent) variable was

binomial (1 = resistant and 0 = susceptible) and the response probability distribution was binomial, the analysis was performed using a generalized linear model with a logit link function and binomial errors.

Statistical analyses were performed using the SAS (2001) GENMOD procedure. Parameters and test statistics were estimated using a Type III analysis (analogous to partial sums of squares analyses of general linear models). We performed separate analyses of the effects for each stripe rust race and a combined analysis using the four isolates. The analyses of each individual isolate entailed estimating the least squares means for each QTL and their interactions, additive effects, additive by additive interaction effects, and likelihood ratio statistics for tests of significance of the effects (p -values were calculated using asymptotic chi-square distributions). The joint analysis entailed estimating the least squares means and test statistics for the effect of stripe rust isolate (I) and interaction effects between I and QTL, in addition to the main and interaction effects across isolates. The probability of resistance to stripe rust was estimated for QTL allele genotypes by $e^p/(1 + e^p)$, where p is the least squares mean for the individual QTL and QTL x QTL interaction.

RESULTS

The reactions of DH lines to each of the four isolates were quite consistent, as shown in Figure 4.1, although there were some cases of DH lines showing differential reactions to isolates (Table 4.1). There were fewer lines with a resistant reaction to isolate BSTR-24, compared to the reaction to inoculation with the other three isolates. The higher number of lines with a resistant reaction was observed for isolate PSH-14. As a result, the same DH lines were not always classified as resistant or susceptible, although the largest groups of lines were those showing a consistent reaction to all the isolates. The high frequency of lines with equivalent reactions to at least three of the isolates (98 out of 115, or 85%) supports the hypothesis that the principal genetic factors determining the resistant phenotype are probably the same for the four isolates. Eighty-four of 115 lines had the same reaction to the three PSH isolates, whereas sixty-five of the eighty-four lines had also the same reaction to BSTR-97. This may be due to minor differences in disease assessment protocols used at the two locations.

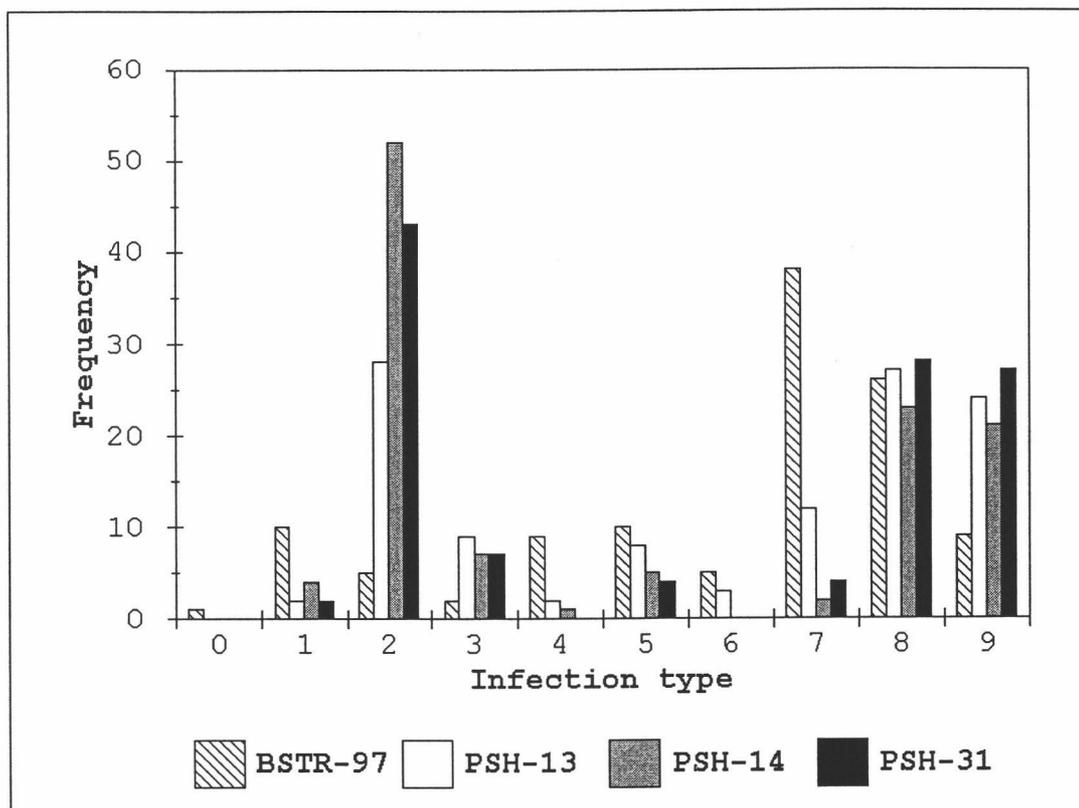


Figure 4.1. Phenotypic distribution of infection type in the MAS-derived doubled haploid population when inoculated at the seedling stage with four different isolates of *Puccinia striiformis* f.sp. *hordei*.

Table 4.1. Numbers of resistant and susceptible MAS-derived doubled haploid lines when inoculated at the seedling stage with four different isolates of *Puccinia striiformis* f.sp. *hordei*, classified according to reaction to each isolate

Isolate				
BSTR-97	PHS-13	PHS-14	PHS-31	No. of DH lines
R	R	R	R	27
R	R	R	S	1
R	R	S	R	0
R	R	S	S	0
R	S	R	R	2
R	S	R	S	5
R	S	S	R	1
R	S	S	S	3
S	R	R	R	16
S	R	R	S	3
S	R	S	R	1
S	R	S	S	1
S	S	R	R	7
S	S	R	S	7
S	S	S	R	3
S	S	S	S	38
39	49	68	57	Total R lines
76	66	47	58	Total S lines

There was a clear pattern of association of resistance vs. susceptibility alleles at QTL4 and QTL5 and seedling reaction to inoculation (Table 4.2). The absence of resistance alleles at both QTL4 and QTL5 was associated with the susceptible phenotype. The effects of resistance vs. susceptibility alleles were greatest in response to inoculation with PSH-14 and PSH-31. The likelihood ratio tests for each individual isolate and the joint analysis of all four isolates confirmed the importance of both QTL in relation to the seedling reaction phenotype (Table 4.3). In all cases, both QTL were significant, no race x QTL interaction was detected, and there was a significant race effect that reflects the higher number of susceptible lines observed after inoculation with BSTR-97.

We also determined the role of QTL7 and the QTL on chromosome 6(6H) in the expression of seedling resistance to the four isolates. QTL7 was not a significant determinant of seedling resistance in the original mapping population (Castro et al., 2002), but because it was a significant determinant of adult plant resistance (Chen et al., 1994) it was a target for MAS in the development of the DH lines used in this study. No effect of QTL7 or QTL7 x race was detected. Regarding chromosome 6(6H), which was a significant determinant of seedling resistance in the two source mapping populations (Castro et al., 2002; Castro et al, chapter 2), D1-72 lacks the Shyri (resistance) alleles at this QTL, and the genotyping with *Bmag173* did not detect Orca alleles. Because the region was not targeted in the MAS scheme, the

absence of Orca alleles means that the four BC₁ plants selected for the final cross carried Harrington alleles at this QTL.

Table 4.2. Numbers of resistant (R) and susceptible (S) MAS-derived doubled haploid lines inoculated at the seedling stage with different isolates of *Puccinia striiformis* f.sp. *hordei*, classified according to their reaction to each one of the races and the alleles present in the QTL regions on chromosomes 4(4H) and 5(1H). Resistance alleles are represented by (+), susceptible alleles by (-).

Alleles in		BSTR-97		PSH-13		PSH-14		PSH-31	
QTL4 ¹	QTL5 ²	R	S	R	S	R	S	R	S
+	+	14	7	18	3	19	2	20	1
+	-	9	11	9	11	16	4	12	8
-	+	13	27	20	20	27	13	23	17
-	-	3	31	2	32	6	28	2	32
Total		39	76	49	66	68	47	56	59

¹ Orca contributes the resistance alleles in QTL4

² D1-72 contributes the resistance alleles in QTL5

Table 4.3. Likelihood ratios for tests of significance of QTL main and interaction effects from separate analyses for each of four stripe rust isolates, and QTL and isolate main and interaction effects for the combined analysis of all isolates. P-values were calculated using asymptotic chi-square distributions.

	Effect	χ^2 statistic	$P < \chi^2$
BSTR-97	QTL4	14.22	0.0002
	QTL5	6.49	0.0109
	QTL4*QTL5	0.98	0.3214
PHS-13	QTL4	20.02	0.0001
	QTL5	24.74	0.0001
	QTL4*QTL5	0.51	0.4769
PHS-14	QTL4	21.82	0.0001
	QTL5	8.86	0.0029
	QTL4*QTL5	1.55	0.2134
PHS-31	QTL4	26.64	0.0001
	QTL5	23.39	0.0001
	QTL4*QTL5	1.06	0.3031
Joint analysis	QTL4	81.86	0.0001
	QTL5	56.96	0.0001
	QTL4*QTL5	3.96	0.0467
	Race	17.87	0.0005
	Race*QTL4	1.51	0.6807
	Race*QTL5	4.51	0.2116
	Race*QTL4*QTL5	0.21	0.9765

As shown in Figure 4.2, the probability of disease occurring was lower than 10% when resistance alleles were present at both QTL (for the joint analysis), and higher than 80% in all analyses when resistance alleles were absent in both QTL. The presence of resistance alleles at only one QTL was associated with intermediate probabilities of disease occurrence.

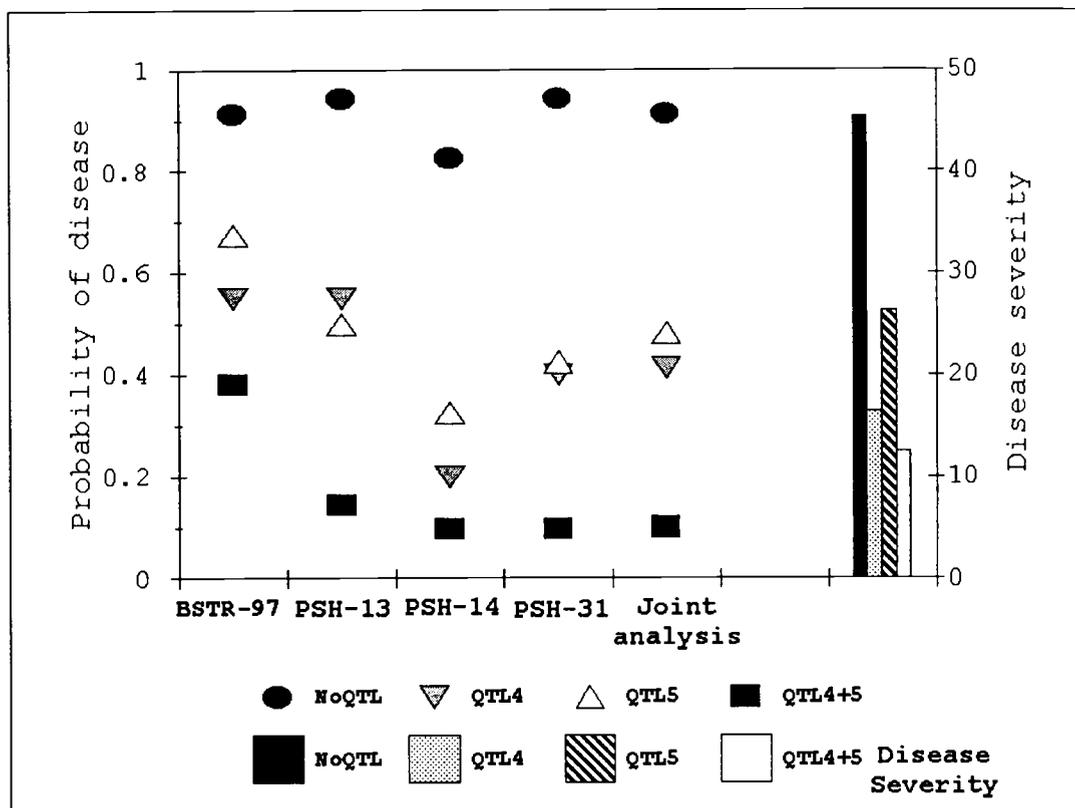


Figure 4.2. Least squares means of the probability of occurrence of the susceptible phenotype in individuals with resistance alleles on QTL4, on QTL5, on both QTL4 and QTL5, and with no resistance alleles for each stripe rust isolate and for all four isolates (left vertical axis). Least squares means of adult plant disease severity for the same individuals in seven environments (right vertical axis) (Castro et al., chapter 3).

DISCUSSION

Our previous findings regarding the effects of QTL4 and QTL5 on seedling and adult plant resistance (Hayes et al., 1996; Castro et al., 2002, chapter 2) have implications for a range of host plant resistance issues, with particular reference to the relationship between quantitative and qualitative resistance and the growth stage specificity of disease resistance. The results presented herein further address these issues and raise additional questions of interest.

Our results validate our earlier reports that alleles at two QTL were necessary in order to achieve BSR resistance at the seedling stage. Alleles at QTL4 and a QTL on chromosome 6(6H) were necessary for resistance at the seedling stage in the Calicuchima x Bowman mapping population, whereas alleles at QTL5 and a QTL on chromosome 6(6H) were necessary in the Shyri x Galena mapping population. In both cases, the resistance fit a complementary gene model: resistance alleles at both QTL were necessary in order to have a probability of 80% or higher of recovering the resistant phenotype. In the current study, we also find that, in a new genetic background, resistance alleles at two QTL are necessary in order to recover the resistance phenotype in high frequency. In terms of allele interaction in a new genetic background, we found significant QTL x QTL interaction in the joint analysis of the four isolates. As shown in Figure 4.2, only in the case of PSH-14 did

alleles at a single QTL have a high probability of resistance and the highest number of resistant lines was observed after inoculation with this isolate.

The results of this study validate the effects of QTL4 and QTL5 as important determinants of BSR resistance at the seedling stage. These results also validate the coincidence between adult plant and seedling resistance QTL as reported by Castro et al. (2002, chapter 2). A comparison of the probabilities of recovering the resistant phenotype in the source mapping populations and in the derived DH lines confirms the consistency of seedling resistance QTL effects (Table 4.4).

However, in the mapping populations, a resistance allele at a QTL on chromosome 6(6H) was necessary for resistance, in conjunction with a resistance allele at either QTL 4 or QTL5. In the DH lines studied in this experiment, resistance alleles were also necessary at two QTL, but the two QTL are on chromosomes 4(4H) and 5(1H). In other words, an allele at QTL4 or QTL5 can substitute for an allele on chromosome 6(6H).

In a previous report (Castro et al., chapter 2) we presented two alternative hypotheses for the necessity of resistance alleles at two QTL in order to recover the resistant phenotype at the seedling stage: (i) alleles at the two QTL control different components of the resistance pathway and (ii) the high inoculum load necessitates multiple resistance genes. Our current results support the second hypothesis although further experiments will be necessary. According to the inoculum threshold-related hypothesis (Luke et al., 1972) incomplete resistance mechanisms

are effective in adult plants under field conditions but are overwhelmed by the higher inoculum load per plant that occurs with greenhouse inoculations of seedlings. Alternatively, QTL5 could determine the same mechanism as the QTL on chromosome 6(6H) reported in the Calicuchima- sib x Bowman population, and QTL4 could determine the same mechanism as the QTL on chromosome 6(6H) detected in the Shyri x Galena population. The inoculum load hypothesis appears to be the most straightforward of the two alternatives.

In terms of race specificity, in the original mapping population we did not detect any QTL5 x race interaction in response to inoculation with three isolates (PSH-1, PSH-13 and PSH-14). For QTL4, only one isolate was used to study seedling resistance in the reference mapping population, so no information on race specificity was previously available. We employed an additional isolate in this study and we did not detect significant race specificity for either QTL4 or QTL5. It is possible, however, that the QTL alleles do show race specificity but we have not sampled a broad enough sample of pathogen virulence to detect it.

The QTL analysis of the two reference mapping populations showed a clear distinction between seedling resistance effects (non-additive) and adult plant resistance effects (additive). As shown in Figure 4.2, in the resistance allele pyramid lines there is a parallel between the probability of disease occurrence at the seedling stage and disease severity at the adult plant stage.

Table 4.4. Comparisons of least squares means for the probability of occurrence of the susceptible phenotype in individuals with different allele configurations at QTL4 and QTL5, in source QTL mapping populations and in MAS-derived DH lines. Comparisons are made with the same isolates. Lines with both QTL are compared with lines with two QTL (one of them not included in this study) in the original reports.

	Isolate	Original report ¹	Pyramid population
QTL4	BSTR-97	0.467	0.550
QTL5	PSH-13	0.665	0.500
	PSH-14	0.667	0.325
Two QTL ²	BSTR-97	0.036	0.381
	PSH-13	0.214	0.143
	PSH-14	0.214	0.095
No QTL	BSTR-97	0.667	0.912
	PSH-13	1.000	0.941
	PSH-14	1.000	0.824

¹ Castro et al., 2002 for BSTR-97 and QTL4; Castro et al., chapter 2, for PSH-13, PSH-14 and QTL5.

² For BSTR-97 in the original report the QTL were QTL4 and another QTL on chromosome 6(6H). For PSH-13 and PSH-14, in the original report the QTL were QTL5 and another QTL on chromosome 6(6H). For the pyramid population the QTL are QTL4 and QTL5.

The results of these experiments also validate the approach that we used to identify the genomic regions that affect a binomially distributed trait, such as seedling resistance to BSR. QTL mapping tools were developed to deal with normally distributed variables (Doerge, 2002), and may not be appropriate for non-normal variables. In a previous report (Castro et al., chapter 2) we proposed and applied a three-step approach using chi-square tests, QTL interval mapping, and candidate gene analysis using generalized linear models to detect the seedling resistance QTL alleles in the reference mapping populations.

At the current level of resolution it is not possible to know if seedling and adult plant resistance QTL represent the effects of the same genes or linked genes. The QTL alleles we targeted comprise large genome regions, averaging 20 cM. Estimates of physical (Mb) to genetic (cM) ratios for these regions of the genome range from less than 1.0 to more than 4.4 Mb/cM (Künzel et al., 2000; Hayes et al., 2000). Resistance genes are known to cluster in plant genomes (Michelmore et al., 1995; Hulbert et al., 2001), so it is also possible that coupling linkages of multiple resistance alleles could underly the coincident QTL.

It is also possible that the difference between seedling and adult plant resistance (complementary gene action for the former, additive gene action for the latter) may be due to phenotyping procedures. Quantitative measurements of resistance at the seedling stage (e.g. latent period or pustule size) and qualitative measures of

resistance at later growth stages (e.g. reaction type) are necessary to resolve this issue.

The results of the first generation of QTL analyses lent support to the idea that relatively few genetic factors were found to be the principal determinants of complex traits (Dekkers and Hospital, 2002). However, the initial enthusiasm for QTL analysis and the potential gains to be achieved by MAS has been tempered by concerns regarding bias in QTL estimation (Beavis, 1998), a paucity of studies validating QTL alleles in different genetic backgrounds and few examples of successful MAS (Dekkers and Hospital, 2002). The small population sizes typically used for QTL detection lead to overestimation of QTL effect and underestimation of QTL number and interaction (Melchinger et al., 1998), in which case, the prospects for MAS are not encouraging (Bernardo, 2001).

We have generated data that lead us to support the application of QTL analysis and MAS. In the case of seedling and adult plant resistance to BSR, results of agricultural significance can be achieved using QTL data to design and implement MAS. A comparison of QTL effects as estimated in the source mapping populations and in the MAS derived lines reveals changes in magnitude of effect. This could be due to bias in estimation of QTL effects in the source mapping populations, or it could be due to uncharacterized interactions of the resistance QTL alleles with the new genetic background. In any event, QTL mapping and MAS were useful and necessary for the development of resistance gene pyramids.

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**MAPPING AND PYRAMIDING OF QUALITATIVE AND
QUANTITATIVE RESISTANCE TO STRIPE RUST IN BARLEY.**

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ABSTRACT

The identification of sources of genetic resistance to plant diseases and the location of the gene or genes controlling resistance are important contributions to the efficient development of new resistant varieties. The combination of different sources and types of resistance in the same genotype should assist in the development of durably resistant varieties. Using a doubled-haploid mapping population of barley, we mapped a qualitative resistance gene (BSTR-1) to barley stripe rust in the line CI10587 (PI 243183) to the long arm of chromosome 1(7H). We combined this gene, through a series of crosses with three mapped and validated barley stripe rust resistance QTL alleles located on chromosomes 4(4H) (QTL4), 5(1H) (QTL5), and 7(5H) (QTL7). Three different barley doubled haploid populations were developed from these crosses, two combining BSTR1 with QTL4 and QTL7, and the third combining BSTR1 with QTL5. Disease severity testing in four environments and QTL mapping analyses confirmed the effects and locations of BSTR1, QTL4 and QTL5, thereby validating the original estimates of QTL location and effect. A new QTL was mapped on chromosome 4(4H), 40 cM proximal to QTL4. QTL alleles were effective in decreasing disease severity in the absence of the resistance allele at BSTR1. Quantitative resistance effects were mainly additive, although magnitude interactions were detected. The lack of effect of QTL7 is consistent with previous validation studies that had shown its limited

effect on reducing disease severity. Our results support the concept that combining qualitative and quantitative resistance in the same genotype is feasible.

INTRODUCTION

Genetic resistance to plant diseases is a major objective of most plant breeding programs. Determining the location of the gene, or genes, controlling resistance can assist in the fast and efficient development of new resistant varieties. The durability and stability of plant disease resistance is also a fundamental issue, as it is an important asset in new cultivars. Johnson (1981) defined “durable” resistance as “resistance that remains effective while a cultivar possessing it is widely cultivated” and added that “no statement or implication about the genetic control of the resistance, its mechanisms, its degree of expression, or its race specificity” are involved in defining durability. Considering that durable resistance is a reasonable and laudable goal, the specific strategies used to achieve this goal must be based on a thorough characterization and analysis of the inheritance of resistance.

The term “qualitative resistance” designates Mendelian genes of large effect that interact on a gene-for-gene basis with the pathogen. The term “quantitative resistance” in this context refers to resistance that shows continuous variation, and is usually incomplete in expression. Qualitative resistance usually shows race specificity, while the race specificity of quantitative resistance is still an unresolved question (Castro et al, chapter 4). Qualitative resistance is easily measured by reaction to seedling inoculation, and its use for the development of new varieties can be straightforward. The use of a gene-for-gene system has the implication that

resistance will not remain effective if the pathogen acquires the corresponding virulence by losing the avirulence allele that elicits resistance (Johnson, 1992). The risks involved in using gene-for-gene resistance are well known (Johnson, 1981; Parlevliet, 1983; Vanderplank, 1963, 1978) and there is evidence that pathogen virulence can evolve more quickly than plant breeders can deploy single resistance genes in new varieties (Parlevliet, 1977). As quantitative resistance has a higher probability of durability (Parlevliet, 1989), it has been proposed as an alternative to qualitative resistance. Its main limitation is that it requires extensive and accurate field-testing, which makes it more difficult to select for in plant breeding programs.

Pyramiding qualitative resistance genes with different races specificities has also been proposed as way to increase the likelihood of resistance durability (Schaffer and Roelfs, 1985). Putting multiple race-specific genes in a single genotype (Huang et al., 1997; McIntosh and Brown, 1997; Mundt, 1991) minimizes the probability that a single mutation can overcome all the resistance genes. Singh et al. (2001) pyramided three bacterial blight resistance genes in rice and demonstrated that this provided a wider spectrum of resistance to the pathogen population than single genes. Pyramiding quantitative resistance may also increase the probability that a variety will show durable resistance. Castro et al. (chapter 3) have combined three resistance alleles at stripe rust resistance QTL in barley in the same genetic background and validated their effects in reducing disease severity.

The combination of both types of resistance genes offers the possibility of exploiting both the complete effect of the qualitative resistance gene, or genes, with the theoretical durability of the quantitative resistance gene, or genes. In other words, one would expect that in the case of a “breakdown” of the qualitative resistance, the quantitative resistance genes present in the genotype would act as an “insurance” policy. Obviously, if both resistances were race-specific, and if both had the same particular race specificity, then the combination of both types of resistance genes would offer no advantage. However, as was mentioned earlier, the race specificity of quantitative resistance has not been clearly elucidated. There is another practical limitation to the development of this kind of heterogeneous resistance gene pyramids. Vanderplank (1968) defined the “Vertifolia” effect as the loss of field (or quantitative) resistance as a consequence of breeding for qualitative race-specific resistance. One of the reasons for the “Vertifolia effect” is that the masking effect of the qualitative resistance eliminates the possibility of selecting simultaneously for both types of resistance, and random drift increases the probability of losing the quantitative resistance during selection. The recent development of abundant polymorphic molecular markers allows for the simultaneous selection for both types of resistance, providing that at least the quantitative resistance gene, or genes, have been mapped.

Stripe rust (caused by the fungal pathogen *Puccinia striiformis* f.sp. *hordei*) is a major disease of barley worldwide, and the development of resistant germplasm is

a major objective of several barley breeding programs. The lack of durability of qualitative resistance in this system has been particularly problematic (Hayes et al., 2001). In a collaborative effort to map and exploit quantitative resistance using molecular tools (reviewed by Hayes et al., 2001), we have used barley germplasm developed by ICARDA/CIMMYT with quantitative resistance (Sandoval-Islas et al., 1998). In the course of this research we have developed qualitative resistance pyramids combining three QTL alleles from two different sources (Castro et al., chapter 3). We were interested in combining these quantitative resistance alleles with a qualitative resistance gene. Our target as a qualitative resistance donor was CI10587 (PI 243183), an accession from the U. S. National Small Grains Collection.

The objectives of this research were to: (i) map, and introgress into an adapted background, the qualitative resistance gene present in CI10587; (ii) validate the estimates of the effects of the qualitative resistance gene and of the three QTL alleles in a new genetic background; and (iii) determine if there were interactions between the qualitative resistance gene and the QTL alleles.

MATERIALS AND METHODS

PLANT MATERIAL

Mapping resistance in CI10587

One-hundred doubled haploid (DH) lines were derived from the F₁ of the cross CI10587 x Galena, using the *Hordeum bulbosum* technique as described by Chen and Hayes (1989). CI10587 (PI 243183) is an accession from the U. S. National Small Grains Collection. It is a two-row, spring habit barley with resistance to stripe rust. Galena is a spring habit, two-row, proprietary malting barley belonging to the Coors Brewing Company. It is susceptible to stripe rust (Toojinda et al., 2000).

Development of resistance gene pyramids

After the phenotyping and genotyping of the CI10587 x Galena population (see below) one DH line (D3-6) was selected and crossed with the variety Baronesse, a high yielding feed barley variety well adapted to the Pacific Northwest. One-hundred doubled haploid lines were derived from the F₁ of this cross, following the procedures already described. Two DH lines (D3-6/B-23 and D3-6/B-61) from this mapping population were selected based on their stripe rust resistance and agronomic trait phenotypes, and crossed with two DH lines from the resistance QTL allele pyramid population described by Castro et al. (Chapter 3). One of these

lines (BCD47) carries stripe rust resistance QTL alleles on chromosomes 4(4H) and 7(5H), while the other (BCD12) carries a stripe rust resistance QTL allele on chromosome 5(1H). Three crosses were made between the quantitative and qualitative resistance sources and three DH populations were obtained from the F₁ of each cross following the procedures described above. Seventy DH lines were derived from the D3-6/B-23 x BCD47 cross (hereafter referred as population BU), seventy-seven DH lines were derived from the D3-6/B-61 x BCD47 cross (hereafter referred as population AJ), and eighty-five DH lines were derived from the D3-6/B-61 x BCD12 cross (hereafter referred as population OP). The germplasm derivation process is shown in Fig. 5.1.

PHENOTYPING

The reaction of CI-10587, Galena and the CI-10587 x Galena population to field inoculum was determined in four tests at the ICARDA/CIMMYT field station located at Toluca, Mexico. The plant material was evaluated in one-row, 3-m plots at two planting dates in 1994 and 1996. Spreader rows, planted at 5.25-m intervals and consisting of a mixture of 15 susceptible genotypes were inoculated twice with infected plants placed in the foliage, and with applications of spores suspended in oil. Infected plants and spores were collected locally. The race composition of this inoculum was not determined. Due to the fact that the stripe rust severity in these lines was either lower than 10% or higher than 80%, their reaction was rated as

resistant and susceptible, on a plot basis. The DH lines from the Baronesse x D3-6 cross were tested at Toluca, Mexico, following the same procedures, in 1997.

BU, AJ, and OP populations were assessed for adult plant resistance in four tests over three years at Toluca, Mexico, following the procedures described above. The DH lines and the parents were planted on one date in 1999 and 2001, and on two dates in 2000. Stripe rust was rated as percentage severity on a plot basis, because a range of disease severities was seen in the germplasm.

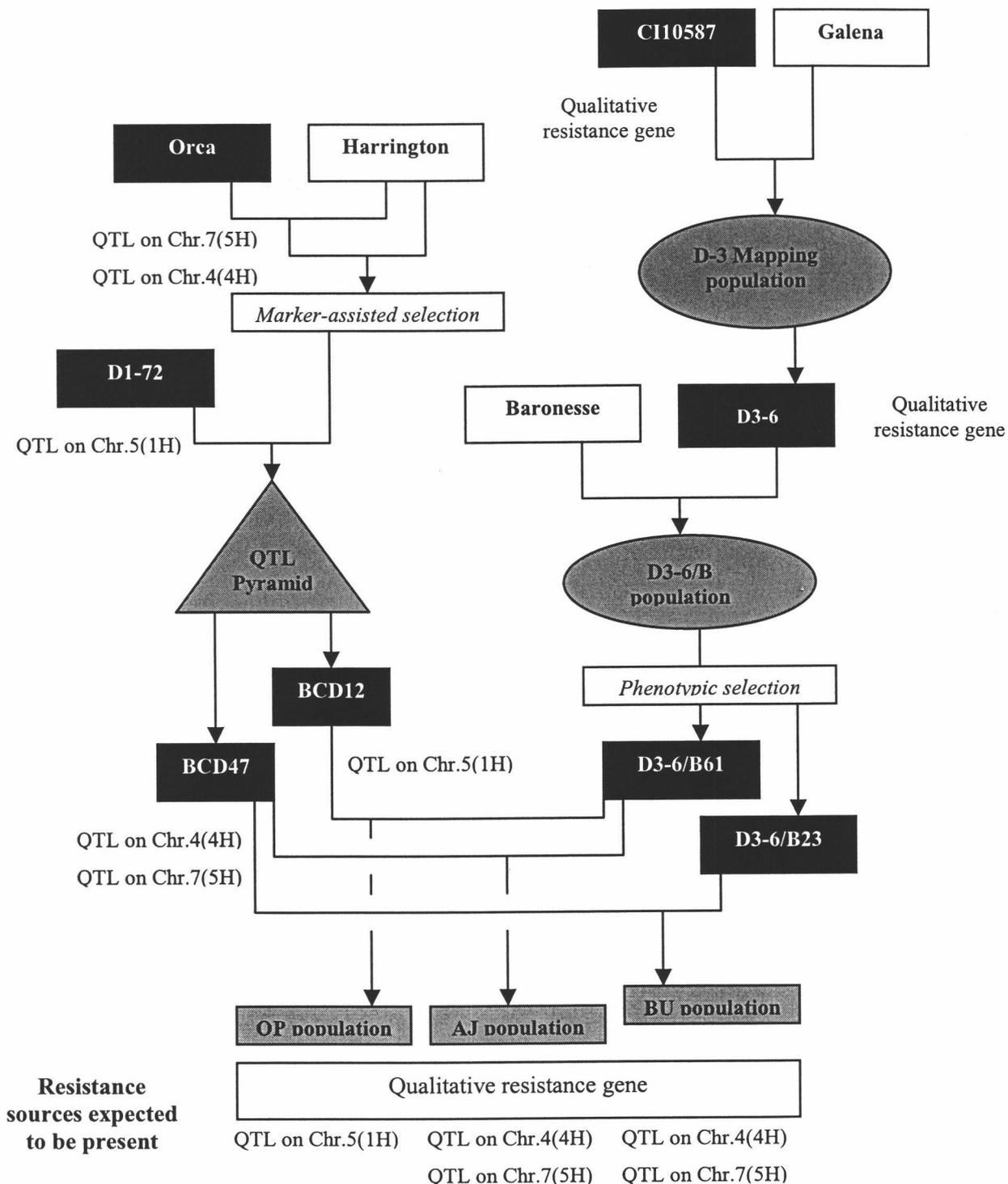


Figure 5.1. Development of the qualitative/quantitative disease resistance populations. Black boxes represent resistance sources and white boxes represent susceptible parents.

GENOTYPING

DNA was extracted from leaf tissue of 2-3 week old plants (one plant per genotype) using the Qiagen DNAeasy 96 Plant Kit. In the CI10587 x Galena population, RFLP markers were first mapped on chromosome 4(4H), 5(1H), and 7(5H) in regions where stripe rust resistance quantitative trait loci (QTL) had been detected in other mapping populations (Chen et al., 1994; Toojinda et al., 2000). When no associations were detected between stripe rust reaction and marker genotypes at these loci, additional RFLPs of known location throughout the genome were used for bulked segregant analysis (BSA) (Michelmore et al., 1991). When a non-random pattern of association of RFLP alleles on the long arm of chromosome 1(7H) with stripe rust reaction was observed, other RFLP loci mapping to this region in other mapping populations were assayed on the 94 DH lines. RFLP markers were assayed as described by Kleinhofs et al. (1993). The RFLP marker nomenclature follows that employed by the North American Barley Genome Mapping Project (Kleinhofs et al., 1993). Sequence tagged site (STS) markers on chromosome 1(7H) were assayed, following the protocols of Mano et al. (1999). SSR markers were added to this map to facilitate alignment with other mapping populations.

The resistance gene pyramid populations (AJ, BU, OP) were genotyped chromosomes using SSR markers of known position on the chromosomes 1(7H), 4(4H), 5(1H) and 7(5H). After screening all available PCR-based markers

mapping to those regions, we were able to map twenty markers in the BU population, twenty-two in the AJ population, and twenty-three in the OP population. SSR were assayed as described by Liu et al. (1996) and Russell et al. (1997). The SSR primer sets were developed and mapped by Ramsay et al. (2000), Liu et al. (1996) and Becker and Heun (1995). The reverse primers were labeled with FAM, TET, NED or HEX fluorescent dyes. DNA amplifications were performed using either a Perkin-Elmer 9600 or MJ Research PTC-100 thermal cyclers. PCR reactions were carried out in a 10 μ l reaction mix containing 37.5 ng of template DNA, 1x PCR buffer, 0.025 units *Taq* DNA polymerase (Qiagen), 0.2 nM dNTPs and 0.1 picomoles of forward and reverse primers. Information on primer sequences, and PCR amplification conditions for each set of primers, are available at <http://www.scri.sari.ac.uk/ssr> (Ramsay et al. 2000), in Liu et al. (1996) and in Becker and Heun (1995). PCR amplified fragments from differentially labeled SSR primers and with non-overlapping fragment sizes were simultaneously analyzed in the same gel lane and separated on an ABI Prism 377 DNA Sequencer at the Oregon State University, Central Service Lab, or on an ABI Prism 3700 DNA Sequencer at OMIC, Inc., Portland, OR. Gene Scan® and Genotyper® Software (Applied Biosystems, Perkin Elmer, Foster City, CA) were used for automated data collection and to determine the allele sizes in base pairs, based on the internal standard.

GENOME MAPPING AND QTL ANALYSIS.

For mapping the gene conferring resistance in CI10587 we directly mapped the trait as a marker in the CI10587 x Galena population. Linkage analyses for all populations were performed using GMendel 3.0 (Holloway and Knapp, 1994) following procedures described by Toojinda et al. (2000).

In the three pyramiding populations (AJ, BU and OP), QTL were mapped using the multi-trait option implemented in MultiQTL version 1.55 (<http://esti.haifa.ac.il/~poptheor/MultiQtl/MultiQtl.htm>). Each dataset was analyzed with 1000 permutations in order to establish the significance of the QTL and a bootstrap simulation (with 1000 samples) was used for the assignment of each significant QTL to a defined marker interval.

Genome regions affecting resistance to stripe rust revealed by the QTL scans were used in performing a QTL analysis analogous to candidate gene analysis, as described by Castro et al. (chapter 2), where the genotypes of the flanking markers are used as independent variables. Recombinant genotypes were not included in the analysis. Therefore, the independent variables had two levels each, with each level corresponding to a parental genotype. The treatment design was a 2 x n factorial, where n is the number of genome regions detected. The difference between parental marker class means estimates the additive effect of the QTL flanked by the markers. Double crossovers between the QTL and marker loci downwardly bias estimates of the effects. Thus, differences between parental marker genotype means

are conservative estimates of the effects of QTL residing in the n chromosomal regions. We performed a joint analysis considering the QTL effects as nested in populations. We also performed the analysis pooling together the AJ and BU populations, as the QTL source was the same (BCD-47). Because the results of this joint analysis were similar to the individual population analyses we present the former. Statistical analyses were performed using the SAS GLM procedure (2001).

RESULTS

MAPPING OF QUALITATIVE RESISTANCE IN CI10587

Across environments, the reactions to stripe rust of the DH lines from the CI10587 x Galena cross were extremely consistent. Based on the reaction data pooled across environments, the ratio of resistant : susceptible scores was 55:45, which fits a 1:1 ratio ($p > 0.30$ from a chi-square test). In a doubled haploid population, the expectation of segregation of alleles at a single locus gives a 1:1 ratio. We systematically tested several RFLP markers of known position through the genome in order to detect associations with the trait. A non-random pattern of association of RFLP alleles on the long arm of chromosome 1 (7H) with stripe rust reaction was observed. Other RFLP loci mapping to this region in other mapping populations were then assayed on the DH lines. We mapped the stripe rust resistance gene between *Ris44* and *ABG461*, and based on known marker positions we located it on the long arm of chromosome 1(7H) (Figure 5.2). In the rest of the paper we will refer to the qualitative resistance gene as BSTR1.

AJ Population OP Population BU Population

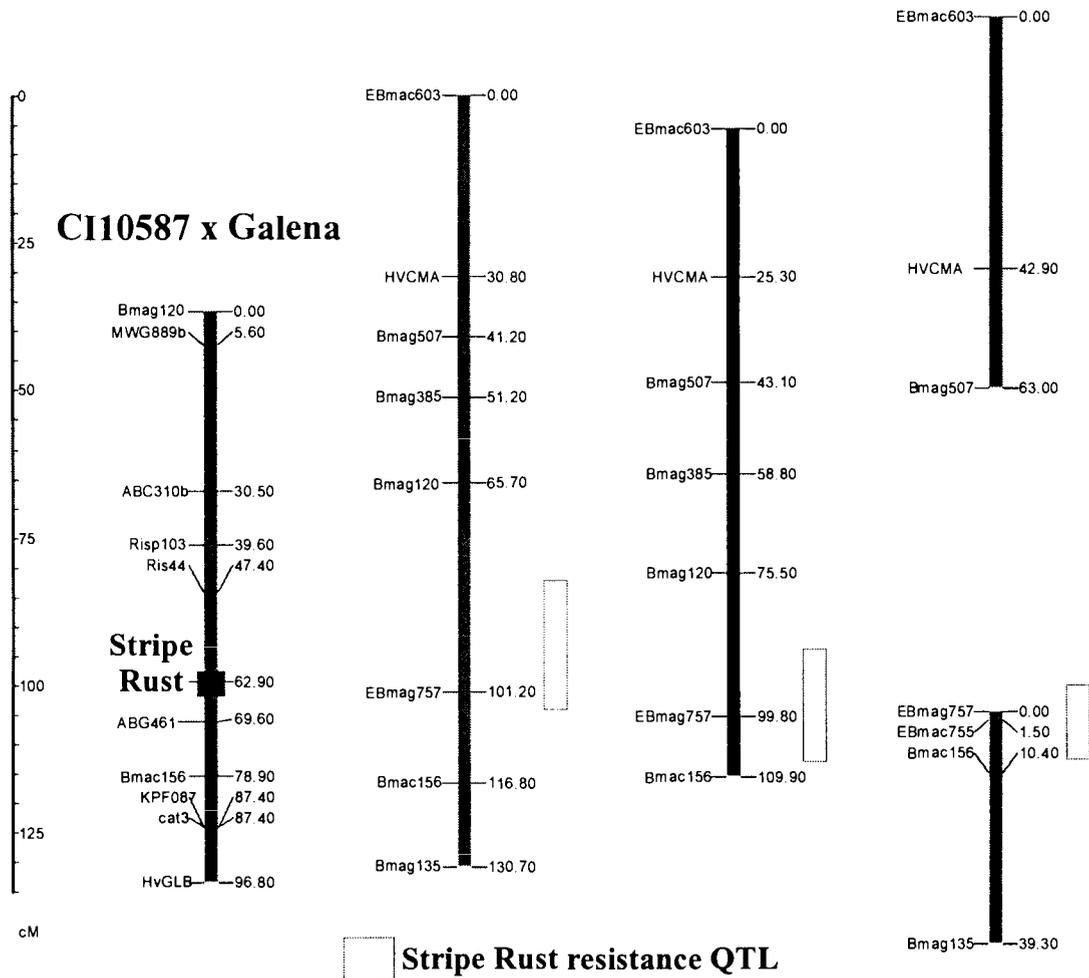


Figure 5.2. Alignment of the chromosome 1(7H) maps of the CI10587 x Galena, AJ, BU and OP populations. The CI10587 x Galena map shows the linkage map location of the stripe rust resistance qualitative gene present in CI10587. The location of the same stripe resistance gene, mapped using QTL analysis procedures in chromosome 1(7H) in each one of the three derived populations are also presented. The gaps in the map of BU population are due to the fact that *Bmag385* and *Bmag120* were monomorphic in this population.

QTL ANALYSIS OF QUALITATIVE AND QUANTITATIVE RESISTANCE PYRAMIDS

The reaction to stripe rust (in terms of susceptible vs. resistant) of the AJ, BU and OP populations was not measured in the field. Rather, in order to confirm the presence of the resistance gene on chromosome 1(7H), we performed QTL analyses of disease severity in the three pyramid populations. This analysis also allowed the estimation of the individual effects of the QTL alleles expected to be present in these populations and their interactions, *inter se* and with BSTR1. As described in the *Materials and Methods*, the genotyping of these populations was restricted to markers on chromosomes 1(7H), 4(4H), 5(1H) and 7(5H).

For the three populations, a significant QTL was detected in chromosome 1(7H) (Table 5.1), in approximately the same region where BSTR1 was expected to map (Figure 5.2). The resistance alleles corresponded to D3-6/B23 (in BU) and D3-6/B61 (in AJ and OP). We considered that BSTR1 is the candidate gene for this QTL. No other QTL was detected in the AJ and BU populations. In the OP population an additional QTL with a smaller effect was detected on chromosome 5(1H). It was located in the expected QTL5 position (Fig. 5.3), since this population was expected to segregate for QTL alleles at this location. No QTL was detected in any population at the QTL4 and QTL7 positions.

Table 5.1. Summary of barley stripe rust severity QTL, corresponding LOD scores and significance level (the QTL location is presented in Fig. 5.2 and Fig. 5.3).

Population	QTL/Gene	Resistance allele	LOD Score	p-value
AJ	BSTR-1	D3-6/B23	6.92	<0.0001
BU	BSTR-1	D3-6/B23	11.95	<0.0001
OP	BSTR-1	D3-6/B61	6.73	0.0009
	QTL5	BCD12	3.41	0.0136

Considering that there could be a masking effect of BSTR1 on the effect of other resistance factors, we performed a second QTL analysis using the subset of lines with susceptible alleles at BSTR1 in each population. The small sizes of the subpopulations (32 in AJ, 36 in BU, 37 in OP) limited the power of the QTL analysis, so we considered any QTL detected as a candidate QTL in the individual analyses. Only if the ANOVA in the complete population confirmed the QTL was it accepted as significant.

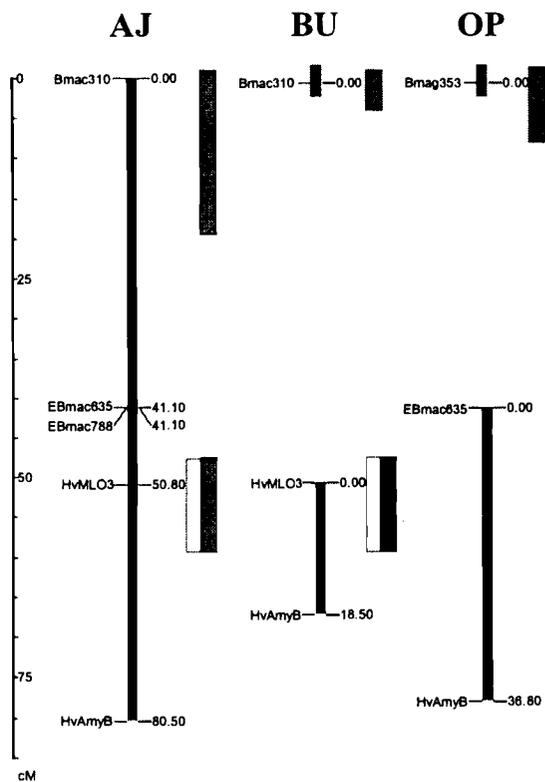
In this analysis, we located stripe rust resistance effects in the QTL4 region in the BU and AJ populations (Fig. 5.3), as well as on the QTL5 region in the OP population. Another QTL candidate was mapped on chromosome 4(4H) in the AJ and BU population. This QTL (hereinafter referred to as QTL4B) was located close to *Bmac310*, more than 40 cM proximal to QTL4. A third QTL candidate was detected in the OP population linked to *Bmag353*, also on chromosome 4(4H). The reported position of *Bmac310* and *Bmag353* is similar (Ramsay et al., 2000). The resistance alleles at these QTL candidates traced to BCD47 (in AJ and BU) and BCD12 (in OP). As the origin was not the same, we will refer to the QTL in OP as QTL4C to distinguish it from QTL4B. A complete map of BCD12 and BCD47 is not available, so it is not possible to determine if these QTL resistance alleles are identical by descent, although it is reasonable to expect so, since we have detected no resistance alleles in CI10587 or Baronesse in that region.

We used all of the QTL candidates from the original mapping populations in a candidate gene analysis, including QTL7. Although we failed to detect QTL effects in that region in any of the populations, this QTL was present in the parental line BCD47 and was considered in the development of these populations. Considering that the same QTL were detected in AJ and BU, and the common origin of these QTL alleles (BCD47), the AJ and BU populations were pooled for a subsequent analysis. The ANOVA results (Table 5.2) confirmed the effects of BSTR1, QTL4, QTL4B, QTL4C and QTL5, and the lack of significance for the QTL7 main effect. The qualitative nature of BSTR1 effect is reflected in the significant interactions with the different QTL. As seen in Figures 5.4 and 5.5, the different QTL alleles show their effects only in the absence of the resistance alleles at BSTR1. The disease severity of lines with resistance alleles at BSTR1 in all the populations was significantly lower than all the other lines. In the absence of BSTR1 resistance alleles, the presence of resistance alleles at QTL4 and QTL4B in AJ and BU, and at QTL5 and QTL4C in OP, significantly decreased disease severity. The significant second and third order interactions (Table 1), except the interactions involving BSTR1, were magnitude interactions that do not lead to changes in estimates of individual effects.

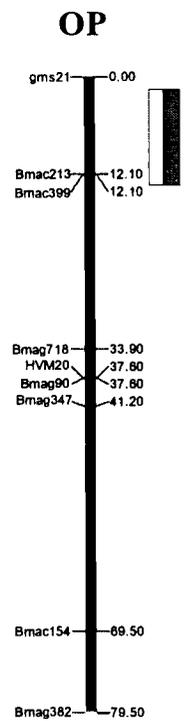
Table 5.2. ANOVA table of the candidate gene analysis for stripe rust resistance. Only QTL main effects and significant QTL x QTL interactions are presented.

	df.	SS	M.S.	F value	Pr>F
Environment	3	33421	11140	37.20	<0.0001
Population	1	4328	4328	14.45	0.0002
BSTR1	1	229276	229276	765.65	<0.0001
QTL4 (AJ/BU)	1	12728	12728	42.50	<0.0001
QTL4B (AJ/BU)	1	1057	1057	3.53	0.0607
QTL7 (AJ/BU)	1	141	141	0.47	0.4920
QTL5 (OP)	1	1508	1508	5.03	0.0251
QTL4C (OP)	1	1820	1820	6.08	0.0139
BSTR1 x QTL4 (AJ/BU)	1	8251	8251	27.55	<0.0001
BSTR1 x QTL4B (AJ/BU)	1	1599	1599	5.34	0.0211
QTL4 x QTL4B (AJ/BU)	1	3593	3593	12.00	0.0006
QTL4 x QTL7 (AJ/BU)	1	2840	2840	9.48	0.0022
BSTR1 x QTL4C (OP)	1	5539	5539	18.50	<0.0001
BSTR1 x QTL4 x QTL4B (A/B)	1	3434	3434	11.47	0.0007
BSTR1 x QTL4 x QTL7 (A/B)	1	2959	2959	9.88	0.0017
QTL4 x QTL4 X QTL7 (A/B)	1	1577	1577	5.27	0.0220
Error	719	214109	299		
	r^2	0.609			

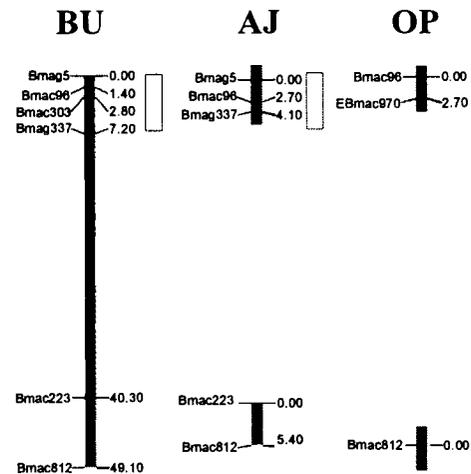
Chromosome 4(4H)



Chromosome 5(1H)



Chromosome 7(5H)



Expected QTL location

Observed QTL

Figure 5.3. Alignment of the maps of chromosomes 4(4H), 5(1H) and 7(5H) maps of AJ, BU and OP populations, showing the expected and actual locations of stripe rust resistance QTL.

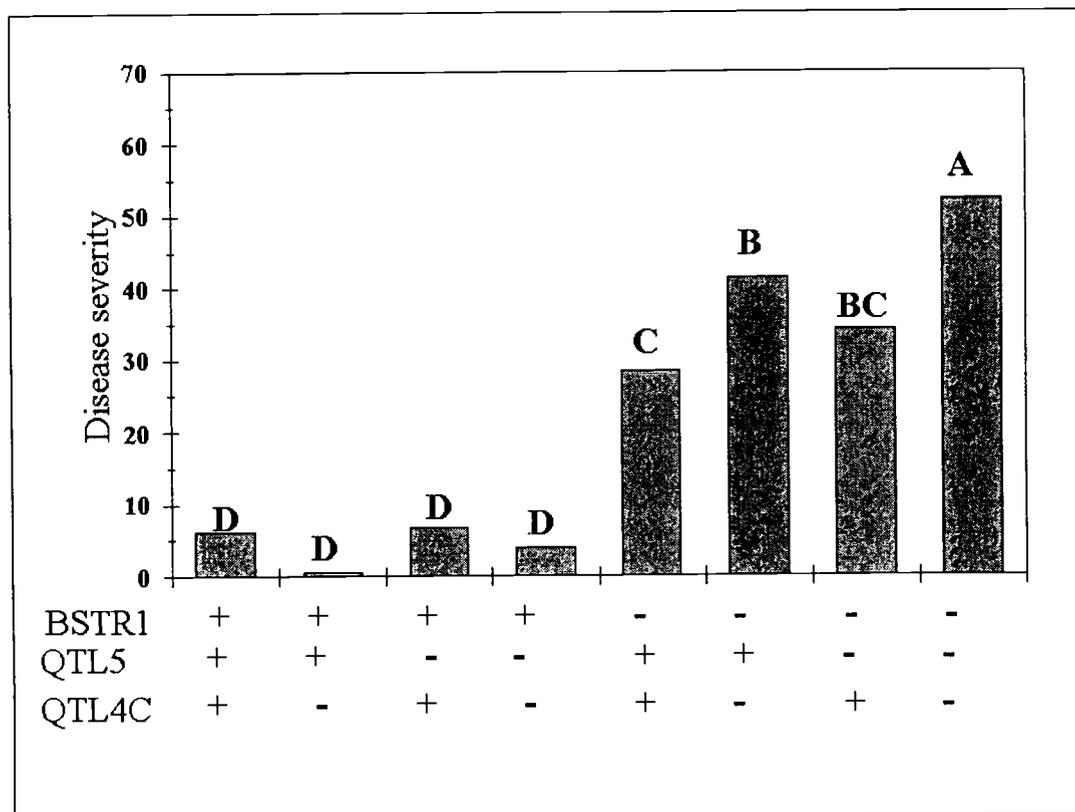


Figure 5.4. Least squares means of disease severity in DH lines of the OP population classified according to the presence or absence of the resistance alleles at BSTR1, QTL5 and QTL4C QTL regions. Bars with the same letter are not significantly different ($p < 0.05$) based on pairwise comparisons.

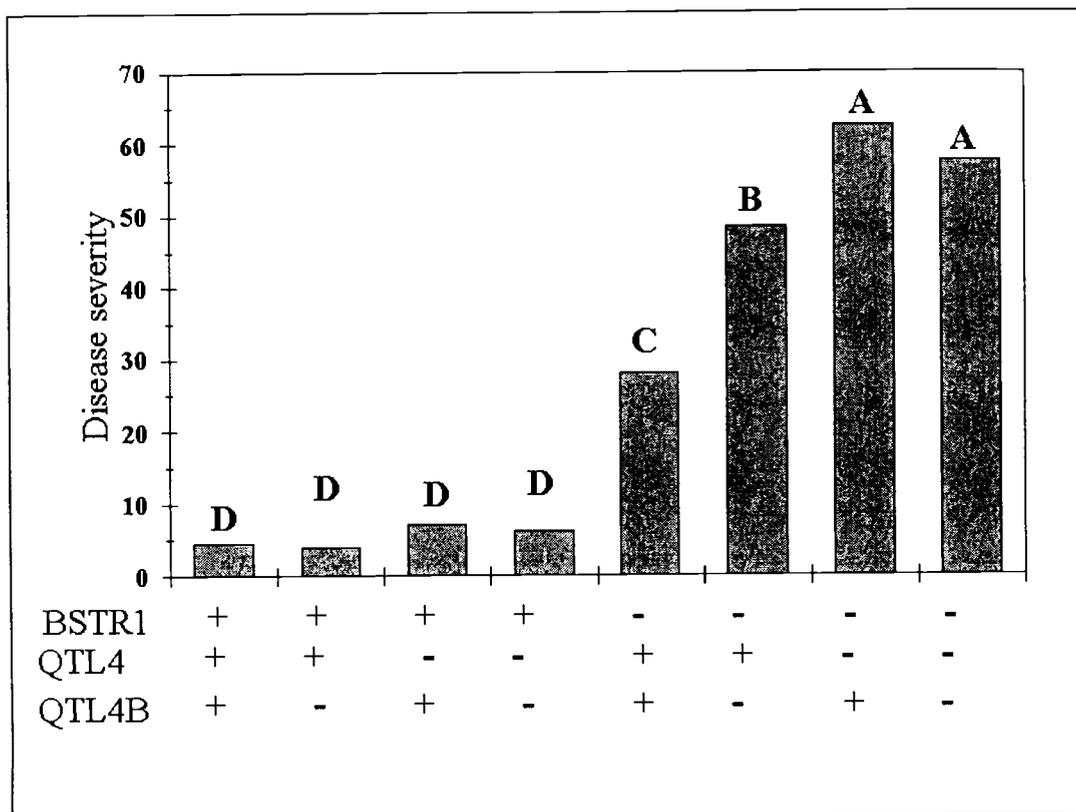


Figure 5.5. Least squares means of disease severity in DH lines of the AJ and BU populations classified according to the presence or absence of the resistance alleles at BSTR1, QTL5 and QTL4B QTL regions. Bars with the same letter are not significantly different ($p < 0.05$) based on pairwise comparisons.

DISCUSSION

We have mapped a gene conferring qualitative resistance to BSR on chromosome 1(7H), and validated its location in three mapping populations segregating for that source of resistance. The only other report of BSR resistance in that region was the one from Thomas et al. (1995), who mapped a BSR resistance QTL. The only BSR qualitative resistance gene mapped before is *Yr4*, which mapped to chromosome 5(1H) (von Wettstein-Knowles, 1992). BSTR1 conferred resistance to races PSH-13, PSH-14, PSH-21, and PSH-31 (R. Line, X. Chen, pers. com.). Further studies are necessary in order to establish the race specificity of BSTR1 and its identity regarding other BSR qualitative resistance genes.

Combining qualitative and quantitative resistance within the same genotype presents a serious challenge to the plant breeder. The complete effectiveness of the qualitative resistance gene acts as a masking factor, precluding phenotypic selection for quantitative resistance genes. The use of molecular marker-assisted selection (MAS) is a solution. However, there are no reports in the literature of interaction between quantitative resistance and qualitative resistance genes. The results of these experiments indicate that there is no negative effect of pyramiding qualitative and quantitative resistance genes. However, there is no way to measure the effectiveness of a qualitative/quantitative resistance gene pyramid except in the presence of a race that is virulent to BSTR1. Currently this virulence pattern is, fortunately, not available. Our results allow us to be cautiously optimistic about the

perspectives of combining qualitative and quantitative resistance in the same genotype. It is clear from Figures 5.4 and 5.5 that the QTL alleles on chromosomes 4(4H) and 5(1H) were indeed effective in lowering disease severity in the absence of the resistance alleles on BSTR1.

The results of QTL analyses of the pyramid populations (AJ, BU and OPS) validate the map location of BSTR1 and also confirm the QTL allele effects reported by Castro et al. (Chapter 3). Of particular interest is the lack of significance of QTL7. The original mapping population estimates of QTL4 and QTL7 (Chen et al., 1994) revealed QTL7 as having a much larger effect than QTL4, but Castro et al. (Chapter 3) have inverted the importance of these QTL alleles based on the estimates of QTL effects in a MAS-derived population. The AJ and BU populations represent a second generation of QTL alleles from the original mapping population and confirmed that these "realized heritability" estimates of QTL effect are indeed more robust estimates of allele value than those obtained in the original mapping population.

QTL effect estimates are reported to be biased, raising concerns about their utility to understand and manipulate genetic determinants of quantitative characters (Beavis, 1998). We have found that QTL effect estimates based on derived populations provide better estimates of QTL effects than source mapping populations.

The detection of a new QTL on chromosome 4(4H) is intriguing, and it helps to explain some of the peculiarities reported regarding stripe resistance QTL effects in this region of the genome. In the original QTL report (Chen et al., 1994), QTL4 had an effect on disease severity in only one environment, and its significance threshold spanned over more than 40cM. Castro et al. (2002) showed that there were non-coincidental seedling resistance and heading date QTL in the same region and hypothesized that the adult plant resistance QTL detected in the source mapping population was actually divisible into two QTL, one coincidental with a seedling resistance QTL, and the other linked to a heading date QTL. Castro et al. (Chapters 3 and 4) validated this hypothesis, with particular reference to the coincidental adult plant and seedling resistance QTL.

The results of the current experiments suggest the presence of another QTL (QTL4B and QTL4C) linked with QTL4. There is evidence that resistance genes in plants occur in clusters (Michelmore, 1995; Kanazin et al., 1996; Ellis et al., 1998) and QTL mapping tools are known to provide poor resolution in the case of linked QTL (Lynch and Walsh, 1997). If the putative multiple QTL could be traced to the same original source, this could explain the poor resolution experienced in determining the precise location of the genetic determinants of stripe rust resistance on chromosome 4(4H). Unfortunately, during the derivation of BCD12 and BCD47, these regions were not genotyped. QTL analysis in a new, larger population of 422 DH lines derived from a cross with BCD47 is under way and

probably will clarify the situation regarding the number of QTL on chromosome 4(4H). Preliminary results (Vales et al., 2002) have shown a significant QTL spanning through QTL4 and QTL4B/C regions.

Our results provide evidence in support of the utility of molecular markers and QTL analysis for understanding and manipulating genes determining qualitative and quantitative resistance to barley stripe rust. We mapped one qualitative resistance gene (BSTR1) and validated its importance, as well as the importance of two QTL (QTL4 and QTL5) that determine quantitative resistance to barley stripe rust in a new genetic background. We also showed that, in the population under study, QTL estimates effects based on derived populations provided a more accurate estimation than estimates obtained from the original mapping populations. We also demonstrated that in the case of these genes, QTL x environment interaction was not important. Thus, our results suggest that molecular markers and QTL analysis can help to identify and manipulate the underlying genetics of an economically important trait.

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CONCLUSIONS

The main topics of this research have been the genetics of resistance to barley stripe rust, the use of molecular techniques, in particular QTL analysis tools to understand it, and, in a broader perspective, the validity of the use of such techniques to dissect and understand quantitatively inherited traits. The information provided by the present study represents a contribution to the advance in the understanding of those issues.

This study has validated the expression of QTL in different genetic backgrounds. Also it has validated that these effects are mostly additive and show no QTL x environment interaction. Their expression was also validated in the presence of a qualitative resistance gene, as shown in Chapter 5. This validation expands to seedling resistance QTL, which had shown complementary action and present a different kind of challenge for their analysis. Results presented in Chapters 2 and validated in Chapter 4 showed that binomially distributed seedling resistance to stripe rust can be analyzed using the combination of QTL mapping tools and logistic regression analysis. They also confirm that regardless of the need of the presence of resistance alleles at two QTL in order to recover the resistant phenotype at the seedling stage, their effects are basically additive in the fact that a QTL from one population can substitute the QTL missing from another population.

The genetics of quantitative traits has remained elusive. Initial QTL analysis lent support to the hypothesis that relatively few genetic factors were the principal determinants of complex traits. However, QTL effect estimation has been considered biased (Beavis, 1998), and the small population sizes used for QTL detection have led to overestimation of QTL effects and underestimation of QTL number and interaction (Melchinger et al., 1998; Utz et al., 1999). Bernardo (2001) even showed that in the hypothetical situation of knowing all the genes affecting a complex trait, that information would not only fail to improve the selection efficiency but rather it would be detrimental. One important exception was however mentioned: the situation in which a limited number of genes explained the complex trait. In that situation, the knowledge of the genes affecting the trait could improve the selection efficiency. That is, according to the presented information, the situation for stripe rust resistance in the populations we studied. As shown in Table 3.2, three QTL explained most of the genetic variance of the trait. The results from Chapters 3 and 5 support the concept that, at least when a limited number of genetic factors control the trait, QTL can indeed be helpful in understanding the genetics of a complex trait. The validation of the expression of QTL in different genetic backgrounds, the additivity of those effects, and the lack of importance of QTL x environment interaction (Chapters 3 and 5) provide conclusive evidence of the utility of QTL analysis in those conditions.

Regarding the bias of QTL effect estimation, Chapter 3 showed that indeed this is the case, but also that the bias was not necessarily downward. Chapter 5 provides the opportunity to compare two possible estimates of the effects of the same QTL: the ones obtained from the original mapping populations and the ones obtained from the population into which those QTL were introgressed. The results showed, beyond the limitations of genome coverage, that the estimates from the derived populations were more accurate than the original ones. If we considered them as a kind of progeny test of QTL effects, the results confirm what classical quantitative genetics had known for decades, - that a progeny test provides a much more precise estimation of the breeding value of a gene. The implications and derivations from this concept are beyond of the scope of this study.

In conclusion, this study has provided significant information regarding the genetics of barley stripe rust resistance, and the use of QTL analysis tools in understanding and exploiting quantitatively inherited traits. We still have a very limited knowledge about the specifics of the processes affected by each stripe rust resistance QTL, which remain as “black boxes” in that regard. Nevertheless, the study has demonstrated that this limitation did not preclude the achievement of selection responses.

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