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Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley

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Abstract Net blotch (caused by *Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) are important foliar diseases of barley in the midwestern region of the USA. To determine the number and chromosomal location of Mendelian and quantitative trait loci (QTL) controlling resistance to these diseases, a doubled haploid population ('Steptoe'/'Morex') was evaluated to the pathogens at the seedling stage in the greenhouse and at the adult plant stage in the field. Alleles at two or three unlinked loci were found to confer resistance to the net blotch pathogen at the seedling stage depending on how progeny exhibiting an intermediate infection response were classified. This result was corroborated in the quantitative analysis of the raw infection response data as 2 major QTL were identified on chromosomes 4 and 6M. A third QTL was also identified on chromosome 6P. Seven QTL were identified for net blotch resistance at the adult plant stage and mapped to chromosomes 1P, 2P, 3P, 3M, 4, 6P, and 7P. The 7 QTL collectively accounted for 67.6% of the phenotypic variance under a multiple QTL model. Resistance to the spot blotch pathogen was conferred by a single gene at the seedling stage. This gene was mapped to the distal region of chromosome 1P on the basis of both qualitative and quantitative data analyses. Two QTL were identified for spot blotch resistance at the adult plant stage: the largest QTL effect mapped to chromosome 5P and the other mapped to chromosome 1P near the seedling resistance locus. Together, the 2 QTL explained 70.1% of the phenotypic variance under a multiple QTL model. On the basis of the chromosomal locations of resistance alleles detected in this study, it should be feasible to combine high levels of resistance to both *P. teres* f. *teres* and *C. sativus* in barley cultivars.

Key words *Hordeum vulgare* · Quantitative trait loci · Molecular mapping · Disease resistance

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Introduction

In the midwest region of the USA, net blotch (caused by *Pyrenophora* Drechs. *teres* f. *teres* Smedeg.) and spot blotch (caused by *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur) are two of the most common foliar diseases of barley (*Hordeum vulgare* L.). These diseases are considered economically important because they can cause marked reductions in both the yield and quality of the crop (Kiesling 1985; Mathre 1982; Nutter et al. 1985; Steffenson et al. 1991). Through a concerted effort of selection and breeding, a high level of resistance to *C. sativus* has been obtained in six-rowed malting barley cultivars (Fetch and Steffenson 1994; Wilcoxson et al. 1990). This resistance, originally derived from the breeding line ND B112 (Wilcoxson et al. 1990), has remained completely effective for over 30 years and is considered durable. Less progress has been made in breeding for resistance to net blotch as all of the major malting and feed cultivars in the midwest are susceptible or moderately susceptible to *P. t.* f. *teres*. At North Dakota State University, efforts to combine high levels of both net and spot blotch resistance in agronomically acceptable cultivars have, thus far, been unsuccessful using ND B112 as the source of resistance and conventional breeding methods. ND B112 was used in this effort because it is one of the few barley genotypes known to possess outstanding resistance to both pathogens under midwestern conditions. Information on the number and chromosomal location of loci controlling resistance to these diseases would greatly facilitate the development of resistant cultivars. In barley and in other crops, progress in determining the chromosomal locations of important disease resistance loci has been accelerated by the devel-

opment of molecular genome maps (Freymark et al. 1993; Heun 1992; Landry et al. 1987, 1992). Several molecular maps have been constructed for different barley populations over the past 5 years (Heun et al. 1991; Giese et al. 1994; Graner et al. 1991; Kleinhofs et al. 1993). One of these, the 'Steptoe'/'Morex' population (Kleinhofs et al. 1993) exhibits polymorphisms in response to infection by both *P. t.* f. *teres* and *C. sativus* at the seedling stage (Steffenson and Dahleen 1991). The 'Steptoe' parent is resistant to *P. t.* f. *teres* and susceptible to *C. sativus*, whereas 'Morex' is resistant to *C. sativus* and susceptible to *P. t.* f. *teres*. ND B112 is the presumed source of spot blotch resistance in Morex (Wilcoxson et al. 1990); the origin of net blotch resistance in 'Steptoe' is not known. The availability of a highly saturated molecular map of the 'Steptoe'/'Morex' population can facilitate detailed analyses on the genetic nature of resistance to these two important fungal diseases of barley. Thus, the objective of this study was to determine the number and chromosomal location of loci controlling net and spot blotch resistance in barley at both the seedling and adult plant stages of development.

Materials and methods

Plant materials, mapping information, quantitative and qualitative trait loci analyses

Molecular map construction and disease evaluations were made on the 'Steptoe'/'Morex' doubled haploid population developed by the North American Barley Genome Mapping Project (NABGMP). This cross represents one of the widest and most agronomically relevant combination of barley cultivars from the USA; 'Steptoe' is a high-yielding and widely adapted feed barley from the western USA, and 'Morex' is the standard of malting quality from the midwest (Kleinhofs et al. 1993). One hundred and fifty doubled haploid lines (DHLs) were produced from F_1 plants of the cross 'Steptoe'/'Morex' using the *Hordeum bulbosum* technique of Chen and Hayes (1989). From this population, a saturated molecular marker map has been constructed (Kleinhofs et al. 1993). The total length of this map is 1,250 cM with an average distance of 4.2 cM between markers. For quantitative trait loci (QTL) analyses, a 123 marker "skeletal" map was generated by selecting loci that were evenly distributed across the seven barley chromosomes (Hayes et al. 1993). The average distance between markers on this skeletal map is 9.6 cM. Quantitative phenotypic data from net and spot blotch disease evaluations were analyzed with the interval mapping procedures of MAPMAKER/QTL (version 1.1b) (Lincoln et al. 1992). QTL effects were considered significant when they exceeded a $\log_{10}$ of the odds ratio (LOD) score of 2.5. This LOD threshold corresponds to an approximate significance level of $P=0.001$ (Lander and Botstein 1989). Heritabilities (narrow sense) for net and spot blotch resistance were calculated based on the average disease severity for individual DHLs at the adult plant stage (Poehlman 1979). For disease evaluations on seedlings, qualitative infection phenotype classes were subjected to chi-square analyses. Single locus data were merged with molecular data from an expanded map of the 'Steptoe'/'Morex' population (Kleinhofs 1994) and analyzed using MAPMAKER (version 2.0) (Lander et al. 1987).

Pathogen isolates and culture conditions

Isolate ND89-19 of *P. t.* f. *teres* and isolate ND85F of *C. sativus* were used in this study because 'Steptoe' and 'Morex' exhibited differential reactions to them in a previous study (Steffenson and Dahleen 1991). On the 22 differential barley genotypes of Steffenson and Webster (1992), isolate ND89-19 exhibits high or compatible infection responses on 'Tifang', 'Canadian Lake Shore', 'Manchurian', 'Ming', 'Kombar', 'Harbin', 'Manchuria', and 'CI 4922' (B. J. Steffenson, unpublished). A set of differential hosts for characterizing pathotypes of *C. sativus* has not been established; however, at North Dakota State University, we have been using several diagnostic barley genotypes to monitor the virulence of this pathogen: 'Bowman', a two-rowed feed barley cultivar; ND 5883, a two-rowed breeding line; and ND B112, a six-rowed breeding line. On these genotypes, isolate ND85F exhibits a low or incompatible infection response on 'Bowman' and ND B112 and a high or compatible infection response on ND 5883 (Fetch and Steffenson 1994). Isolates ND89-19 and ND85F were originally derived from single conidia and were stored on silica gel in glass vials at 4°C until needed. Inoculum for inoculation was produced by placing, several silica gel crystals with adsorbed conidia in petri dishes containing either V-8 juice agar (for *P. t.* f. *teres*) or YpG agar (for *C. sativus*) (Tuite 1969). The cultures were then incubated at 21°C with a 12-h photoperiod (150–270 $\mu Em^{-2}s^{-1}$). After 2 weeks of incubation, conidia were harvested by adding about 2 ml of distilled water to the plate and then gently scraping the surface of the culture with a rubber spatula. This conidial suspension was filtered through four layers of cheesecloth to remove mycelial fragments and adjusted to the desired concentration based on hemacytometer conidia counts.

Inoculation and incubation conditions

For seedling evaluations, four to six seeds of parents and DHLs were sown in plastic cones (3.8-cm diameter and 21-cm length) filled with a peat moss:perlite (3:1) potting mix and grown at 22°–26°C in a greenhouse. Fertilization was provided at planting with water-soluble (15-0-15, N-P-K) and controlled release (14-14-14, N-P-K) formulations (Steffenson et al. 1993). When the second leaves of the plants were fully expanded (14 days after planting), inoculations were made with conidial suspensions of the individual pathogens using an atomizer pressured by an air pump at 414 kPa. Inoculations with isolate ND89-19 of *P. t.* f. *teres* and ND85F of *C. sativus* were made using a concentration of 5,000 and 8,000 conidia/ml, respectively. The volume of the inoculum suspension applied to each plant was approximately 0.15 ml. To facilitate even distribution and adherence of conidia, we added 10 µl of Tween® 20 (polyoxyethylene-20-sorbitan monolaurate) for every 100 ml of the inoculum suspension. Plants were allowed to dry slightly after inoculation before being placed in chambers maintained at near-saturation by periodic mistings from ultrasonic humidifiers. After a 16-h infection period in complete darkness, the plants were allowed to dry slowly for approximately 4 h before being returned to the greenhouse. Assessments of the infection response (IR) were made 9–11 days post-inoculation using the rating scale of Tekauz (1985) for net blotch and that of Fetch and Steffenson (1994) for spot blotch. The experiment was conducted in a randomized complete block design with two replicates and was repeated twice.

Parents and DHLs were also evaluated to the net and spot blotch pathogens in the field at Langdon and Fargo, North Dakota, respectively. The host entries were sown in hill plots (8–15 seeds/hill) spaced 0.3 m apart in paired rows. Susceptible barley genotypes (cv 'Hector' for net blotch and line ND 5883 for spot blotch) were planted around the paired rows of hill plots to increase disease development in the nurseries. When most of the DHLs were at the mid-tillering stage of development, the susceptible spreader plants were inoculated with barley straw infected with either isolate ND89-19 of *P. t.* f. *teres* or ND85F of *C. sativus*. This infected barley straw was taken from the previous season's crop at the respective locations. Assessments of disease severity (percentage of leaf area affected by disease) were made at the mid-dough stage of development using standard disease area diagrams [Burleigh and Loubane (1984) for net blotch and James (1971) for spot blotch]. The experimental design was a randomized complete block with three replications. Evaluations for net blotch reaction were made in 1991 only and for spot blotch both in 1991 and 1992.

Results

Net blotch resistance at the seedling stage

In the greenhouse evaluations, 'Steptoe' exhibited low IRs (2–3) to the net blotch pathogen, whereas 'Morex' exhibited high IRs (7–9). Three distinct phenotypic classes were observed in the progeny to *P. t.* f. *teres*: one comprised of individuals exhibiting low IRs (2–3); a second, individuals exhibiting intermediate IRs (4–5); and a third, individuals exhibiting high IRs (6–9). These results were consistent between replicates and experiments. The number of DHLs exhibiting low, intermediate, and high IRs was 112, 23, and 15, respectively. This segregation fits a 6:1:1 ratio (χ^2=1.72, *P*=0.42, *df*=2), suggesting the involvement of three genes for resistance, one of which confers an intermediate reaction. If the progeny are grouped into just two phenotypic classes, those exhibiting the distinct low IR of 'Steptoe' versus all other higher IRs, a segregation ratio of 112:38 results. This segregation fits a 3:1 ratio (χ^2=0.01, *P*=0.92, *df*=1), indicating that two genes are involved in conferring the low IR of 'Steptoe'.

In the QTL analysis, two chromosome regions were identified as contributing to net blotch resistance at the seedling stage: one near the centromeric region of chromosome 4 flanked by the marker pair *ABG3/ABG484* (LOD=11.1, r^2=0.31) and the second on the minus (M) or long arm of chromosome 6 flanked by the marker pair *ksuD17/ksuA3D* (LOD=4.5, r^2=0.14) (Fig. 1). In the multiple QTL model, these 2 loci accounted for 47.0% of the phenotypic variation. A third QTL was identified on the plus (P) or short arm of chromosome 6 between the markers *ABG458* and *Rrn1* (Fig. 1). This QTL had a LOD score of 3.5 and a single locus r^2 of 0.10. The inclusion of this third QTL in the multiple QTL model increased the amount of phenotypic variation explained only slightly – to 49.6%. 'Morex' contributed the higher value (susceptibility) alleles for all of the QTL (1.42 for the one on chromosome 4; 0.95 for 6M, and 0.82 for 6P).

Net blotch resistance at the adult plant stage

The terminal net blotch severity on 'Steptoe' and 'Morex' was 23.6±3.9% and 35.0±5.1%, respectively (Fig. 2). Transgressive segregation was observed in the DH population as disease severity ranged from 7.5% to 93.3% with a mean of 31.0%. The frequency distribution of net blotch severity in the population was skewed to the left because a greater number of individuals exhibited lower levels of disease. Seven QTL were identified for net blotch resistance at the adult plant stage. These QTL, listed in decreasing order of LOD score magnitude, mapped to the following intervals: *BCD129/Glx* on chromosome 1P (LOD=7.1, r^2=0.21), *ABA303/ABC171* on chromosome 3P (LOD=5.4, r^2=0.20), *ABG2/ABG459* on chromosome 2P (LOD=4.7, r^2=0.15), *His4B/ABG4* on chromosome 3M (LOD=4.6, r^2=0.16), *ABG395/Rrn2* on chromosome 7P (LOD=3.5, r^2=0.11), *ABG387B/ABG458* on chromosome 6P (LOD=3.3, r^2=0.10), and *ABG3/ABG484* on chromosome 4 (LOD=2.6, r^2=0.08) (Fig. 1). The 7 QTL collectively accounted for 67.6% of the phenotypic variance under the multiple QTL model. 'Morex' contributed the higher value (susceptibility) alleles for the QTL on chromosomes 1P (17.8), 3P (17.4), 4 (10.7), and 6P (12.1), whereas 'Steptoe' contributed the higher value alleles for QTL on chromosomes 2P (14.9), 3M (15.4), and 7P (12.8). Narrow-sense heritability for net blotch resistance was 0.92.

Spot blotch resistance at the seedling stage

'Morex' displayed low IRs (3–4, occasionally 5) and 'Steptoe' high IRs (7–9, occasionally 6) to isolate ND85F of *C. sativus*. Infection responses in the DH population varied almost continuously across the rating scale; however, most DHLs exhibited reactions similar to that of the parents. These IRs were generally stable between replicates within an experiment and between experiments. Individual DHLs were separated into two general categories of resistant or susceptible based on lesion size and type. Progeny exhibiting necrotic lesions less than 4 mm in length and little or no chlorosis were classified as resistant (IRs 1 through 5 according to Fetch and Steffenson 1994). Those progeny exhibiting necrotic lesions greater than 4 mm in length and moderate to extensive chlorosis were classified as susceptible (IRs 6 through 9 according to Fetch and Steffenson 1994). On the basis of this classification scheme, the DHLs segregated 76:74 resistant:susceptible (χ^2=0.03, *P*=0.87, *df*=1 for a 1:1 ratio), indicating the presence of a single resistance gene. When the spot blotch disease reaction is treated as a qualitative trait (i.e., a single locus with two alleles where IRs of 1–5=resistant and 6–9=susceptible) and analyzed in MAPMAKER, the resistance locus maps 2.0 cM proximal to *ABC167A* and 4.2 cM distal to *ABG380* on chromosome 1P of the 'Steptoe'/'Morex' map (Fig. 3). When the raw IR scores of individual DHLs were treated as quantitative data, a large-effect QTL (LOD=37.6, r^2=0.71) was detected in the *WG789A/ABG380* interval on chromosome 1P of the skeletal map (Figure 1). 'Steptoe' contributed the higher value (susceptibility) allele (2.7) at this locus.

Spot blotch resistance at the adult plant stage

In the field nurseries, high disease levels were observed during both years. 'Steptoe' and 'Morex' exhibited mean disease severities of 68.9% and 23.3% in 1991 and 58.8% and 4.8% in 1992, respectively. Pooled means for the parents over the 2-year study were 60.2±4.8% for 'Steptoe' and 11.5±2.9% for 'Morex'. Disease severity in the DH population ranged from 8.3% to 90.0% (mean=41.1%) in 1991 and 1.7% to 78.3% (mean=33.9%) in 1992 (Fig. 4). Although the disease pressure was higher in the first year of the experiment, the correlation between years for severity ratings on individual DHLs was high (r=0.83 with

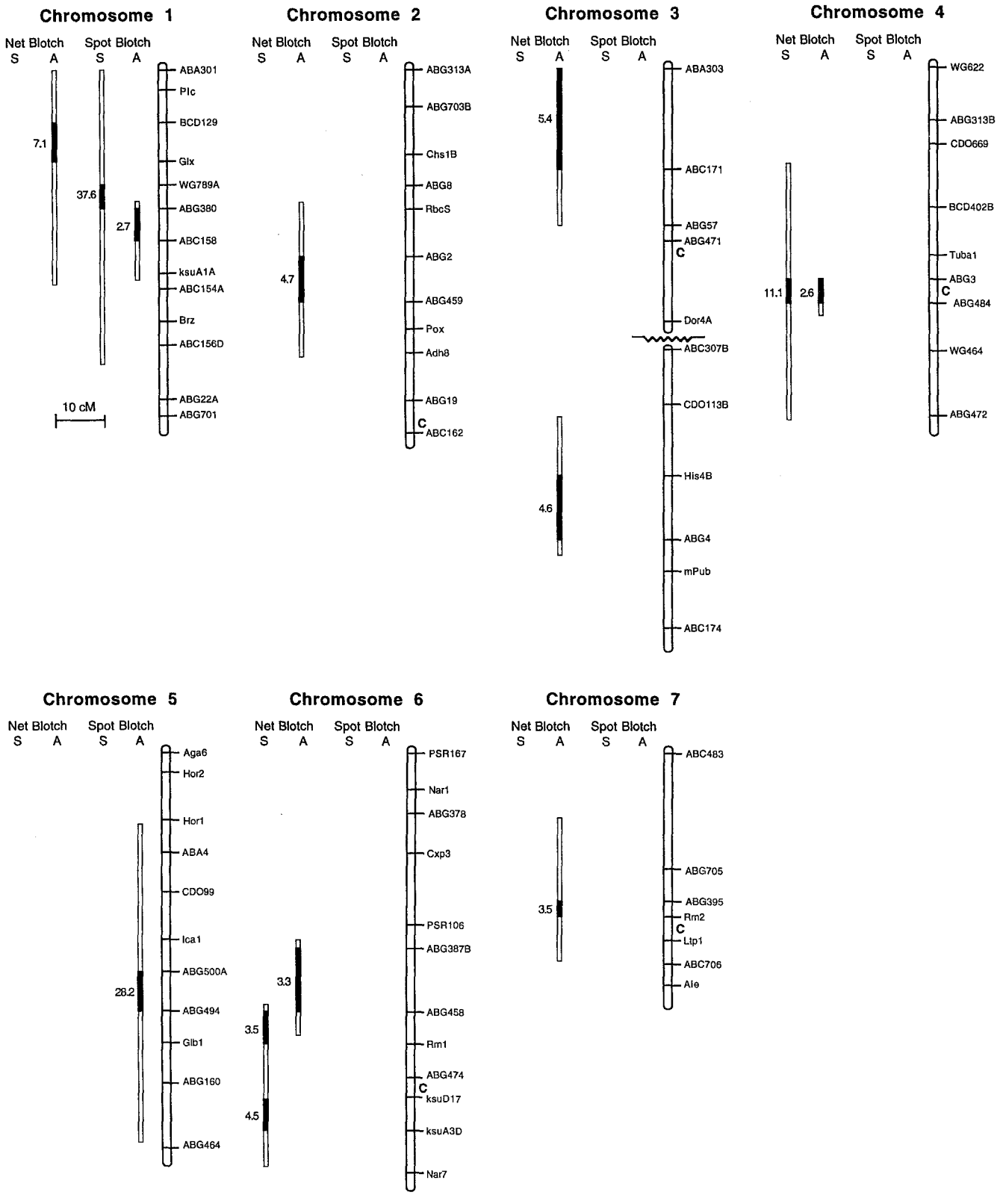

Fig. 1 Partial genetic linkage maps of barley chromosomes 1P, 2P, 3P/3M, 4P/4M, 5P, 6P/6M, and 7P/7M based on the 'Steptoe'/'Morex' population. *Vertical bars* correspond to the regions of the chromosome where significant QTL effects (LOD>2.5) were identified for net blotch and spot blotch resistance. The *black regions within the bars* indicate the intervals where the highest LOD scores (the maximum LOD is given to the *left*) were found (*C* centromere, *S* seedling, *A* adult)

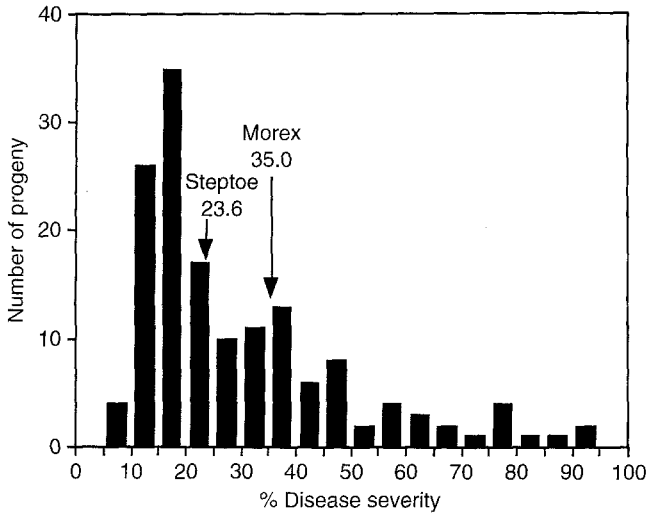

Fig. 2 Frequency distributions for net blotch disease severity on 150 doubled haploid lines derived from the cross 'Steptoe'/'Morex'. Data are from the 1991 field experiment

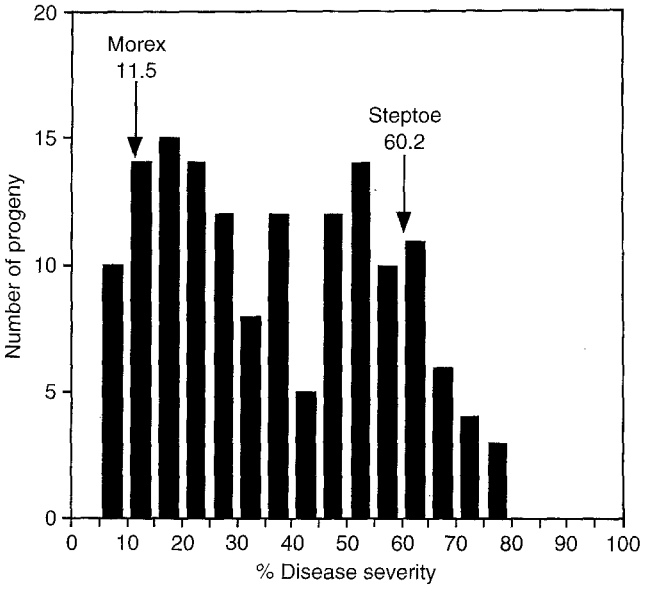

Fig. 4 Frequency distributions for spot blotch disease severity on 150 doubled haploid lines derived from the cross 'Steptoe'/'Morex'. Pooled data from the 1991 and 1992 field experiments are shown

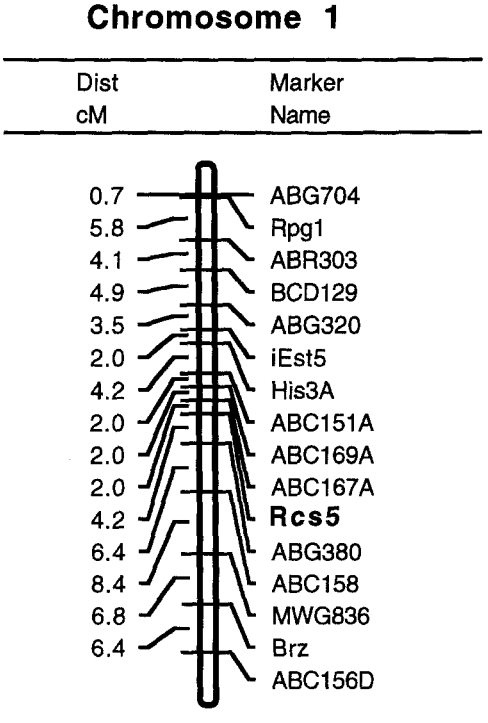

Fig. 3 Partial genetic linkage map of chromosome 1P showing the *Rcs5* locus (in *bold*) for seedling resistance to *Cochliobolus sativus*

P=0.0001). Two QTL were identified for spot blotch resistance at the adult plant stage (Fig. 1). The largest QTL effect mapped to the *ABG500A/ABG494* interval of chromosome 5P (LOD=28.2, r^2=0.62). A second QTL of lesser magnitude mapped to the *ABG380/ABC158* interval of chromosome 1P (LOD=2.7, r^2=0.09). Together, the 2 QTL explained 70.1% of the phenotypic variance under the multiple QTL model. 'Steptoe' contributed the higher value (susceptibility) alleles for both of the QTL (32.3 for the one on chromosome 5P and 12.0 for 1P). Narrow-sense heritability for spot blotch resistance was 0.91.

Discussion

The availability of a well-characterized molecular map of the 'Steptoe'/'Morex' population facilitated the identification of Mendelian and quantitative trait loci conferring net blotch and spot blotch resistance in barley at the seedling and adult plant stages of development. In the case of seedling resistance, similar genetic conclusions were obtained from both qualitative and quantitative data analyses. On the basis of phenotypes alone, 2 or 3 unlinked genes were found to confer seedling resistance to the net blotch pathogen depending on the classification of the infection response data. This result was corroborated in the quantitative analysis of the raw infection response data as 2 major QTL were identified on chromosomes 4 and 6M. A third smaller effect QTL also was identified on chromosome 6P. From the qualitative analysis of the spot blotch infection response data, a single locus for resistance was identified. This result was again confirmed in the quantitative analysis of the data as a single large effect QTL (LOD=37.6) was identified. The resistance gene mapped to the same relative position on chromosome 1P when mapped as a single locus or as a QTL (Figs. 1 and 3). This example demonstrates the power by which a QTL can be resolved into a Mendelian trait locus based on a saturated molecular linkage map as was previously reported (Lander and Botstein 1989; Paterson et al. 1988).

Despite the economic importance of both net blotch and spot blotch on barley, there are limited reports on the mapping of genes for resistance. Using trisomic analysis, Bockelman et al. (1977) mapped the *Rpt1* gene from 'Tifang' to chromosome 3, the *Rpt2* gene from CI 9819 to chromosome 5, and the *Rpt3* gene from CI 7584 to chromosome 2. The 2 or 3 QTL identified for seedling resistance to *P. t.* f. *teres* in this study are not allelic with these loci. Additional studies are needed to elucidate the relation of these QTL with a putative fourth resistance gene designated *Rpta* by Khan and Boyd (1969). Four genes for resistance to *C. sativus* (*hl1*, *hl2*, *hl3*, and *hl4*, designated here as *Rcs1–Rcs4*) are listed for barley by Søgaard and von Wettstein (1987). The existence as well as chromosome location for the first three named genes is based on tenuous correlative data. Griffee (1925) reported that resistance to the spot blotch pathogen in the cultivar 'Svanhals' was generally correlated with the *V/v(hex-v)* locus on chromosome 2, the *B/b* locus on chromosome 5, and the *R/r* locus on chromosome 7. Based on these loose correlations and the independent assortment of the three morphological characters, Griffee (1925) inferred that three unlinked genes control resistance to spot blotch at the adult plant stage. The designation of the fourth gene, *hl4*, is based on the genetic data of Arny (1951). In this investigation, no correlation was found between spot blotch resistance and 8 different marker loci. Gonzalez Ceniceros (1990) identified two genes for resistance to *C. sativus* in the cultivar 'Bowman'; the respective genes were associated with the *gs2* locus on chromosome 3 and *v3* locus on chromosome 5. The seedling resistance gene identified in this study is different from those previously described by Gonzalez Ceniceros (1990) and Griffee (1925) based on chromosome location and is tentatively designated *Rcs5*. The same is true for the QTL identified on chromosome 5 in this study. This assumption is based on the relative proximal position of the QTL to *Hor1* on chromosome 5P. The morphological markers (*v3* and *B/b*) found associated with spot blotch resistance by Gonzalez Ceniceros (1990) and Griffee (1925) map at a relatively distal position on chromosome 5M.

From disease evaluations of DHLs as seedlings in the greenhouse and as adult plants in the field, resistance genes were detected that function at different ontogenetic stages in plant development. In seedlings, resistance to *P. t.* f. *teres* was controlled by loci on chromosomes 4 and 6, whereas adult plant resistance was controlled primarily by loci on chromosomes 1, 2, and 3. A similar situation was observed in response to *C. sativus*, as seedling resistance was governed by a locus on chromosome 1P and adult plant resistance primarily by a locus on chromosome 5P. Several rust resistance genes have been reported to function at different stages of plant development in wheat (Roelfs 1988). The examples described in this study provide unequivocal evidence for the presence of seedling and adult plant resistance genes to net and spot blotch of barley. It is important to note that these resistance genes were detected in response to the same isolates of the pathogen in both the greenhouse and field experiments. Loci from the same general chromosomal region also contributed to resistance at both stages of plant development. Examples include the *ABG3/ABG484* interval on chromosome 4 for net blotch resistance and the intervals adjacent to *ABG380* on chromosome 1P for spot blotch resistance. It is not known whether these effects are due to linkage or pleiotropy. Segmental isolines or other genetic stocks will be required to resolve this question.

For over 30 years, six-rowed barley cultivars in the midwest region of the USA have remained completely resistant to *C. sativus* despite being cultivated over large areas (over 1.4 million ha) where the pathogen is endemic and the environment favorable for disease. This resistance is durable according to the definition of Johnson (1984). Information regarding the number of genes controlling durable resistance is of paramount importance to breeders who wish to manipulate this trait in their programs. On the basis of the results with 'Morex' from the field, it appears that durable spot blotch resistance is conferred by two genes, one of major effect on chromosome 5P and the other of relatively minor effect on chromosome 1P. This result is in agreement with Wilcoxson et al. (1990) who found that resistance was due to one or two genes in M33, a breeding line derived from ND B112. Simultaneous improvement in resistance to the two diseases in agronomically acceptable germplasm has been difficult using ND B112 as the parental source and conventional breeding procedures. The mapping data generated from this study on the 'Steptoe'/'Morex' population indicates that it should be feasible to combine high levels of resistance to *P. t.* f. *teres* and *C. sativus* in barley cultivars because the alternative favorable alleles do not map to the same chromosomal regions. Of equal importance are the presence of repulsion linkages between resistance loci and agronomic/quality traits. Hayes et al. (1993) recently described QTL for yield, malting quality parameters (grain protein, alpha amylase, diastatic power, and malt extract), and several agronomic characters (lodging, plant height, and heading date) in the same 'Steptoe'/'Morex' population evaluated in this investigation. In comparing their data with those from this study, we found that nearly every major QTL for yield, malting quality and agronomic traits mapped to different locations than those for net and spot blotch resistance. It should, therefore, be feasible to develop agronomically superior germplasm with high levels of resistance to *P. t.* f. *teres* and *C. sativus* in this genetic background. Studies are now in progress to validate the use of molecular markers for selecting these traits in experimental breeding populations.

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