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Comprehensive genetic analyses reveal differential expression of spot blotch resistance in four populations of barley

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Abstract Spot blotch, caused by *Cochliobolus sativus*, is an important disease of barley in the Upper Midwest region of the United States. The resistance of six-rowed malting cultivars like Morex has remained effective for over 40 years and is considered durable. Previous research on Steptoe/Morex (S/M), a 6×6-rowed doubled haploid (DH) population, showed that seedling resistance is controlled by a single gene (*Rcs5*) on chromosome 1(7H) and adult plant resistance by two quantitative trait loci (QTL): one of the major effect on chromosome 5(1H) explaining 62% of the phenotypic variance and a second of minor effect on chromosome 1(7H) explaining 9% of the phenotypic variance. To corroborate these results in a 2×6-rowed DH population, composite interval mapping (CIM) was performed on Harrington/Morex (H/M). As in the S/M population, a single major gene (presumably *Rcs5*) on chromosome 1(7H) conferred resistance at the seedling stage. However, at the adult plant stage, the results were markedly different as no chromosome 5(1H) effect whatsoever was detected. Instead, a QTL at or near *Rcs5* on chromosome 1(7H) explained nearly all of the phenotypic variance (75%) for disease severity. To determine whether this result might be due to the genetic background of the two-rowed susceptible parent Harrington, we analyzed another DH population that included the same resistance donor (Morex) and another six-rowed susceptible cultivar Dicktoo (D/M). Three QTL conferred seedling resistance in the D/M population: one near *Rcs5* on chromosome 1(7H) explaining 30%, a second near the centromere of chromosome 1(7H) explaining 9%, and a third on the short arm of chromosome 3(3H) explaining 19% of the phenotypic variation. As in the H/M population, no chromosome 5(1H) QTL was detected for adult plant resistance in the D/M population. Instead, three QTL on other chromosomes explained most of the variation: one on the short arm of chromosome 3(3H) explaining 36%, a second on the long arm of chromosome 3(3H) explaining 11%, and a third at or near *Rcs5* on chromosome 1(7H) explaining 20% of the phenotypic variation. These data demonstrate the complexity of expression of spot blotch resistance in different populations and have important implications in breeding for durable resistance.

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Introduction

Spot blotch, caused by *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.), is one of the most devastating foliar diseases of barley in the Upper Midwest region of United States. Under epidemic conditions, yield losses as high as 35% can occur in susceptible barley cultivars (Clark 1979). Six-rowed malting cultivars bred for the Upper Midwest region possess high levels of spot blotch resistance derived from the breeding line NDB112 (Steffenson et al. 1996; Wilcoxson et al. 1990). This resistance has remained effective for over 40 years and is considered one of the best examples of durable resistance in cereals (Steffenson et al. 1996; Steffenson 2000).

To elucidate the genetics of durable spot blotch resistance in six-rowed malting cultivars, Steffenson et al. (1996) utilized the Steptoe/Morex (S/M) doubled haploid (DH) population developed by the North American Barley Genome Project (NABGP). Morex is a resistant midwestern six-rowed malting cultivar, and Steptoe is a susceptible six-rowed feed cultivar bred for

the western United States. A single gene (designated *Rcs5*) located at the telomeric region of chromosome 1(7H) was found to confer spot blotch resistance at the seedling stage in the S/M population (Steffenson et al. 1996). Two quantitative trait loci (QTL) conferred adult plant resistance: one of major effect on chromosome 5(1H) explaining 62% of the phenotypic variation and a second of minor effect on chromosome 1(7H) explaining 9% of the variation. The minor effect QTL on chromosome 1(7H) mapped to the same region as *Rcs5*. These results clearly indicate that durable spot blotch resistance in midwestern six-rowed cultivars is oligogenic. Preliminary analysis of a DH population involving Morex and the two-rowed susceptible malting cultivar Harrington (H/M) revealed no chromosome 5(1H) effect at the adult plant stage as was found in the S/M population (Steffenson 2000). Instead, a major effect QTL at or near *Rcs5* on chromosome 1(7H) explained most of the phenotypic variance. These data raise several interesting and important questions regarding the expression of spot blotch resistance in different populations of barley. Thus, the objective of this study was to characterize the genetic basis and expression of spot blotch resistance in barley populations involving different six-rowed and two-rowed parents.

Materials and methods

Plant materials

To study the genetics and expression of spot blotch resistance in different genetic backgrounds, four DH populations were used: S/M (150 DH lines), which was previously characterized by Steffenson et al. (1996), Harrington/Morex (H/M, 140 DH lines), Harrington/TR306 (H/T, 150 DH lines), and Dicktoo/Morex (D/M, 92 DH lines). The S/M (Hayes et al. 1993b; Kleinhofs et al. 1993), H/M (Marquez-Cedillo et al. 2000), and H/T (Kasha et al. 1995; Mather et al. 1997; Tinker et al. 1996) populations were all developed by the NABGP and used for QTL analysis of malting and agronomic traits. The D/M population was developed at Oregon State University for studying the genetic basis of cold hardiness (Hayes et al. 1992, 1993a, 1997). The durable spot blotch resistance of Morex was derived from NDB112 (Steffenson et al. 1996), and its resistance level is typical of other midwestern malting barley cultivars released over the last 40 years. The susceptible parents involved in crosses with Morex are diverse. Steptoe is a six-rowed feed barley cultivar released from the Washington State Experiment Station in 1973, Harrington is a susceptible two-rowed malting cultivar released from the University of Saskatchewan, Saskatoon, Canada in 1981, and Dicktoo is a six-rowed feed cultivar that was first selected in North Dakota and released from the Dickinson Experiment Station in 1952 as a winter barley cultivar for Nebraska. TR306 is a two-rowed breeding line that possesses some spot blotch resistance. It was developed by the Crop Development Centre in Saskatchewan, Saskatoon, Canada in 1987. The origin of spot blotch resistance in TR306 is not known, but is probably not derived from NDB112. TR306 does, however, have Peatland in its pedigree. Peatland was reported to possess a moderate level of spot blotch resistance (Wiebe and Reid 1961) and may be the source of resistance in TR306.

Pathogen isolate and inoculum production

Pathotype 1 (isolate ND85F) of *C. sativus* (Valjavec-Gratian and Steffenson 1997) was used in all disease phenotyping tests. This isolate was also used in previous studies involving the S/M and H/M populations (Steffenson et al. 1996; Steffenson 2000). Inoculum for seedling tests was increased and prepared according to the methods of Fetch and Steffenson (1999). For the field experiments, inoculum was produced by initially inoculating the susceptible barley line ND5883 with a foliar suspension of isolate ND85F. Inoculum was applied to plants at the booting to early heading stage (Growth Stage [GS] 13 on Zadoks' scale [Zadoks et al. 1974]) using a pressurized CO_2 sprayer. At the end of the season, the spot blotch-infected straw of ND5883 was harvested, bundled, and stored until the following spring. The infected barley straw was then used as the inoculum source for the field nurseries as previously described (Steffenson et al. 1996).

Inoculation and disease assessment

The H/M, H/T, and D/M populations were evaluated for their reaction to spot blotch at the seedling stage in greenhouse and at the adult plant stage in the field according to the methods of Fetch and Steffenson (1999) and Steffenson et al. (1996), respectively. For seedling evaluations, five seeds of the parents and DH progeny were inoculated with isolate ND85F (0.15–0.30 ml/plant of a 8,000 conidia/ml stock solution) when the second leaves of plants were fully expanded, about 14 days after planting. Assessments of the infection response (IR) were made 9–11 days postinoculation using the rating scale of Fetch and Steffenson (1999). This one to nine rating scale is based on the lesion size and the degree of associated chlorosis.

The seedling experiments were conducted in a randomized complete block design with two replicates and were repeated twice. Evaluations for the H/M, H/T, and D/M populations were performed in 1996, 1998, and 2003, respectively. Data for the S/M population were collected in 1991–1992 and reported by Steffenson et al. (1996). Three checks (NDB112, Bowman, and ND5883) were included in three to four replications within each experiment to verify the purity and virulence of the pathotype 1 isolate and assess the relative level of disease in different inoculation tests.

Parents and DH progeny were also evaluated to spot blotch at the adult plant stage in the field. Evaluations of the H/M, H/T, and D/M populations were made in 1995–1996, 1998–1999, and 2002–2003, respectively. Data for the S/M population were collected in 1991–1992 and reported by Steffenson et al. (1996). Field tests from 1991 to 1999 were made at the North Dakota Agricultural Experiment Station in Fargo, and those from 2002 to 2003 were made at the Minnesota Agricultural Experiment Station in St. Paul. In Fargo, the host entries were sown in hill plots (8–15 seeds/hill) spaced 0.3 m apart in paired rows. In St. Paul, the host entries were planted in paired 1-m rows (15–25 seeds/row) spaced 0.3 m apart. The susceptible barley line ND5883 was planted around the paired rows of test entries to increase disease development in the nurseries. When most of the progeny were at the midtillering stage of development, the susceptible spreader plants of ND5883 were inoculated with barley straw infected with isolate ND85F of *C. sativus*. This infected barley straw was taken from the previous season's crop at the respective locations and was verified to contain pathotype 1 of *C. sativus* by virulence tests using single spore isolates. Assessments of disease severity (percentage of leaf area affected by disease from 0% to 100%) were made using standard disease area diagrams (James 1971). Disease assessments were made twice during the growing season, three times for late maturing progeny. The terminal disease severity data that were used in QTL analyses were made at the middough stage of development (Zadoks GS 85-86). IR assessments were made according to the rating scale of Fetch and Steffenson (1999). This adult plant IR rating scale consists of four classes (resistant [R], moderately resistant [MR], moderately susceptible [MS], susceptible [S]) and is based on the lesion size and the degree of associated chlorosis. The experimental design was a randomized complete block with two replications. The experiment was repeated a second season. As in the seedling tests, the three checks of NDB112, Bowman, and ND5883 were included to verify the purity and virulence of the pathotype 1 isolate and assess the relative level of disease across different experiments.

Linkage map construction and QTL analyses

Molecular marker maps were previously developed for S/M (Hayes et al. 1993b; Kleinhofs et al. 1993), H/M (Marquez-Cedillo et al. 2000), H/T (Kasha et al. 1994, 1995), and D/M (Hayes et al. 1997; Pan et al. 1994). The molecular marker data for the base maps of S/M (129 markers), H/M (117 markers), H/T (107 markers), and D/M (127 markers) were obtained from the Graingenes website (http://wheat.pw.usda.gov/ggpages/maps.shtml). In preparing the molecular data for QTL analyses in this study, we added previously published data for three molecular markers: one (ABG494) on chromosome 5(1H) and two (ABC167A and WG789A) on chromosome 1(7H) of the S/M base map in regions where the major effect and minor effect QTL for adult plant spot blotch resistance were described by Steffenson et al. (1996). This was done to further refine the QTL region during composite interval mapping (CIM) analysis, a technique not used in the original study by Steffenson et al. (1996). Data previously collected for the S/M population (Steffenson et al. 1996) were reanalyzed in this study using the CIM algorithm of QTL Cartographer (Version 2.0, Basten et al. 2001). Linkage analyses for molecular marker data obtained from the Graingenes website were conducted using MapMaker (Version 2.0; Lander et al. 1987). Our MapMaker analysis of D/M data confirmed the same map distances as published by Hayes et al. (1997), but resulted in a reverse orientation of chromosome 1(7H). We retained our orientation of chromosome 1(7H) because it was consistent with two barley consensus maps (Langridge et al. 1995; Qi et al. 1996). The base map of the D/M population was constructed on data for the first 92 of 100 total progeny because the last eight had incomplete molecular marker data (Hayes et al. 1997). Another nine progeny (DM10, 16, 20, 43, 50, 55, 66, 72, 76) were omitted from seedling and adult stage QTL analyses because they exhibited very late maturity in the field. Our MapMaker analyses confirmed the marker order of previously published base maps of the H/M and H/T populations; thus, we utilized the previously published map distances and chromosome orientations for our QTL analyses.

The most common IR observed on the second leaves of each DH line in the respective populations was averaged over all replicates and used in the seedling QTL analysis. In the field, the terminal disease severity (%) on flag-2 leaves was averaged over all replicates and used in the adult plant QTL analysis. QTL analyses were performed by QTL Cartographer (Version 2.0) software (Basten et al. 2001) using the CIM algorithm (Zeng 1994). One thousand permutations at a significance level of $\alpha = 0.05$ were performed to estimate the appropriate LOD thresholds as suggested by Doerge and Churchill (1996). Forward–backward regression was done to select cofactors before performing QTL detection by CIM. The standard method (Model 6) with window size of 10.0 cM was selected for CIM analysis. Bin locations of QTL were estimated based on the "chromosome BIN locator of barley genes and markers" available at http://barleygenomics.wsu.edu. For AFLP markers in the D/M map, bin estimations were based on the nearest anchoring RFLP marker.

Progeny were classified into qualitative categories of resistant and susceptible to determine if they followed a Mendelian inheritance pattern. At the seedling stage, progeny exhibiting IRs 1 through 5 were classified as resistant, and those exhibiting IRs 6 through 9 were classified as susceptible according the criterion used previously by Steffenson et al. (1996). At the adult plant stage, progeny with mean disease severity scores within 1.5 or 2.0 standard deviations of the resistant parent were classified as resistant, and those with mean disease

severity scores within 1.5 or 2.0 standard deviations of the susceptible parent were classified as susceptible. The resistant and susceptible classes did not overlap using this criterion, except in the H/M population. In this population, the 1.5 standard deviation criterion had 30% severity as the upper limit for the resistant class and 24% severity as the lower limit for the susceptible class. Fifteen progeny fell within this overlapping interval of 24–30%. IR is highly correlated with disease severity. Therefore, we classified these 15 progeny into resistant and susceptible classes based on the other disease assessment parameter of IR. Progeny exhibiting IRs of R and MR were classified as resistant, whereas those exhibiting IRs of MS and S were classified as susceptible. All of the chi-square tests were applied for an expected ratio of 1:1 with df = 1.

Results

Genetics and expression of spot blotch resistance at the seedling stage

The IRs exhibited by the parents and progeny were generally in agreement between replicates within an experiment and also between experiments. Morex and TR306 exhibited low IRs (3–4, occasionally 5), Dicktoo intermediate IRs (5–6), Harrington intermediate to high IRs (5–7), and Steptoe high IRs (7–8) to pathotype 1 (isolate ND85F) of *C. sativus*. The frequency distributions for the average IR of S/M, D/M, H/M and H/T progeny are given in Fig. 1a. Progeny from the four populations were separated into two general categories of resistant (IRs 1–5) or susceptible (IRs 6–9) based on the IR. Using this criterion, Steffenson et al. (1996) identified a single gene for seedling resistance (*Rcs5*) in the S/M population. A single gene was also identified in the H/M and H/T populations from chi-square analysis ($P = 0.05$) of segregating progeny (Table 1). Most progeny in the D/M population exhibited intermediate reactions (Fig. 1a); thus, their segregation pattern was not consistent with a single gene ratio (Table 1).

Qualitative data (i.e., a single locus with two alleles) for the S/M, H/M, and H/T populations were analyzed in MapMaker. In the H/M population, the resistance locus (designated in Fig. 2 as *RcsMx*-S) mapped to the same bin interval (bins 2–4) of chromosome 1(7H) as *Rcs5* in the S/M population (see Fig. 3 in Steffenson et al. 1996). Morex contributed the resistance allele in both the S/M and H/M populations. In the H/T population, the resistance locus (designated in Fig. 2 as *RcsTR*-S) mapped 21.1 cM proximal to *aHis3A* and 9.1 cM distal to ABG380 on chromosome 1(7H), a position in the same bin interval (bins 2–4) as *Rcs5* in the S/M population. TR306 contributed the resistance allele in the H/T population.

The mean of the most common IR of progeny in the respective populations was subjected to QTL analysis. In each population, a large effect QTL (*Rcs-qtl-7H-2-4* denoting *Resistance* to *C. sativus-QTL-chromosome-chromosome bin numbers*) was detected on the short arm of chromosome 1(7H) in the *Rcs5* region, i.e., just distal to ABG380 (Table 2; Fig. 3). The percent phenotypic variation explained by this QTL was 67, 30, 75, and 85% for the S/M, D/M, H/M, and H/T populations, respectively. Morex contributed the resistance allele in the S/M, D/M, and H/M populations and TR306 in the H/T population. In addition to this major effect QTL, several minor effect QTL were also detected in the S/M, D/M, and H/T populations. The second QTL (*Rcs-qtl-3H-11-12*) detected in the S/M population lies on the long arm of chromosome 3(3H) ($r^2 = 0.04$). Morex contributed this resistance allele. Two additional QTL were detected in the D/M population: one (*Rcs-qtl-3H-4-6*) in the ABG460–saflp55 interval on the short arm of chromosome 3(3H) ($r^2 = 0.19$) with resistance allele contributed by Morex and the other (*Rcs-qtl-7H-7*) in saflp76–saflp246 interval at the centromeric region of chromosome 1(7H) ($r^2 = 0.09$) with resistance allele contributed by Dicktoo. The other QTL (*Rcs-qtl-7H-7*) detected in the H/T population was near the centromere of chromosome 1(7H) ($r^2 = 0.04$) as was found in the D/M population. This resistance allele was contributed by Harrington. The chromosomal location, percent phenotypic variation explained, donor parent for the resistance allele, LOD scores, and marker intervals for all QTL conferring seedling resistance are summarized in Table 2.

Genetics and expression of spot blotch resistance at the adult plant stage

In the field nurseries, uniform and high disease levels were attained in all experiments. This allowed for the reliable classification of progeny without the chance for disease escapes. Frequency distributions for the average spot blotch severity of D/M, H/M, and H/T progeny are given in Fig. 1b and for S/M progeny in Fig. 4 of Steffenson et al. (1996). Steptoe and Morex exhibited mean disease severities of $60.2 \pm 4.8\%$ and $11.5 \pm 2.9\%$ over all replications (Steffenson et al. 1996). The corresponding mean disease severities for Dicktoo and Morex were $57.9 \pm 8.9\%$ and $20.2 \pm 9.9\%$, for Harrington and Morex $67.0 \pm 28.9\%$ and $12.7 \pm 11.6\%$, and for Harrington and TR306 $68.8 \pm 21.9\%$ and $20.0 \pm 5.0\%$, respectively.

The number of resistant and susceptible progeny based on the qualitative data analyses in the H/M and H/T populations was consistent with a 1:1 Mendelian segregation ratio, indicating that a single gene confers adult plant resistance (Table 1). MapMaker analysis positioned this single gene (designated in Fig. 2 as *RcsMx*-A and *RcsTR*-A for H/M and H/T, respectively) in the same bin interval (bins 2–4) of chromosome 1(7H) as the seedling resistance locus in the S/M (i.e., *Rcs5*), H/M, and H/T populations. The number of resistant and susceptible progeny observed in the S/M and D/M populations did not fit a single gene ratio. The S/M

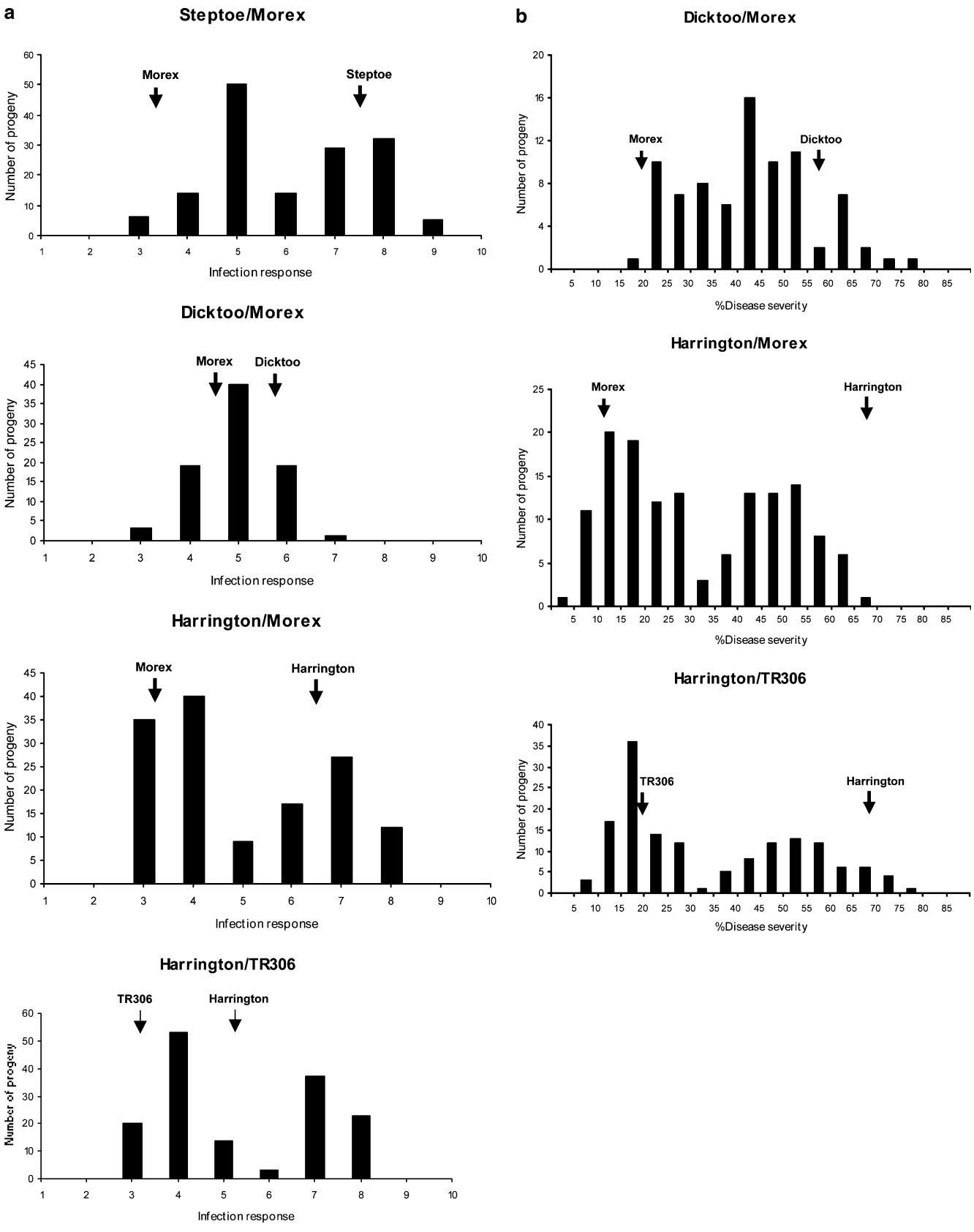

Fig. 1 **a** Frequency distributions for the mean of the most common infection response of progeny from the S/M, D/M, H/M, and H/T populations at the seedling stage, and **b** frequency distributions for the average terminal disease severity (%) of progeny in D/M, H/M, and H/T populations at the adult plant stage

Table 1 Segregation ratios of spot blotch resistant and susceptible progeny from the S/M, D/M, H/M, and H/T populations at the seedling and adult plant stages

Population	Seedling stage			Adult stage		
	Number of resistant: susceptible progeny	χ^2	*P* value	Number of resistant: susceptible progeny	χ^2	*P* value
S/M	76:74[a]	0.03	0.87	31:51[b]	–	–
D/M	62:20[c]	21.51	0.00	29:53[c]	7.02	0.01
H/M	78:62	1.83	0.18	74:66	0.46	0.50
H/T	87:63	3.84	0.05	83:67	1.71	0.19

[a]Data from Steffenson et al. (1996)
[b]Sixty-eight progeny from the S/M population fell between two standard deviations of the parents and could not be classified into the resistant or susceptible groups
[c]One progeny did not produce sufficient seed for the disease evaluation

population had 68 progeny that fell between the two standard deviations of the parents and further classification based on IR did not result in a statistically acceptable 1:1 ratio. Progeny from the D/M population were classified into resistant and susceptible groups based on the two standard deviation criterion of the parents; however, the numbers were again not consistent with a single gene ratio (Table 1).

Since the number of resistant and susceptible progeny observed in the S/M and D/M populations did not fit a single gene ratio (Table 1), QTL analyses were performed to identify and position the resistance loci. In the S/M population, two QTL were identified by CIM analysis: one (*Rcs-qtl-1H-6-7*) of major effect ($r^2 = 0.62$) in the ABG500A–ABG452 interval at the centromeric region of chromosome 5(1H) and a second (*Rcs-qtl-7H-2-4*) of minor effect ($r^2 = 0.12$) in the WG789A–ABG380 interval of the short arm of chromosome 1(7H) (Table 3; Fig. 3). Both resistance alleles were contributed by Morex. Additionally, two new minor effect QTL were identified that were not originally reported in the S/M population by Steffenson et al. (1996). One resistance allele (*Rcs-qtl-2H-3-5*) was contributed by Morex and mapped to the RbcS–ABG459 interval on the short arm of chromosome 2(2H) ($r^2 = 0.04$), and the second (*Rcs-qtl-3H-2-4*) was contributed by Steptoe and mapped to the ABC171–MWG584 interval on the short arm of chromosome 3(3H) ($r^2 = 0.06$) (Table 3; Fig. 3). Three QTL were detected at the adult plant stage in the D/M population: one (*Rcs-qtl-3H-2-4*) in the saflp119–saflp54 interval of the short arm of chromosome 3(3H) explaining 36%, the second (*Rcs-qtl-3H-9-11*) in the saflp35–saflp53 interval of the long arm of chromosome 3(3H) explaining 11%, and a third (*Rcs-qtl-7H-2-4*) in the saflp139–ABC167 interval near *Rcs5* on the short arm of chromosome 1(7H) explaining 20% of the phenotypic variation (Table 3; Fig. 3). All three resistance alleles in the D/M population were contributed by Morex.

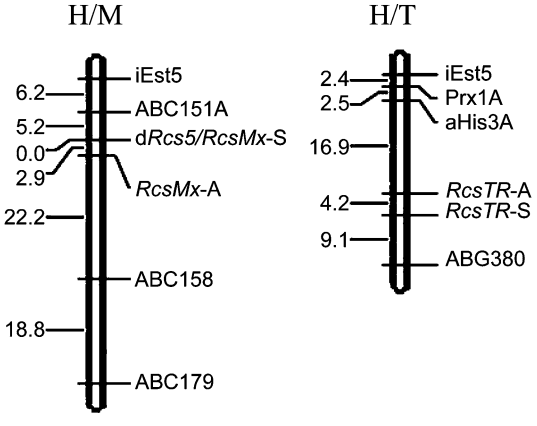

Fig. 2 Partial genetic linkage map of chromosome 1(7H) of the H/M and H/T populations showing loci conferring seedling (*RcsMx*-S and *RcsTR*-S) and adult plant (*RcsMx*-A and *RcsTR*-A) resistance to spot blotch. Marker d*Rcs5* in the H/M population is based on data for spot blotch resistance used in this study, but was previously published in the Graingenes website by A. Kleinhofs. The map distances in centimorgans (cM) were calculated using the Kosambi function

In addition to the qualitative (Mendelian) analysis described above, we also subjected the mean terminal disease severity data from the H/M and H/T populations to QTL analysis. In the H/M population, a major effect QTL (*Rcs-qtl-7H-2-4*) explaining 75% of the phenotypic variation was identified on the short arm of chromosome 1(7H) in same region as *Rcs5* (Table 3; Fig. 3). This resistance allele was contributed by Morex. Two other minor effect QTL (2–3% of the variance) were mapped at the centromeric region of chromosome 2(2H) (*Rcs-qtl-2H-7-8*) and on the long arm of chromosome 3(3H) (*Rcs-qtl-3H-9-11*). (Table 3; Fig. 3). These resistance alleles were contributed by Harrington and Morex, respectively. Similar to the H/M population, a major effect QTL (*Rcs-qtl-7H-2-4* explaining 77% of the variation) was identified in the *Rcs5* region on the short arm of chromosome 1(7H) in the H/T population with the resistance allele contributed by TR306 (Table 3; Fig. 3). A minor effect QTL (*Rcs-qtl-5H-10-11* explaining 5% of the variance) was also identified in the H/T population and was positioned in the RZ404–MWG781 interval on the long arm of chromosome 7(5H). This resistance allele was contributed by Harrington and was not identified in any other population. The chromo-

Table 2 Chromosomal location, percent phenotypic variation explained, donor parent for the resistance allele, LOD scores, and marker intervals for all QTL conferring seedling spot blotch resistance in the S/M, D/M, H/M, and H/T populations

	Chromosome	*Rcs-qtl-7H-2-4* 1(7H)	*Rcs-qtl-7H-7* 1(7H)	*Rcs-qtl-3H-4-6* 3(3H)	*Rcs-qtl-3H-11-12* 3(3H)
S/M	Variation	67%			4%
	Donor	Morex			Morex
	LOD	38.36			4.35
	Interval	WG789A–ABG380			CDO113B–*His4B*
D/M	Variation	30%	9%	19%	
	Donor	Morex	Dicktoo	Morex	
	LOD	10.05	3.68	6.96	
	Interval	ABC167–ABC158	saflp76–saflp246	ABG460–saflp55	
H/M	Variation	75%			
	Donor	Morex			
	LOD	54.21			
	Interval	ABC151A–ABC158			
H/T	Variation	85%	4%		
	Donor	TR306	Harrington		
	LOD	60.95	3.88		
	Interval	*aHis3A*–ABG380	MWG511–Vatp57A		

somal location, percent phenotypic variation explained, donor parent for the resistance allele, LOD scores, and marker intervals for all QTL conferring adult plant resistance are summarized in Table 3.

Discussion

The genetic basis and expression of durable resistance is an important aspect in breeding research. In this study, one of our primary objectives was to characterize the genetic basis and expression of durable spot blotch resistance in different barley populations. The spot blotch resistance in midwestern six-rowed cultivars has remained effective for over 40 years and is considered one of the best examples of durable resistance in cereals. Previous research on one of these cultivars (Morex) demonstrated that durable adult plant resistance in the field is controlled primarily by a major effect QTL on chromosome 5(1H), along with a minor effect QTL on chromosome 1(7H) (Steffenson et al. 1996). Analysis of the same population for seedling resistance revealed only the presence of a single major gene *Rcs5* on the short arm of chromosome 1(7H). The adult plant resistance QTL identified on chromosome 1(7H) was thought to be due to *Rcs5* or possibly another closely linked locus (Steffenson et al. 1996).

To validate the expression of the major effect QTL on chromosome 5(1H) in another population, seedling and adult plant evaluations were performed on a population (H/M) with a different susceptible parent, the two-rowed malting cultivar Harrington. As in the S/M population, a single major gene (presumably *Rcs5*) on chromosome 1(7H) conferred resistance at the seedling stage (Fig. 2). However, at the adult plant stage, the results were markedly different as no chromosome 5(1H) effect whatsoever was detected (Table 3; Fig. 3). Instead, a QTL at or near *Rcs5* on chromosome 1(7H) explained nearly all of the variance (75%) for disease severity (Steffenson 2000; this study). The lack of expression of the chromosome 5(1H) QTL was initially thought to be associated with the two-rowed genetic background of the susceptible parent Harrington, because previous experience from the two-rowed barley breeding program in North Dakota showed that it was difficult to incorporate high levels of spot blotch resistance into this row type. Thus, one additional population involving Morex (i.e., D/M) was tested for its reaction to spot blotch. In this case, the susceptible parent was the six-rowed feed cultivar Dicktoo. Analysis of the D/M population served to test whether the chromosome 5(1H) QTL first identified in the S/M population would again be expressed in a different 6×6-rowed cross, but again no chromosome 5(1H) QTL was detected (Table 3; Fig. 3). QTL were, however, detected on the short arm of chromosome 3(3H), the long arm of chromosome 3(3H), and the short arm of chromosome 1(7H) near *Rcs5*. Thus, in three populations involving the same resistance source (all crosses with Morex were made with the same single plant source) and different susceptible parents, three different major effect QTL for resistance were identified—all of which were contributed by the common resistance source Morex.

The markedly different patterns of expression found for the chromosome 5(1H) QTL in the S/M, H/M, and D/M populations were dramatic and surprising, but the basis for them is not known. Previous studies have documented the potential problems that can occur in QTL detection (Bernardo 2002), especially in mapping populations that are small (i.e., $n < 200$). For example, Melchinger et al. (1998) and Beavis (1994) found that the number of QTL detected was higher in large maize populations ($n = 344$ and 400) than in smaller derived subsets ($n = 107$ and 100, respectively) of the same populations. These researchers also reported that only a few of the identified QTL were common in all derived subsets. In another study, Beavis et al. (1991) reported that only one out of five to six plant height QTL that

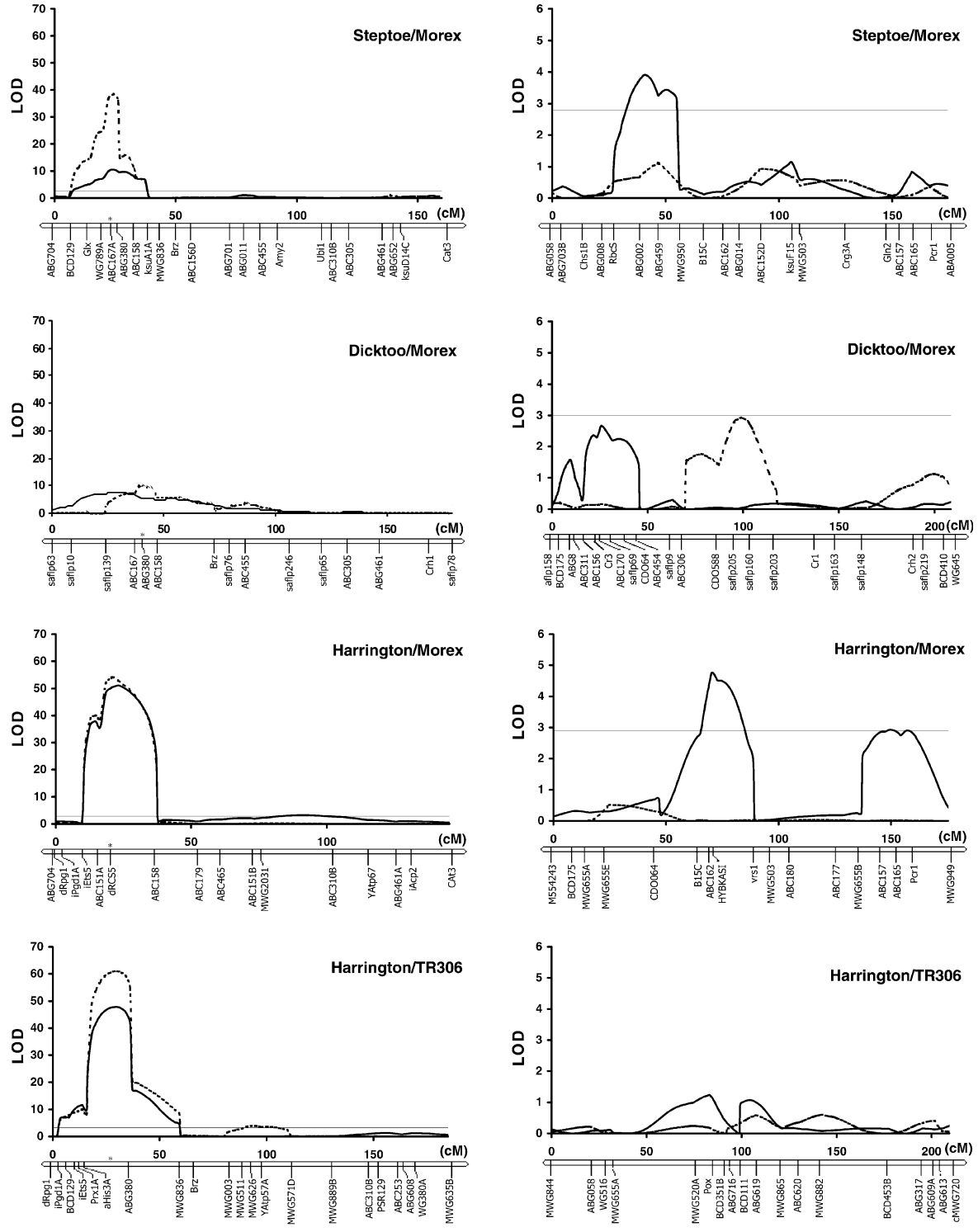

Fig. 3 LOD score profiles of QTL for spot blotch resistance in the S/M, D/M, H/M, and H/T populations. The plots show the locations of the putative QTL identified for resistance to *C. sativus* pathotype 1 based on mean quantitative trait data over all replications at the seedling (*dashed line*) and adult (*solid line*) plant stages. The LOD score scan was obtained by composite interval mapping using QTL Cartographer. The critical LOD values were determined by 1,000 permutations at $\alpha = 0.05$ as suggested by Doerge and Churchill (1996). The LOD thresholds shown in the figures were the most conservative among thresholds determined for the seedling and adult stage QTL. *Asterisks* on the chromosome 1(7H) scans indicate the putative location of *Rcs5* in different populations. Marker dRCS5 in the H/M population is based on data for spot blotch resistance used in this study, but was previously published in the Graingenes website by A. Kleinhofs

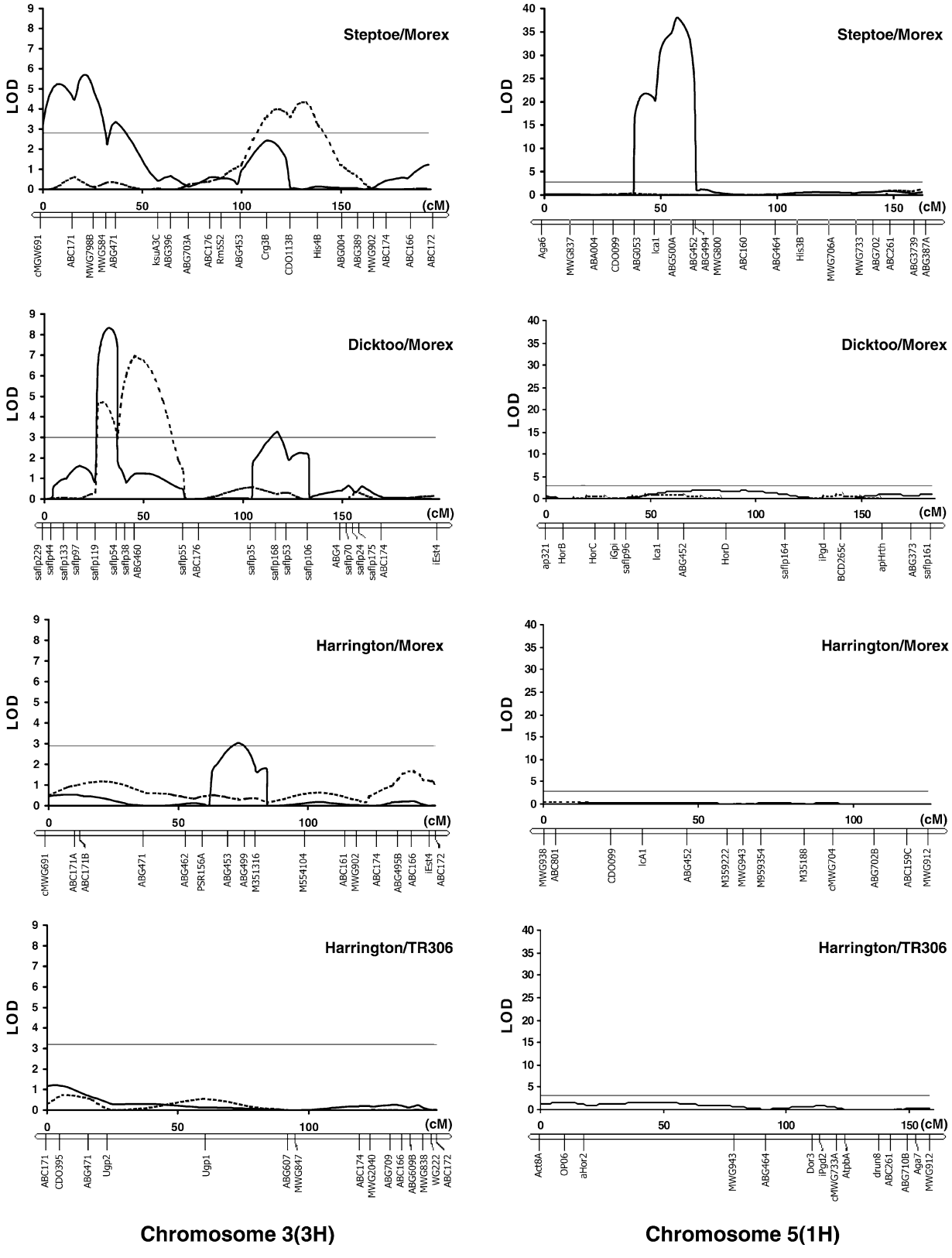

Fig. 3 (Contd.)

were identified in individual maize populations (each with $n = 112$) mapped to the same location of the same chromosome when a common female parent was used. Relatively small population sizes were used in this study (82–150 progeny). However, it is unlikely that the absence of the major effect chromosome 5(1H) QTL was

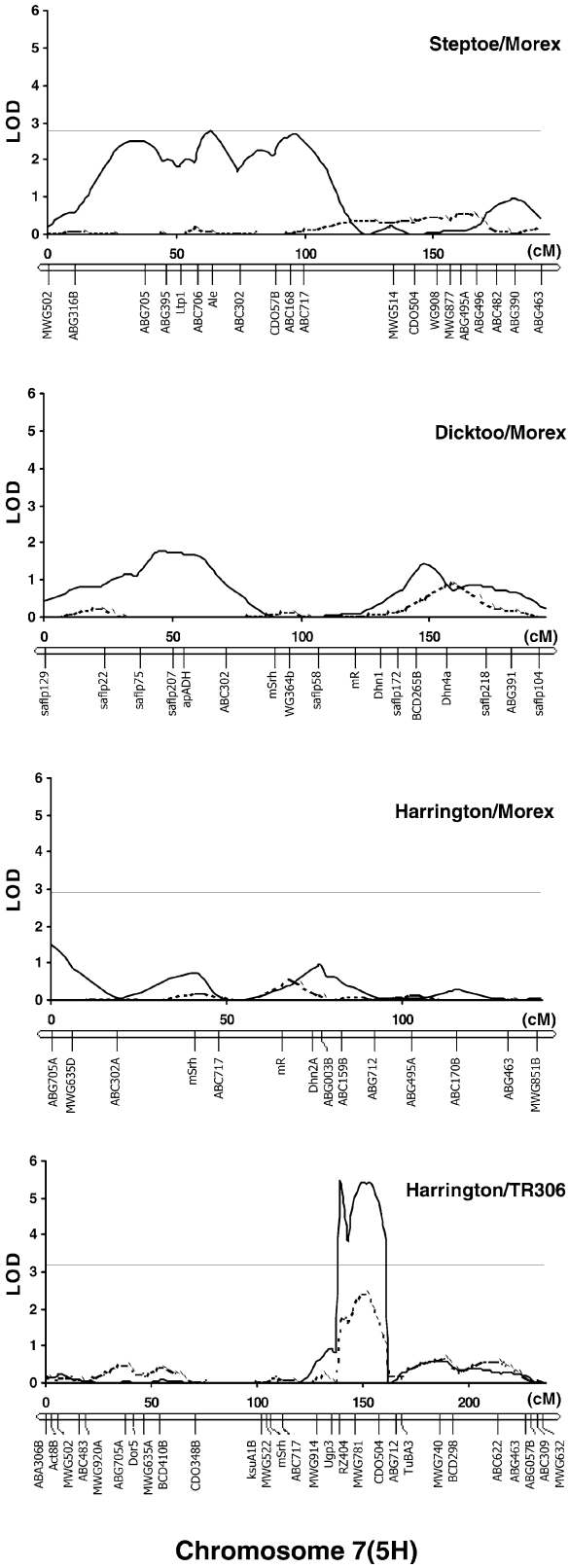

Fig. 3 (Contd.)

due to sampling error variation in the small mapping populations of H/M and D/M. Although the chromosome 5(1H) QTL contributed a very large phenotypic effect in S/M, we nevertheless investigated the possibility that it might be spurious by conducting an additional CIM analysis at the highly stringent significance level of $\alpha = 0.0001$. This analysis confirmed the same chromosomal position and phenotypic variance ($r^2 = 0.62$) for this QTL (data not shown). We also reevaluated the S/M population at St. Paul and obtained the same results previously reported in Steffenson et al. (1996). Epistasis between resistance loci could be a contributing factor for the lack of expression of the chromosome 5(1H) QTL in the H/M and D/M populations. To explore this possibility, we performed multiple interval mapping (MIM) analysis in QTL Cartographer to determine whether epistasis was present between QTL detected previously by CIM in the four populations. The analysis revealed an epistatic interaction between the adult plant QTL on chromosome 1(7H) (*Rcs-qtl-7H-2-4* at the *Rcs5* region in Table 3) and the one on chromosome 5(1H) (*Rcs-qtl-1H-6-7* in Table 3) in the S/M population, but the amount of variation explained was very low (4.4%). We also investigated possible QTL×E interactions using simple interval mapping (SIM) for multiple environments available in NQTL, a Windows version of MQTL software (Tinker and Mather 1995). The test statistic of 0.3 (a statistic similar to LOD score) for the QTL×E interaction was well below the threshold value of significance (1.6), indicating that the QTL×E interaction was not significant for *Rcs-qtl-1H-6-7*. Additionally, when CIM analyses of QTL data for individual years were performed, the QTL mapped to the same location (i.e., *Rcs-qtl-1H-6-7*) and had very similar LOD values. This result is further evidence for a nonsignificant QTL×E interaction.

Additional research is being advanced to help elucidate the underlying basis for the differential expression of the chromosome 5(1H) QTL in different genetic backgrounds. We selected two H/M progeny with the Morex resistance allele for the chromosome 5(1H) QTL and the most complete Harrington chromosome complement for all other regions, including the *Rcs5* region. These progeny were crossed to the susceptible cultivar Steptoe and will be phenotyped in the field to determine if the chromosome 5(1H) Morex allele suppressed in the original cross with Harrington will now be expressed in the new cross with Steptoe. Similarly, a H/M progeny with the Morex resistance allele at *Rcs5* and the most complete Harrington chromosome complement for all other regions was selected and crossed to Steptoe. The resulting population will be phenotyped to determine if the Morex *Rcs5* allele that conferred both seedling and adult plant resistance in the original cross with Harrington would now do the same in the cross with Steptoe. We are also developing near-isogenic lines for the chromosome 5(1H) QTL and *Rcs5* in both six-rowed and two-rowed genetic backgrounds. These lines will be a valuable tool for studying the expression of the resistance loci in uniform genetic backgrounds.

Many of the QTL detected for adult plant resistance in this study were specific to a particular genetic back-

Table 3 Chromosomal location, percent phenotypic variation explained, donor parent for the resistance allele, LOD scores, and marker intervals for all QTL conferring adult plant spot blotch resistance in the S/M, D/M, H/M, and H/T populations

		Rcs-qtl-7H-2-4	*Rcs-qtl-2H-3-5*	*Rcs-qtl-2H-7-8*	*Rcs-qtl-3H-2-4*	*Rcs-qtl-3H-9-11*	*Rcs-qtl-1H-6-7*	*Rcs-qtl-5H-10-11*
	Chromosome	1(7H)	2(2H)	2(2H)	3(3H)	3(3H)	5(1H)	7(5H)
S/M	Variation	12%	4%		6%		62%	
	Donor	Morex	Morex		Steptoe		Morex	
	LOD	10.54	3.90		5.68		37.94	
	Interval	WG789A–ABG380	RbcS–ABG459		ABC171–MWG584		ABG500A–ABG452	
D/M	Variation	20%			36%	11%		
	Donor	Morex			Morex	Morex		
	LOD	7.53			8.33	3.29		
	Interval	saflp139–ABC167			saflp119–saflp54	saflp35–saflp53		
H/M	Variation	75%		3%		2%		
	Donor	Morex		Harrington		Morex		
	LOD	51.06		4.77		3.04		
	Interval	ABC151A–ABC158		B15C–HVBKASI		ABG453–ABG499		
H/T	Variation	77%						5%
	Donor	TR306						Harrington
	LOD	47.77						5.41
	Interval	*aHis3A*–ABG380						RZ404–MWG781

ground. In contrast, the *Rcs5* region of chromosome 1(7H) contributed universally to both seedling and adult plant resistance in all four populations. The expression pattern of this resistance varied depending on the population involved. At the seedling stage, *Rcs5* (or a closely linked locus) segregated in a clear Mendelian fashion and conferred low IRs of 2–4 in both the H/M and H/T populations. A similar result was obtained in the S/M population, except that the low phenotype included more lines with IR 5 (B. Steffenson, unpublished). The significant contribution of this locus was also verified in the QTL analysis of data as the percent variation explained ranged from 67% to 85% in the three populations (Table 2). Although the other QTL were detected in the S/M and H/T populations, they accounted for only a small portion of the overall variation (4%). In the D/M population, segregation of progeny at the seedling stage did not conform to a single gene ratio. Although quantitative analysis positioned a major effect QTL in the *Rcs5* region, it explained only 30% of the phenotypic variation (Table 2). Two other QTL on chromosomes 1(7H) and 3(3H) were identified in the D/M population and contributed 9% and 19% of the phenotypic variation, respectively. The *Rcs5* region was also significant in conferring adult plant resistance. *Rcs5* (or a closely linked locus) segregated in a clear Mendelian fashion and conferred low disease severity in both the H/M and H/T populations (Table 1; Fig. 2). The significant contribution of this locus was also confirmed in the quantitative analysis of data as a QTL in the region explained 75–77% of the phenotypic variation (Table 3). Thus, in the H/M and H/T populations, the *Rcs5* region contributed to a high level of both seedling and adult plant resistance. In the S/M population, the region contributed to a high level of seedling resistance (specifically *Rcs5*) and a relatively low level of adult plant resistance (Steffenson et al. 1996). Finally, in the D/M population, the region contributed to a moderate level of resistance at both the seedling and adult plant stages. The major QTL contributing to adult plant resistance in S/M was on chromosome 5(1H) and in D/M on chromosome 3(3H) (Table 3; Fig. 3). Thus, *Rcs5* (or possibly other closely linked loci acting alone or in concert) can vary markedly in the level of resistance conferred in different populations and at different ontogenetic stages.

Relatively few studies have been advanced on the genetics and mapping of spot blotch resistance in barley. Five major Mendelian genes for resistance (seedling or adult plant) to *C. sativus Rcs1* to *Rcs5*) have been described in barley (Søgaard and von Wettstein 1987; Steffenson et al. 1996), but several are based on tenuous correlative data. Griffee (1925) reported that spot blotch resistance in the cultivar Svanhals was generally correlated with the *vrs1* (formerly *V*/*v*) locus on chromosome 2(2H), the *Blp* (formerly *B*/*b*) locus on chromosome 5(1H), and the *raw1* (formerly *R*/*r*) locus on chromosome 7(5H). Based on these loose correlations and the independent assortment of the three morphological characters, Griffee (1925) inferred that three unlinked genes (*Rcs1 Rcs3* formerly *hl1 hl3*) control resistance to spot blotch at the adult plant stage. The designation of the fourth gene *Rcs4* (formerly *hl4*) is based on the genetic data of Arny (1951). In that investigation, no correlation was found between the spot blotch resistance and eight 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 *gsh2* (formerly *gs2*) locus on chromosome 3(3H) and *vrs3* (formerly *v3*) locus on chromosome 5(1H). The seedling resistance gene identified by Stef-

fenson et al. (1996) was different from those previously described by Gonzalez Ceniceros (1990) and Griffee (1925) based on chromosome location and was designated *Rcs5*. The same was true for the major effect QTL identified on chromosome 5(1H) by Steffenson et al. (1996). Another gene conferring spot blotch resistance (at both the seedling and adult plant stages) was recently identified in the Calicuchima-sib/Bowman-BC population and was mapped to the telomeric region of the short arm of chromosome 5(1H). Since no other gene was reported in this region, the locus was designated *Rcs6* (Bilgic et al., in press).

In this study, three putative new loci were identified for spot blotch resistance based on the comparison of marker loci positions used in QTL scans (Fig. 3) and barley consensus maps (Franckowiak 1997; Langridge et al. 1995; Qi et al. 1996): one conferring seedling resistance at the centromeric region of chromosome 1(7H) (i.e., *Rcs-qtl-7H-7* in Table 2); one conferring adult plant resistance on the short arm of chromosome 2(2H) (*Rcs-qtl-2H-3-5* in Table 3); and one conferring both seedling and adult plant resistance on the short arm of chromosome 3(3H) (i.e., *Rcs-qtl-3H-4-6* in Table 2 and *Rcs-qtl-3H-2-4* in Table 3). The assertion that these loci are new is based on the fact that no QTL for resistance were previously reported at the centromeric region of chromosome 1(7H) and also on the associations found between spot blotch resistance and various morphological markers on chromosomes 2(2H) and 3(3H) (*vrs1* and *gsh2*, respectively) by previous researchers (Gonzalez Ceniceros 1990; Griffee 1925). The resistance loci reported by these workers map to a more distal position on the long arms of chromosome 2(2H) and chromosome 3(3H). The remaining seven QTL reported in this study appear to map in genomic regions where spot blotch resistance loci were previously described. These include seedling/adult plant resistance QTL *Rcs-qtl-7H-2-4* (Table. 2, 3) mapping to the *Rcs5* region where Steffenson et al. (1996) and Steffenson (2000) reported an effect; seedling resistance QTL *Rcs-qtl-3H-11-12* (Table 2) and adult plant resistance QTL *Rcs-qtl-3H-9-11* (Table 3) mapping near the region of *gsh2* on chromosome 3(3H) where Gonzalez Ceniceros (1990) reported an effect; adult plant resistance QTL *Rcs-qtl*-2H-7-8) (Table 3) mapping near the *vrs1* region on chromosome 2(2H) where Griffee (1925) reported an effect; adult plant resistance QTL *Rcs-qtl*-1H-6-7 (Table 3) mapping near ABG500A where Steffenson et al. (1996) reported an effect; and adult plant resistance QTL *Rcs-qtl-5H-10-11* (Table 3) mapping near *raw1* where Griffee (1925) reported an effect. Obviously, it is difficult to state with certainty whether all of the QTL reported in this study are indeed allelic with previously reported loci. Allelism tests will be required to resolve this question, but may be limited in some cases by difficulties in phenotyping.

The H/T population was included in this study because TR306 was previously reported to possess spot blotch resistance (B. Steffenson, unpublished) and was paired with Harrington, a susceptible parent common to the H/M population. Even though the origin of the spot blotch resistance in TR306 was not known, we thought analysis of the H/T population might contribute new information regarding the expression of spot blotch resistance in barley. Mendelian analysis of the H/T population revealed that a single gene conferred resistance at both seedling and adult plant stages. Moreover, this single gene mapped to the *Rcs5* region as was reported in the S/M and H/M populations. It is not known whether TR306 carries *Rcs5* or another closely linked gene. This question can only be resolved when the proper allelism tests are conducted.

Over the past 40 years, breeders have been very successful in retaining the chromosome 5(1H) resistance QTL in their six-rowed malting germplasm, presumably by fixing the resistance allele in elite parents and practicing occasional phenotypic selection. It appears that this resistance is highly expressed in the six-rowed genetic backgrounds of the major malting barley breeding programs in the Upper Midwest. This resistance may, however, be completely suppressed when introgressed into more diverse two- or six-rowed genetic backgrounds (e.g., H/M and D/M populations) as was clearly demonstrated in this study. Molecular markers for the chromosome 5(1H) resistance QTL are being developed. Their utility in marker assisted selection may be limited given the suppression that occurs in crosses with both two- and six-rowed susceptible parents. In the future, we will employ marker assisted selection to verify that parents used in the breeding program carry the resistance allele at the chromosome 5(1H) QTL and then continue to screen advanced breeding lines in the field to ensure that the resistance is expressed in the current breeding background.

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