

# Coincident QTL Which Determine Seedling and Adult Plant Resistance to Stripe Rust in Barley

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## ABSTRACT

Barley stripe rust (caused by *Puccinia striiformis* Westend. 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 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. Quantitative trait loci (QTL) effects and significance were estimated by means of 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 × 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.

BARLEY STRIPE RUST is an important disease of barley. 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 USA, BSR was first observed in Texas in 1991 (Marshall and Sutton, 1995). By 1995, the disease was reported throughout the western USA. 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 USA 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 1960s. Consid-

erable variation has since been reported in pathogen isolates collected in the USA (Chen et al., 1995; Marshall and Sutton, 1995; Roelfs and Huerta-Espino, 1994). Chen et al. (1995), on the basis of 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 USA 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 nonrace specific and expressed only at the adult plant stage (Milus and Line, 1986a,b). 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 USA. 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* cor-

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**Abbreviations:** BSR, barley stripe rust; cM, centimorgan; DH, doubled haploid; QTL, quantitative trait loci; sCIM, composite interval mapping.

responding 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-yr period may be grounds for describing it as having durable resistance. Accordingly, one approach to develop resistant varieties for the U.S. Pacific Northwest would be to introgress quantitative resistance genes from the unadapted ICARDA/CIMMYT germplasm.

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 cultivar 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, on the basis of 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, b). 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 on the basis of the reaction of seedlings to inoculation with defined isolates under controlled environmental 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 cultivar 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 resis-

tance 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  $F_1$ -derived doubled haploid (DH) lines were produced from the cross of Shyri  $\times$  Galena as described by Toojinda et al. (2000), using the *Hordeum bulbosum* L. 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 DH 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 d after inoculation on the basis of a 0-to-9 scale (0: complete resistance; 9: complete susceptibility) as described by Line et al. (1974).

### 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 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 by means of the interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures of MQTL (Tinker and Mather, 1995), regression procedures, and the single and multivariate options implemented in MultiQTL 1.55 <http://esti.haifa.ac.il/~poptheor/MultiQtl/MultiQtl.htm>; verified April 30, 2002).

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 centimorgan (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 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 to test for QTL  $\times$  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  $2 \times n$  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 by 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  $\mu$  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

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

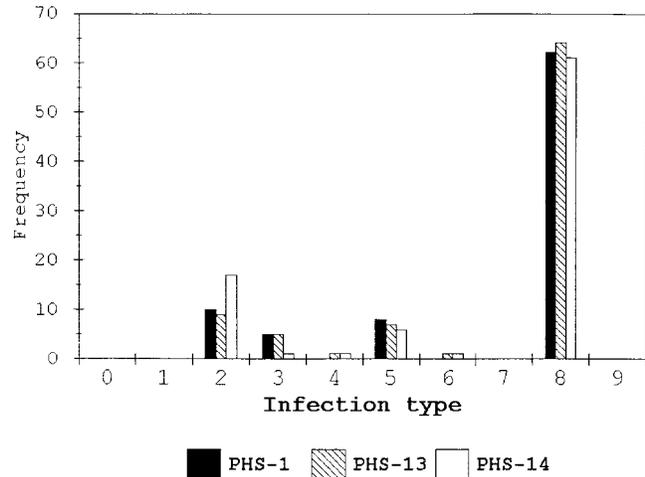
and  $[\mu(1 - \mu)]/n$ , respectively. We performed statistical analyses using the SAS (2001) GENMOD procedure. Parameters and test statistics were estimated by a Type III analysis (analogous to partial sums of squares analyses of general linear models). We performed separate analyses of the effects 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 by means of 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  $\times$  QTL interaction.

**RESULTS**

**Seedling Resistance–Susceptibility Phenotypic Data**

When the Shyri  $\times$  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 (Fig. 1) fit a 3:1 (susceptible:resistant) ratio (Table 1). In a DH 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 1), the DH lines in each group were not always the same (Table 2). This could be due to misclassification of the resistance phenotype, or, as elaborated upon in the *Discussion*, to linked, race-specific resistance genes.



**Fig. 1. Phenotypic distribution of infection type in the Shyri  $\times$  Galena DH mapping population when inoculated at the seedling stage with three North American races of *Puccinia striiformis* f. sp. *hordei*.**

**Mapping Resistance Genes**

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) by all QTL analysis procedures. These QTL will be referred to as QTL5 and QTL6 in the remainder of this manuscript (Table 3). No significant QTL  $\times$  race interaction was detected.

Using MultiQTL, we detected two significant QTL at the previously mentioned positions on chromosomes

**Table 1. Numbers of resistant and susceptible doubled haploid lines in the Shyri  $\times$  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	<i>P</i>
	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. Numbers of resistant and susceptible doubled haploid lines in the Shyri  $\times$  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  $\leq 5$ ; susceptible score  $\geq 6$ ).**

Race			No. of DH lines
PSH-1	PSH-13	PSH-14	
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

**Table 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 multitrait procedure of MultiQTL.**

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

**Table 4. Numbers of resistant (R) and susceptible (S) doubled haploid lines in the Shyri × 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

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 3). In both cases, the alleles associated with the higher level of resistance came from Shyri (Table 4).

The locations of the seedling resistance QTL on the Shyri × Galena map are presented in Fig. 2, on the basis of the QTL peaks detected by 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) (Fig. 3). Because of the presence

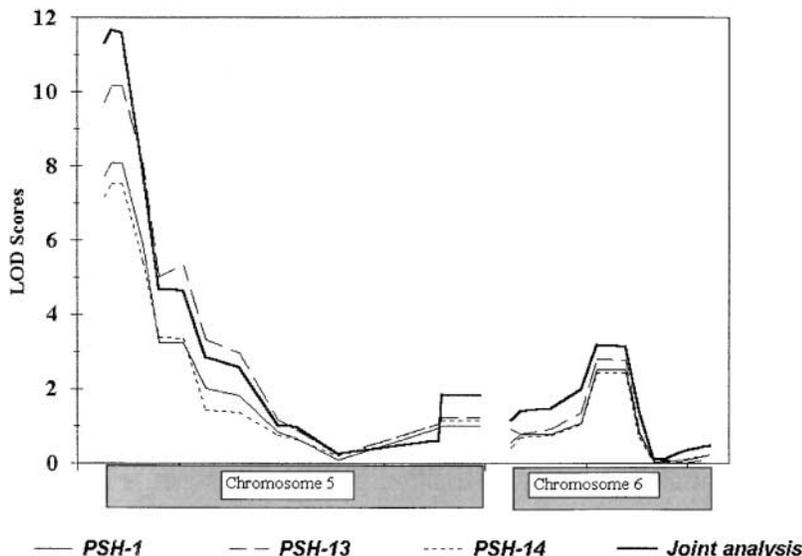
of crossovers in the QTL5 and QTL6 regions nine DH lines (seven for QTL5 and two for QTL6) were not included in the subsequent analyses.

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 5). No significant QTL × race interaction was detected in the joint analysis. No QTL5 × QTL6 interaction was detected in any of the analyses. As shown in Fig. 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.

### 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 with the present level of resolution, however, 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.,



**Fig. 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 × Galena population.**

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 × Galena population, the stripe rust resistance phenotype at the adult plant stage, under field conditions, is quantitative while the resistant phe-

notype 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-yr period and because this cultivar 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.

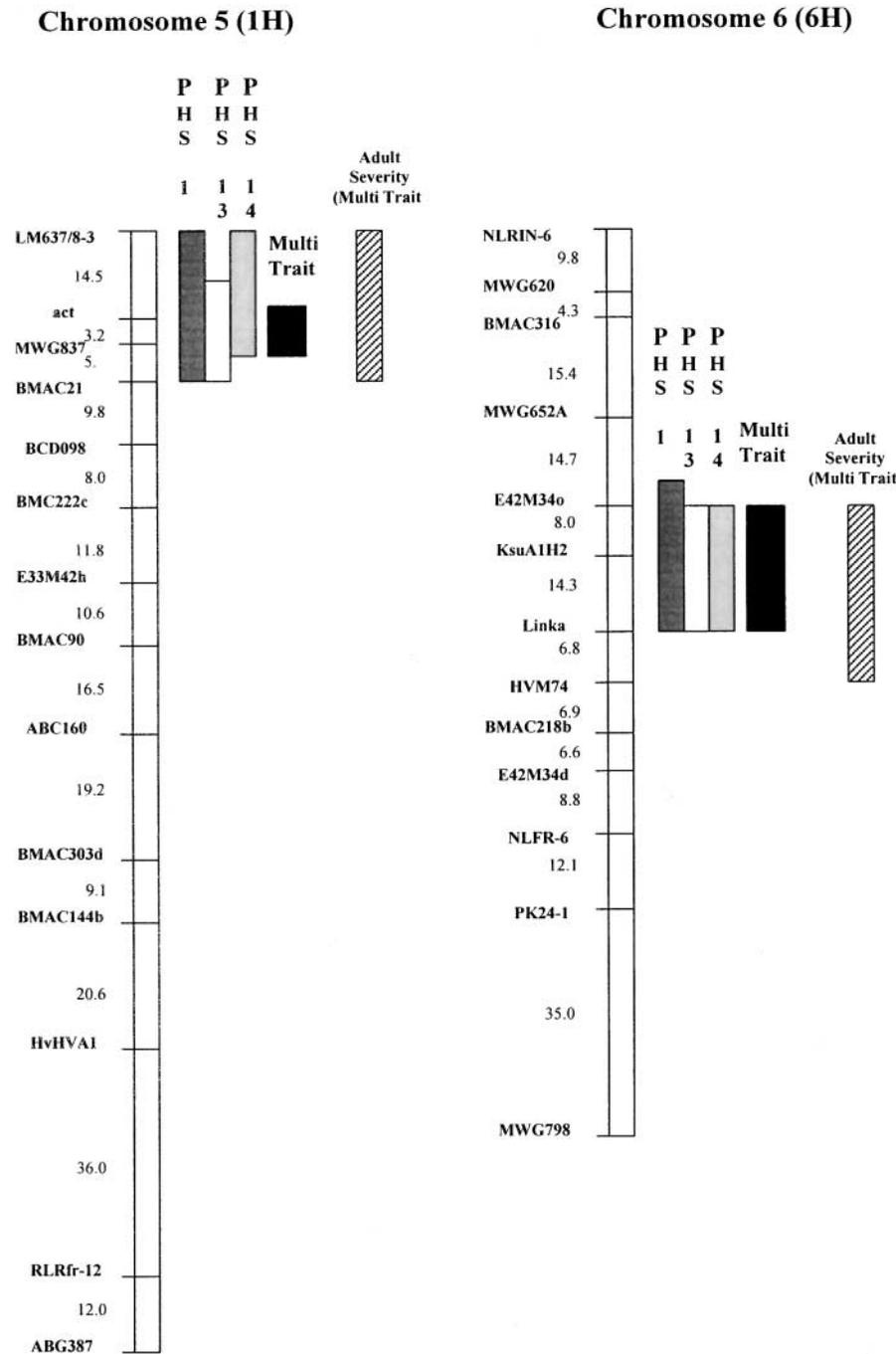


Fig. 3. Linkage maps of chromosomes 5(1H) and 6(6H) of the Shyri × Galena population based on Toojinda et al.(2000), showing seedling resistance QTL to each of the three isolates (PHS-1, PHS-13, PHS-14), the combined seedling reaction data (multi trait) and the adult-plant resistance QTL reported by 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.

**Table 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 by means of asymptotic chi-square distributions).**

	Effect	$\chi^2$ statistic	<i>P</i> < $\chi^2$
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

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 × race interaction could be interpreted as evidence for non-race specificity of the seedling resistance QTL. However, this interpretation cannot account for the DH lines that were resistant to only one or two of the three

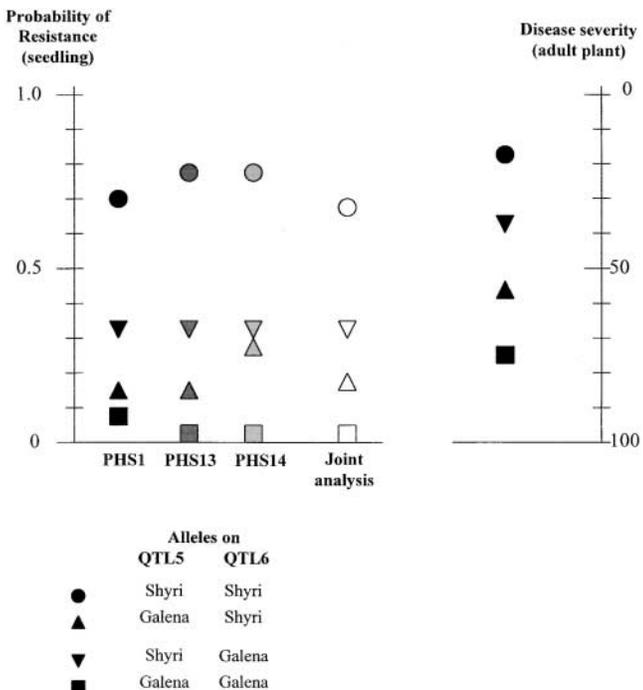
isolates (Table 2) or the lack of significance of the QTL6 effect for PSH-1, and it does not support the definition of adult plant quantitative resistance, *sensu* Chen and Line (1995) and Sandoval-Islas et al. (1998). Shyri was not among the ICARDA/CIMMYT accessions studied by the latter authors.

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 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 (Fig. 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 × 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,



**Fig. 4. Least square means of the probability of occurrence of the resistant 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 square means of adult plant disease severity for the same individuals in four environments (right vertical axis) (Marquez-Cedillo et al., 2001).**

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 which 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% (Fig. 4).

Regarding inoculum load, Luke et al. (1972) reported "threshold-related" late resistance to crown rust (caused by *Puccinia coronata* Corda) in oat (*Avena sativa* L.), 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), 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., 2002b). 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 it is hoped will prove useful as a model for developing cultivars with durable resistance and for integrating molecular and epidemiological approaches to understanding plant disease resistance.

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#### REFERENCES

- Backes, G., A. Granner, B. Foroughi-Wehr, G. Fischbeck, G. Wenzel, and A. Jahoor. 1995. Localization of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 90:294–302.
- Berger, R.D. 1977. Application of epidemiological principles to achieve plant disease control. *Annu. Rev. Phytopathol.* 15:165–184.
- Calhoun, D.S., S. Arhana, and H.E. Vivar. 1988. Chemical control of barley stripe rust, a new disease for North America. *Barley Newsl.* 32:109–112.
- Castro, A., A. Corey, T. Filichkin T, P.M. Hayes, J.S. Sandoval-Islas, and H.E. Vivar. 2000. Stripe rust resistance QTL pyramids in barley. Volume II: 86–88. *In* S. Logue (ed.) *Proceedings of the International Barley Genetics Symposium, VIII, Adelaide, South Australia.* 22–27 Oct. 2000 Adelaide Univ., Glen Osmond, South Australia.
- Castro, A., P.M. Hayes, T. Filichkin, and C. Rossi. 2002a. Update of barley stripe rust resistance QTL in the Calicutima-sib × Bowman mapping population. *Barley Genetics Newsl.* (in press).
- Castro, A., X. Chen, A. Corey, T. Filichkin, P.M. Hayes, M. Johnson, S. Sandoval-Islas, and H. Vivar. 2002b. Stripe rust resistance QTL pyramids in barley. Abstracts, p. 179. *In* Plant and Animal Genome X, San Diego. 12–16 Jan. 2002. see also <http://www.intl-pag.org/10/> (verified April 19, 2002).
- Chen, F.Q., and P.M. Hayes. 1989. A comparison of *Hordeum bulbosum* - mediated haploid production efficiency in barley using *in vitro* floret and tiller culture. *Theor. Appl. Genet.* 77:701–704.
- Chen, F.Q., D. Prehn, P.M. Hayes, D. Mulrone, A. Corey, and H.E. Vivar. 1994. Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). *Theor. Appl. Genet.* 88:215–219.
- Chen, X.M., and R.F. Line. 1992. Inheritance of stripe rust resistance in wheat cultivars used to differentiate races of *Puccinia striiformis*. *Phytopathology* 82:633–637.
- Chen, X.M., and R.F. Line. 1995. Gene action in wheat cultivars for durable, high temperature, adult-plant resistance and interaction with race-specific, seedling resistance to *Puccinia striiformis*. *Phytopathology* 85:633–637.
- Chen, X.M., and R.F. Line. 1999. Recessive genes for resistance to *Puccinia striiformis* f. sp. *hordei* in barley. *Phytopathology* 89:226–232.
- Chen, X.M., R.F. Line, and H. Leung. 1995. Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. *Phytopathology* 85:1335–1342.
- Chen, X.M., R.F. Line, and H. Leung. 1998. Resistance gene analogs associated with a barley locus for resistance to stripe rust. Abstracts, p. 124. *In* Plant and Animal Genome X, San Diego. 12–16 Jan. 2002. see also <http://www.intl-pag.org/10/> (verified April 19, 2002).
- Dubin, H.J., and R.W. Stubbs. 1985. Epidemic spread of barley stripe rust in South America. *Plant Dis.* 70:141–144.
- Ellis, J.G., G.J. Lawrence, W.K. Peacock, and A.J. Pryor. 1998. Approaches to cloning plant genes conferring resistance to fungal pathogens. *Annu. Rev. Phytopathol.* 26:245–263.
- Flor, H.H. 1946. Genetics of pathogenicity in *Melampsora lini*. *J. Agric. Res.* 73:335–357.
- Hayes, P.M., D. Prehn, H.E. Vivar, T. Blake, A. Comeau, I. Henry, M. Johnston, B. Jones, and B. Steffenson. 1996. Multiple disease

- resistance loci and their relationship to agronomic and quality loci in a spring barley population. *J. Agric. Genomics* 2. ([www.ncgr.org/jag/](http://www.ncgr.org/jag/); verified April 19, 2002)
- Hayes, P.M., A. Castro, A. Corey, T. Fillichkin, M. Johnson, C. Rossi, S. Sandoval, I. Vales, H.E. Vivar, and J. Von Zitzewitz. 2001. Collaborative stripe rust resistance gene mapping and deployment efforts. p. 47–60. *In* H.E. Vivar and A. McNab (ed.) *Breeding barley in the new millennium: Proceedings of an international symposium*. Mexico, DF. CIMMYT.
- Hayes, P.M., A. Castro, L. Marquez-Cedillo, A. Corey, A. C. Henson, B. Jones, J. Kling, D.E. Mather, I. Matus, C. Rossi, and K. Sato. 2000. A summary of published barley QTL reports. <http://www.css.orst.edu/barley/nabgmp/qtlsum.htm> (verified April 19, 2002).
- Kanazin, V., L.F. Marex, and R.C. Shoemaker. 1996. Resistance gene analogs are conserved and clustered in soybean. *Proc. Natl. Acad. Sci. (USA)* 93:11746–11750.
- Knott, D.R., and R.G. Anderson. 1956. The inheritance of rust resistance. I. The inheritance of stem rust resistance in ten varieties of common wheat. *Can. J. Agric. Sci.* 36:174–195.
- Knzel, G., L. Korzun, and A. Meister. 2000. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* 154: 397–412.
- Line, R.F., and A. Qayoum. 1991. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968–87. p. 44. USDA-ARS, Tech. Bull. 1788.
- Line, R.F., C.F. Konzak, and R.E. Allan. 1974. Evaluating resistance to *Puccinia striiformis*. Induced mutations for disease resistance in crop plants. *Int. Atom. Energy Agency* 180:125–132.
- Luke, H.H., W.H. Chapman, and R.D. Barnett. 1972. Horizontal resistance of Red Rustproof oats to crown rust. *Phytopathology* 62:1246–1248.
- Marquez-Cedillo, L., T. Toojinda, and P.M. Hayes. 2001. The Shyri × Galena mapping population. <http://wheat.pw.usda.gov/ggpages/SxG/> (verified April 19, 2002).
- Marshall, D., and R.L. Sutton. 1995. Epidemiology of stripe rust, virulence of *Puccinia striiformis* f. sp. *hordei*, and yield loss in barley. *Plant Dis.* 79:732–737.
- McCullagh, P., and J.A. Nelder. 1989. *Generalized linear models*. Chapman and Hall, London.
- McIntosh, R.A., and C.R. Wellings. 1986. Wheat rust resistance – the continuing challenge. *Austr. J. Plant Pathol.* 15:1–8.
- Michelmore, R. 1995. Molecular approaches to manipulation of disease resistance genes. *Annu. Rev. Phytopathol.* 15:393–427.
- Milus, E.A., and R.F. Line. 1986a. Number of genes controlling high-temperature, adult-plant resistance to stripe rust in wheat. *Phytopathology* 76:93–96.
- Milus, E.A., and R.F. Line. 1986b. Gene action for inheritance of durable, high-temperature, adult-plant resistance to stripe rust in wheat. *Phytopathology* 76:435–441.
- Nelder, J.A., and R.W.M. Wedderburn. 1972. Generalized linear models. *J. Roy. Statist. Soc.* 135:370–384.
- Qi, X., R.E. Nicks, P. Stam, and P. Lindhout. 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. *Theor. Appl. Genet.* 96:1205–12135.
- Roelfs, A.P., and J. Huerta-Espino. 1994. Seedling resistance in *Hordeum* to barley stripe rust from Texas. *Plant Dis.* 78:1046–1049.
- SAS. 2001. Statistical analysis system online documentation. Cary, NC.
- Sandoval-Islas, J.S., L.H.M. Broers, H.E. Vivar, and K.S. Osada. 1998. Evaluation of quantitative resistance to yellow rust (*Puccinia striiformis* f. sp. *hordei*) in the ICARDA/CIMMYT barley breeding program. *Plant Breed.* 117:127–130.
- Singh, R.P., and R.A. McIntosh. 1984. Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. I. Genetic and linkage studies. *Can. J. Genet. Cytol.* 26:723–735.
- Spaner, D., L.P. Shugar, T.M. Choo, I. Falak, K.G. Briggs, W.G. Legge, D.E. Falk, S.E. Ullrich, N.A. Tinker, B.J. Steffenson, and D.E. Mather. 1998. Mapping of disease resistance loci in barley on the basis of visual assessment of naturally occurring symptoms. *Crop Sci.* 38:843–850.
- Steffenson, B.J., P.M. Hayes, and A. Kleinhofs. 1996. Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. sp. *teres*) and spot blotch (*Cochlibus sativus*) in barley. *Theor. Appl. Genet.* 92:552–558.
- Thomas, W.T.B., W. Powell, R. Waugh, K.J. Chalmers, U.M. Barua, P. Jack, V. Lea, B.P. Forster, J.S. Swanston, R.P. Ellis, P.R. Hanson, and R.C.M. Lance. 1995. Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 91:1037–1047.
- Tinker, N.A., and D. Mather. 1995. Methods for QTL analysis with progeny replicated in multiple environments. <http://www.ncgr.org/jag/papers95/paper195/jqtl15.html> (verified April 19, 2002).
- Toojinda, T., L.H. Broers, X.M. Chen, P.M. Hayes, A. Kleinhofs, J. Korte, D. Kudrna, H. Leung, R.F. Line, W. Powell, L. Ramsay, H.E. Vivar, and R. Waugh. 2000. Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). *Theor. Appl. Genet.* 101:580–589.
- Van Silfhout, C.H. 1993. Durable resistance in the pathosystem: Wheat – stripe rust. p. 135–145. *In* Th. Jacobs and J.E. Parlevliet (ed.) *Durability of disease resistance*. Kluwer Academic Publishers, the Netherlands.
- Von Wettstein-Knowles, P. 1992. Cloned and mapped genes: Current status. p. 73–98. *In* P.R. Shewry (ed.) *Barley: Genetics, biochemistry, molecular biology and biotechnology*. CAB Int., Wallingford, UK.