Novel Septoria Speckled Leaf Blotch Resistance Loci in a Barley Doubled-Haploid Population

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ABSTRACT

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The genetics of resistance to Septoria speckled leaf blotch (SSLB), caused by *Septoria passerinii*, was studied in the Leger × CIho9831 barley doubled-haploid population. The 140 lines in the population segregated as 102 resistant and 38 susceptible, approximating a 3:1 ratio. A recombination map was developed using diversity arrays technology and other molecular markers. Quantitative trait locus (QTL) analysis demonstrated that resistance is primarily conferred either by having the

CIho9831 allele at a QTL on 6HS or by having the CIho9831 allele at both of two QTLs on 3H and 2HL. In addition, ≈1/16 of the lines were resistant for unidentified reasons. This model predicts a resistant/susceptible ratio of 11:5, which fits the phenotypic observations. Minor QTLs were detected on 2HS and 1H. DNA sequences of linked markers suggest that the 6HS, 3H, and 2HS QTLs are part of resistance gene clusters and that the 6HS and 3H QTLs share homology. The 6HS QTL is identical to or closely linked to the SSLB resistance locus *Rsp4* and the 1H QTL to the *Rsp2* or *Rsp3* locus. The 3H and 2HS QTLs are unique and offer new opportunities for pyramiding resistance genes through marker-assisted breeding for resistance to *S. passerinii*.

Septoria speckled leaf blotch (SSLB), caused by the fungus *Septoria passerinii* Sacc., has reemerged as a major disease of barley (*Hordeum vulgare* L.) in the Upper Midwest region of the United States and in the Canadian Prairie Provinces (22). The disease remains a serious problem (28) because most of the malting and feed barley varieties grown in North America are susceptible (31). The most effective response to this challenge is the development of cultivars with genetic resistance. Several sources of resistance to *S. passerinii* have been identified in cultivated barley as well as in wild barley accessions (31). Identifying unique sources of resistance is facilitated by the observation that there is a strong correlation between adult resistance in the field and seedling resistance in the greenhouse or growth cabinet (31).

Breeding barley for resistance would also be facilitated by knowledge of the number and chromosomal locations of resistance genes in these unique sources and by the identification of associated molecular markers that could be used for molecular marker-assisted selection (MAS) breeding. Various restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), and diversity arrays technology (DART) markers have been linked to the *Rsp1*, *Rsp2*, and *Rsp3* resistance genes. *Rsp1* was shown to map to barley chromosome 3HS and *Rsp2* and *Rsp3* to two closely linked loci on 1HS (18,36). A sequence-characterized amplified region (SCAR) marker was developed for *Rsp2* and *Rsp3* (36) and sequence-

tagged site (STS) markers linked to the three resistance loci have been developed for MAS breeding purposes (19). Recently, a new resistance locus, *Rsp4*, was mapped to chromosome 6H (28) and a novel resistance quantitative trait locus (QTL), *QrSp-2H*, to 2HL (34). Resistance loci from the wild species *H. vulgare* subsp. *spontaneum* C. Koch and *H. bulbosum* L. have been mapped to 2H and 6H (35) and to 4H (30), respectively. To date, five resistance loci have been mapped in barley and three others have been identified in related *Hordeum* spp. It is probable that other resistance loci await discovery.

‘CIho9831’ is resistant to *S. passerinii* at both the seedling and adult stages (31). CIho9831 has been tested with STS markers and found to be positive for one of two markers developed for *Rsp1*, negative for both markers developed for *Rsp2*, and negative for the single marker developed for *Rsp3* (19). These results showed that CIho9831 may or may not carry *Rsp1* but is unlikely to carry *Rsp2* or *Rsp3*. However, the results must be interpreted with caution because the authors found that, although the *Rsp3*-associated marker was highly diagnostic across a range of germplasm, the other STS markers were much less predictive. Thus, it is unclear whether CIho9831 contains a combination of recognized resistance genes or one or more novel resistance genes.

A doubled-haploid (DH) population has been developed from a cross between ‘Leger’ (a six-rowed Canadian barley cultivar susceptible to SSLB) and CIho9831. This population has already been characterized for resistance to net blotch (13,23) and Fusarium head blight (6) as well as for other traits, including kernel size and shape (10), chemical composition (9), salt tolerance (21), and agronomic traits (15,32).

The objective of the present study was to use SSLB phenotype and molecular marker mapping data to characterize a DH population derived from the cross Leger × CIho9831 in order to determine the number of loci contributing to the resistance of CIho9831 to SSLB, their chromosomal locations, and whether these loci are unique or correspond to known resistance genes.

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*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains one supplemental table.

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MATERIALS AND METHODS

Germplasm and subpopulations. Leger is an elite, high-yielding, six-rowed spring barley cultivar well adapted to Eastern Canada (8). CIho9831 is a two-rowed barley (GRIN database, United States Department of Agriculture–Agricultural Research Service; www.ars-grin.gov). Leger was crossed with CIho9831 and 155 DHs were developed using the *H. bulbosum* technique (5). As genotyping of the DH lines proceeded, it became apparent that the DH lines constituted three sets. The earliest and the latest produced DHs shared common CIho9831 (CI, male) alleles; however, the middle set of DHs had a different CI allele at some loci. All DHs had the same Leger (L) allele at all loci. Genotyping multiple Leger seed and multiple CIho9831 seed revealed that Leger was homogeneous but CIho9831 contained two genotypes. With reference to the mapping population, we will refer to the large subpopulation (LS), the small subpopulation (SS), and the whole population (WP), which is a composite of these two highly related half-sib populations.

Disease evaluations. The parents and the progeny population (consisting of 155 DH lines) were tested for resistance to *S. passerinii* at the seedling stage in the greenhouse. Plant growth conditions, inoculum preparation, and inoculation methods were as described by Toubia-Rahme and Steffenson (30,31). Parental plants and plants from the DH lines were grown in plastic pots (10 by 10 cm) filled with a commercial potting mixture (3:1 peat moss/perlite) (number 1 Sunshine Mix; Fisons Horticulture, Inc., Vancouver, BC, Canada). Slow-release (14-14-14, N-P-K, 2 g/pot) and water-soluble (15-0-15, N-P-K, N rate of 536 ppm) fertilizers were added at the time of planting. Plants were grown in a greenhouse at $20 \pm 3^\circ\text{C}$ with 14 h of supplemental lighting (230 to $270 \mu\text{E m}^{-1} \text{s}^{-1}$ supplied by 1,000-W metal halide lights). Seedlings were inoculated with the *S. passerinii* isolate (ND97-15) 10 to 12 days after planting, when the second leaves were fully expanded. Inoculum production was conducted using the protocols described by Toubia-Rahme et al. (30,31). Pycnidiospore suspension (5×10^5 pycnidiospores/ml) was applied on seedlings at a rate of ≈ 0.5 ml/plant, using an atomizer pressured by an air pump at 60 kPa. Inoculated seedlings were incubated at 21°C in the dark and 25°C in the light for 72 h in chambers maintained near-saturation by periodic misting with ultrasonic humidifiers. The first 40 h of incubation were in darkness, followed by a photoperiod of 5 h (150 to $270 \mu\text{E m}^{-1} \text{s}^{-2}$), a dark period of 22 h, and a photoperiod of 5 h. Plants were allowed to dry off slowly before being transferred to the greenhouse under the same conditions previously described. In addition to routine watering, plants were misted twice a day to enhance infection and production of pycnidia. Infection responses (IRs) were assessed on the second leaves of seedlings 20 days after inoculation using a 0-to-5 rating scale, where 0, 1, 2, and combinations thereof were considered indicative of resistance and 3, 4, 5, and combinations thereof were considered indicative of susceptibility (31). Lines that exhibited yellowing but no or few pycnidia were scored as resistant. Resistant ('Atlas', plant introduction [PI] 539108) and susceptible ('Betzes', PI 129430) checks were included in all experiments. The experiment was repeated twice.

Genotyping with molecular markers and construction of recombination map. Genomic DNA was extracted from young leaves using the cesium chloride technique, as described previously (23), or with the Dellaporta method (7). The population was genotyped with DArT markers by Triticarte (www.triticarte.com.au), a subsidiary of Diversity Arrays Pty Ltd. (www.diversityarrays.com). In an effort to obtain many markers polymorphic across the WP, the array (version 1.7) containing DArT markers polymorphic in Australian and European germplasm as well as in wild barley accessions was used. Six parental samples and 88 DH lines were genotyped using a 96 well plate with two blank control wells.

Data were analyzed for 87 DH lines, consisting of 57 lines from the LS and 30 lines from the SS.

The initial DArT marker map was built using 496 DArT markers, which were polymorphic across the WP, showed normal segregation, and had both a quality score and a call rate above 80%, as recommended by Triticarte and by Wenzl et al. (33). The map was constructed using Mapmaker v. 2.0 (16) for the Apple Macintosh computer by grouping the markers at increasing logarithm of the odds (LOD) values (LOD 3.0 to 10.0, $\theta = 0.40$), ordering them using the "Compare" and "Try" commands, testing marker order using the "Ripple" command, and then mapping using the Kosambi mapping function. For coincident markers, the marker with the highest LOD score was retained.

The DArT map was enhanced with 37 additional molecular markers and 2 morphological markers to fill gaps, improve resolution in specific regions, or cross-reference other studies. RAPD markers were employed as in Molnar et al. (23) and the morphological markers have been reported by Choo et al. (5). STS markers from A. Graner and T. Blake were used as described by Graner et al. (11) and Larson et al. (17), respectively. Polymerase chain reaction (PCR) products were separated on 2% agarose gels or 5% Nusieve gels and stained with ethidium bromide. PCR conditions for SSR markers were the same as those described previously (2,20,26) and SSR PCR products were then separated on a 6% denaturing polyacrylamide gel and silver stained according to Bassam and Caetano-Anolles (1). AFLP markers were generated by following the manufacturer's instructions (Gibco/BRL/Invitrogen), which included labeling fragments with P^{33} , separation on 6% polyacrylamide gel, and exposure to X-ray film.

QTL analysis. First, a correlation analysis was performed between the phenotypic data and each individual marker using the NQTL computer program (formerly named MQTL) (29). In all, 10,000 permutations of the data were used to calculate the threshold value, which must be exceeded by the test statistic (TS) to declare a QTL to be significant. To search for additive or epistatic interactions, the peak marker for the strongest QTL was fixed and the full marker dataset reanalyzed to identify markers giving strong correlation.

Second, the recombination map was used for simplified interval mapping (SIM) QTL analysis performed with NQTL. The phenotypic trait means of each DH line were analyzed with an experiment-wide error rate of 5%. As before, 10,000 permutations were used to calculate the threshold. Regions with a TS above the threshold were considered a QTL. The major QTL was then anchored and the map was rescanned for regions that had additive or epistatic effects.

Third, simplified composite interval mapping (sCIM) was done on the recombination map using 14 background markers, 2 per chromosome. Analysis parameters were 10,000 permutations, 5% error rate, and a walking speed of 5 centimorgans (cM).

The DNA sequences of many of the DArT markers are available from Diversity Arrays Pty. Ltd. (<http://www.diversityarrays.com/sequences.html>) and were used to annotate markers near each QTL using BLASTN, available on the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/>).

RESULTS

Disease assessment. The parents Leger and CIho9831 exhibited a clear differential reaction to *S. passerinii*. Leger was susceptible (IR of 4 to 5), whereas CIho9831 was highly resistant (IR of 0). Of the 155 DH lines tested, 13 lines exhibited different reactions between the two experiments and were omitted. Two additional lines were assessed on just a single plant and were also omitted from the calculation of the segregation ratio. The remaining 140 homozygous DH lines exhibited a ratio of resistant/susceptible (R/S) of 102:38 (2 lines with IR of 2.5 scored as

S) (Fig. 1). This segregation ratio fits a 3:1 ratio ($\chi^2 = 0.343$, $P = 0.56$, $df = 1$), suggesting that the resistance of CIho9831 to *S. passerinii* could be governed by two independently segregating genes, either one of which confers resistance.

DArT marker map. High-quality DArT markers were used to construct a recombination map of the whole Leger × CIho9831 population (Fig. 2). In total, 1,083 DArT markers were screened and 496 were polymorphic across the WP, of which 334 had known map locations. Because many DArT markers co-mapped, only the one with the highest LOD score was retained during the map-building process. Complete linkage groups were obtained corresponding to chromosomes 2H, 5H, 6H, and 7H. In the case of chromosomes 3H and 4H, the WP DArT markers fell into three small linkage groups, which were easily joined by the addition of a few non-DArT markers. A similar situation arose on chromosome 1H, where mapping the WP DArTs left two large gaps, which were easily filled with either WP non-DArT markers or subpopulation-specific DArT markers. Both versions of 1H are shown in Figure 2; the first version was used for the QTL analysis and the second version facilitates convenient comparison to the DArT consensus map. The two versions of the final full map include 163 (with 1H map) or 180 (with 1H* map) DArT markers. The final map also includes 37 non-DArT molecular markers and two morphological markers (row number, lemma color). Total length of the map is 1,246 cM, highly consistent with the 1,161 cM of the DArT consensus map (33) and other published studies.

The Leger × CIho9831 map was almost identical to the published barley DArT consensus map (33), with a few exceptions (Supplemental Table 1). Each difference was individually tested and, in all cases, for the present data, the marker order shown on this population's DArT map was preferred over the consensus order with respect to LOD values and length of the linkage group. Most differences were simple flips in marker order for tightly linked markers, and such differences are often observed in recombination mapping. In addition, three more DArT markers mapped to different chromosomes on the present map than on the consensus map. Based on the low frequency of such observations and the high quality of the data, it is possible that these are members of duplicated loci.

QTL analysis of SSLB phenotype data. The results of QTL analysis for resistance to SSLB are summarized in Figures 2 and 3 and Tables 1 and 2. Although QTL analysis was performed for all 36 combinations of disease rating (experiments 1, 2, and mean) by the three populations (WP, LS, and SS) by four QTL techniques (SIM, sCIM, additive, and epistatic), to be conservative in the analysis, only those combinations that detected a QTL (i.e., a TS above threshold) using the mean disease rating data are listed in Tables 1 and 2.

QTL analysis consistently showed a major QTL on the short arm of chromosome 6H at the AFLP marker E35616, and close to DArT markers bPb-7165 and bPb-5252 and SSR marker BMAG500 (Table 1; Figs. 2 and 3A and B). Experiment 1 data had a higher TS than experiment 2 and explained more of the phenotypic variation than experiment 2 (62 compared with 53%) (data not shown). This QTL was detected in the WP (61%) as well as in both the LS (61 to 64%) and the SS (58%) by using both SIM and sCIM. The CI allele from CIho9831 increased resistance to *S. passerinii* and this was reflected by a decrease in disease rating of 1.87 to 2.78 units, on a scale of 0 to 5 (Table 1).

A second large-effect QTL was identified on chromosome 3H by sCIM in the WP and by testing in the WP and in the LS for epistatic or additive interactions with the chromosome 6H QTL (Tables 1 and 2; Figs. 2 and 3B to D). The QTL peaked near one of four closely linked markers (UMB301, bPb-3025, bPb-2018, and ABG070), depending on the particular analysis. It explains 12% of the phenotypic variation, and the CI allele at this locus decreased the IR by 1.17. The combination of the chromosome

6H and 3H QTLs explained, in total, 70% of the phenotypic variation. For lines that have the CI allele at the chromosome 6H locus, having the CI allele at the chromosome 3H locus increases resistance to SSLB and reduces the IR by 2.32 to 2.36 units.

Two smaller QTLs were detected on opposite arms of chromosome 2H by testing for epistatic effects when the main QTL on 6H was used as a cofactor (Table 2; Figs. 2 and 3D). This first QTL on 2HS peaked at DArT marker bPb-1212, depending on the particular analysis; the peak of the second QTL on 2HL is located at one of the tightly linked DArT markers bPb-8117, bPb-5755, and bPb-2971. For lines that have the CI allele at the 6H QTL, having CI alleles at these two QTLs increases resistance to SSLB and decreases the IR by 1.62 and 1.76 to 2.81 units, respectively. The combination of 6H, 3H, 2HL, and 2HS QTLs explains 74% of the phenotypic variation.

Consideration of the epistatic interaction between the 6H QTL and either the 3H, 2HS, or 2HL QTL suggests that the CI/CI and CI/L predicted genotypic classes have similar IR values (Table 2). As a result in each pairing, only three IR phenotypes are observed, indicating that the 6H QTL exhibits dominant epistasis over the other three QTL.

In the present barley population, a minor QTL with epistatic effects was detected on the short arm of chromosome 1H near DArT markers bPt-0005 (7.2 cM) and bPb-5877, but only in the SS. For lines that have the L allele at the 6H QTL, having the CI allele at the 1H QTL unexpectedly and dramatically decreased resistance and increased IR by 3.94 units (Table 2). In the Leger × CI9831 population, DArT marker bPt-0005 co-maps with SCAR marker E-ACT/M-CAA-170a (data not shown), developed by Zhong et al. (36) for selecting the *Rsp2* SSLB resistance allele. In addition, this QTL is in the region of the *hor2* hordein locus.

Analysis of genotypic classes. To better understand the role of each of these five QTLs in generating the resistance phenotype, the different genotypic classes were examined and a subset presented in Table 3. Approximately half of the lines in the population have the CI allele at the peak marker for the 6H QTL and mixed alleles (m) at the other four QTLs (genotype Rmmmm) and all are resistant, indicating that the 6H QTL is a single major resistance gene. There is relatively little effect on IR of having resistance alleles at the other four QTLs for lines that already have the CI resistance allele at the 6H QTL. The other half of the population is composed of lines having the L susceptibility allele at the 6H QTL (Smmmm), and these are mixed for both resistance and susceptibility. For these lines, if one also considers their genotype at the peak marker for the 3H QTL, then those with the L allele (SSmmmm) are all susceptible but those with the CI allele (SRmmmm) are mixed. The similar situation holds true for the 2HL

Fig. 1. Histogram of the Septoria infection response rating of 140 doubled-haploid lines from the Leger × CIho9831 population relative to parental averages.

QTL, where most (R/S, 1:11) of those with the L allele (SmSmm) are susceptible but those with the CI allele (SmRmm) are mixed. The impact of the 3H QTL on IR ($3.09 - 1.99 = 1.1$) (Table 3) is also very similar to that of the 2HL QTL ($3.09 - 2.1 = 0.99$), by this analysis. Eight of nine lines having CI alleles at both the 3H and 2HL QTLs (SRRmm) are resistant. The nine lines have an average IR of 0.95, very close to that predicted for an additive interaction between the 3H and 2HL QTLs ($3.09 - 1.1 - 0.99 = 1.0$). Similarly, the 2HS QTL appears additive with 3H and 2HL QTLs but with smaller impact on IR. To address the latter two points, the WP was divided into two halves, based on lines having the L or CI allele at the major 6H QTL, and then QTL analysis was performed independently in each half. No additional QTL were detected in the half of the population with the CI allele at the 6H QTL. In the half of the population having the L allele at the

Fig. 3. Quantitative trait loci (QTLs) probability scans showing the variation in test statistic (TS) along the maps of the seven chromosomes. Results are shown for analyses in the whole population using the following: **A**, simplified interval mapping; **B**, simplified composite interval mapping; **C**, additive interaction with the 6H QTL; and **D**, epistatic interaction with the 6H QTL. Horizontal lines show the respective TS thresholds for declaring a QTL.

6H QTL, QTL analysis detected both the 3H and the 2HL QTLs, and they were strongly epistatic (data not shown). It would appear that there are at least two classes of resistant lines. The first consists of lines having the CI allele at the 6H QTL (Rmmmm) and the second of those that have the L allele at the 6H QTL but have CI alleles at both the 3H and 2HL QTLs (SRRmm) and, ideally, also at the 2HS QTL (SRRRm). All other genotypic classes at the 6H, 3H, 2HL, and 2HS loci are susceptible or a mix of resistant and susceptible. One-half and one-eighth of the DH lines are expected to have the Rmmmm and SRRmm genotypes, respectively, and be resistant. However, these two genotypes do not account for all the resistant lines. There are 12 resistant lines plus 23 susceptible lines plus 3 lines which scored resistant in one experiment and susceptible in the other experiment within the Smmmmm half of the population, and 8 of these resistant lines have the SRRmm genotype. The remaining four resistant lines are resistant due to one or more unidentified factors. Because eight resistant lines have genotype SRRmm, which is expected in 1/8 of DH lines in a balanced population, we can estimate that the mechanisms responsible for the additional four resistant lines would be expected in $\approx 1/16$ of the DH lines. A complex genetic model (three loci or two genotypes + unidentified factor) gives an expected R/S ratio of 11:5, which is not significantly different from the observed R/S ratio of 102:38 (χ^2 value = 1.099, $P = 0.2944$, $df = 1$).

Genic environment of the chromosome 6H and 3H QTLs.

The DNA sequences of several of the DArT markers mapped in Leger $\times$ CIho9831 and linked to the 3H and 6H QTLs are available on the Diversity Arrays Pty. Ltd. website. Additional sequenced DArT markers mapping to these two genomic regions are identified on the DArT consensus map (33). The sequences of such DArT markers were screened by BLASTN analysis against the NCBI nucleotide database.

The closest DArT marker to the peak of the 6H QTL is bPb-7165, which has homology to a wheat chromosome 3B-specific BAC clone containing an *Hv-pg4* homolog and HGA-like sequences. Those *Hv-pg4* and HGA-like sequences have homologs at the barley leaf rust resistance *Rph7* multigenic locus (Table 4). Co-mapping DArT markers bPb-8398 and bPb-8492 have high homology to the barley stem rust resistance gene *Rpg1* and the wheat leaf rust resistance gene *Lr1*, respectively. Other closely linked DArT markers (bPb-9749 and bPb-9807) also have homologies to various disease resistance genes (Table 4). This observation suggests that the 6H QTL and the previously reported *Rsp4* locus, which maps to the same region, are members of a cluster of resistance genes and resistance gene analogs. Within this cluster, more detailed allelic or sequencing studies are required to determine whether the Leger $\times$ CIho9831 QTL and *Rsp4* are allelic or at tightly linked loci.

TABLE 1. Map positions of quantitative trait loci (QTLs) detected by simple interval mapping (SIM), simplified composite interval mapping (sCIM), or additive interaction (Add) analysis and their effects detected for resistance to Septoria speckled leaf blotch in the Leger $\times$ CIho9831 doubled-haploid population

Chr. ^a	Markers at QTL peak (or marker/marker flanking peak)	Position of QTL peak (cM)	TS (or LOD) of peak marker ^b	Threshold for TS (or LOD) to declare a QTL ^b	QTL support interval (cM/cM)	Population variation explained (%)	Population ^c	QTL method	Effect on IR of CI allele ^d
6H	E35616	29.9	71.5 (15.7)	13.0 (2.9)	27.1/30.4	61	WP	SIM	-2.74
6H	E35616	29.9	89.2 (19.6)	15.3 (3.4)	29.9/37.3	61	WP	sCIM	-2.67
6H	E35616	29.9	47.1 (10.4)	13.3 (2.9)	23.0/25.3	64	LS	SIM	-2.78
6H	E35616	29.9	62.9 (13.8)	14.7 (3.2)	23.0/39.3	61	LS	sCIM	-2.78
6H	E35616 and BMAG500	29.9, 33.4	24.9 (5.5)	13.7 (3.0)	14.3/10.1	58	SS	SIM	-2.61
6H	BMAG500/bPb-5252	38.4	29.9 (6.6)	14.5 (3.2)	15.0/12.9	58	SS	sCIM	-1.87
3H	bPb-2018	16.4	20.7 (4.6)	15.3 (3.4)	2.8/2.3	12	WP	sCIM	-1.17
3H	bPb-2018	16.4	18.2 (4.0)	13.3 (2.9)	7.8/2.3	12	WP	Add	-1.17

^a Chromosome.

^b Logarithm of the odds (LOD) values estimated by multiplying the test statistic (TS) $\times$ 0.22 (29); cM = centimorgans.

^c QTL detection in whole population (WP) or in large (LS) or small (SS) subpopulation.

^d IR = infection response and CI = CIho9831 allele.

One of the peak markers for the 3H QTL is bPb-3025. The sequence of the co-mapping marker bPb-1137 is highly homologous to that of the 212-kb barley contig 211252, which spans the barley *Rph7* leaf rust resistance locus and has been shown to contain four members of the HGA gene family and six other genes, including *Hv-pg4* (4). The proximal flanking DArT marker bPt-1350 and the distal flanking SSR marker UMB301 both have homology to wheat leaf rust resistance kinases, suggesting that they are part of a disease resistance cluster at this genomic region.

DISCUSSION

Subpopulations. This study showed an unexpected structure within the DH mapping population, which was easily detected because the DH lines were numbered in chronological order of their production. The origin of the two subpopulations could be traced to the use of two genotypes of the male parent CIho9831. This reinforces the desirability of using DH parents when developing a mapping population. However, this is seldom the case and, as a result, we expect that the present barley mapping

population is not unique in having such structure. Because of the structure, extra care was required when analyzing the mapping and QTL data to ensure that population structure did not affect results. In the present study, the same QTLs were identified by using separate QTL analysis in each subpopulation and in the combined population, with a few minor exceptions, and analysis in the combined population was generally more robust. This is expected because most QTLs are in genomic regions in which the two subpopulations are indistinguishable.

Genetic models. Septoria IR rating of 140 DH lines was largely bimodal (Fig. 1). The observed R/S ratio of 102:38 closely matched a 3:1 ratio. Qualitative genetic analysis suggested that two resistance loci might be responsible for resistance in the Leger × CIho9831 population. Quantitative genetic analysis suggested a more complex genetic model. A major QTL for resistance to SSLB was detected on chromosome 6HS and a second major QTL on 3H. The combined additive effect of these genes explained 70% of the phenotypic variation. Several additional QTLs were also detected, the most important of which was on 2HL. Analysis of genotypic classes indicated that most resistant

TABLE 2. Quantitative trait loci (QTLs) (epistatic) map positions and their effects detected for resistance to Septoria speckled leaf blotch in the Leger × CIho9831 doubled-haploid population

Chr. ^a	Marker at peak ^b	Position (cM) ^c	TS (LOD) ^d	Threshold TS (LOD) ^{d,e}	Support (cM) ^f	Pop. ^g	IR scores ^h				Effect ⁱ
							CI/CI	CI/L	L/CI	L/L	
3H	bPb-3025	18.7	32.2 (7.1)	13.7 (3.0)	4.9/5.0	WP	0.55	0.62	2.19	4.55	-2.36
3H	ABG070/UMB301	8.6	21.0 (4.6)	20.2 (4.4)	0/10.1	LS	0.74	0.69	2.33	4.65	-2.32
2HS	bPb-1212	22.4	15.4 (3.4)	13.7 (3.0)	4.9/5.0	WP	0.70	0.44	2.71	4.33	-1.62
2HL	bPb-8117/bPb-2971	165.7	14.3 (3.2)	13.7 (3.0)	0/0	WP	0.62	0.54	2.32	4.08	-1.76
2HL	bPb-8117	160.7	19.6 (4.3)	16.9 (3.7)	27.7/0	SS	0.43	0.36	1.31	4.12	-2.81
1H	bPt-0005/bPb-5877	6.2	18.0 (4.0)	16.9 (3.7)	5.0/0	SS	0.44	0.32	3.94	0	3.94

^a Chromosome.

^b Marker at QTL peak (or marker/marker flanking peak).

^c Position of QTL peak; cM = centimorgans.

^d Logarithm of the odds (LOD) values estimated by multiplying the test statistic (TS) × 0.22 (29).

^e Threshold for TS (or LOD) to declare a QTL.

^f QTL support interval (cM/cM).

^g QTL detection in whole population (WP) or in large (LS) or small (SS) subpopulation.

^h Infection response (IR) scores of genotypic classes for epistasis between 6H QTL and tested QTL. Allele designations are Leger (L) and CIho9831 (CI).

ⁱ Effect on IR of CI at test locus.

TABLE 3. Genotypic classes, Infection response (IR) of class, and distribution of lines per class

Genotypic classes at QTL ^a					IR, class phenotypes, and ratio of lines ^{b,c}						
					Whole population		Large subpopulation		Small subpopulation		Overall
6H	3H	2HL	2HS	1H	IR	Phenotype	IR	Phenotype	IR	Phenotype	Phenotype
R	...	...	...	...	0.46	R (47R)	0.69	R (30R)	0.35	R (17R)	R
R	R	...	...	...	0.53	R (22R)	0.69	R (11R)	0.36	R (11R)	R
R	R	R	...	...	1.08	R (10R)	0.64	R (6R)	0.43	R (4R)	R
R	R	R	R	...	0.83	R (6R)	0.90	R (5R)	0.50	R (1R)	R
R	R	R	R	R	1.03	R (4R)	1.03	R (4R)	–	nil	R
S	S	S	S	S	4.00	S (1S)	–	nil	4.00	S (1S)	S
S	S	...	...	...	4.95	S (12S)	4.84	S (8S)	4.26	S (4S)	S
S	...	S	...	...	4.05	M(1R, 1R/S, 11S)	4.13	M (1R, 1R/S, 7S)	3.89	S (4S)	M/S
S	...	...	...	...	3.09	M (12R, 3R/S, 23S)	3.26	M (8R, 3R/S, 16S)	2.74	M (4R, 7S)	M
S	R	...	...	...	1.99	M (12R, 3R/S, 9S)	2.04	M (8R, 3R/S, 7S)	1.51	M (4R, 2S)	M
S	...	R	...	...	2.10	M (8R, 5S)	2.69	M (4R, 4S)	1.15	M (4R, 1S)	M
S	...	...	R	...	2.51	M (8R, 7S)	2.98	M (4R, 5S)	1.67	M (4R, 2S)	M
S	...	...	...	R	3.09	M (5R, 1R/S, 8S)	3.88	M (2R, 1R/S, 7S)	1.31	M (3R, 1S)	M
S	R	R	...	...	0.95	M (8R, 1S)	1.45	M (4R, 1S)	0.44	R (4R)	M/R
S	R	...	R	...	2.00	M (8R, 6S)	2.53	M (4R, 5S)	1.05	M (4R, 1S)	M
S	R	...	...	R	1.49	M (6R, 1R/S, 2S)	2.54	M (2R, 1R/S, 2S)	0.44	R (4R)	M/R
S	...	R	R	...	1.39	M (7R, 2S)	2.15	M (3R, 2S)	0.44	R (4R)	M/R
S	...	R	...	R	2.36	M (5R, 1R/S, 4S)	3.19	M (2R,1R/S, 3S)	1.31	M (3R,1S)	M
S	R	R	R	...	0.94	M (7R, 1S)	1.44	R (3R,1S)	0.44	R (4R)	M/R

^a R alleles are contributed by CIho9831 at 6H, 3H, 2HL, and 2HS quantitative trait loci (QTLs) and by Leger at the 1H QTL. S and ... (mixed) alleles at QTL are also indicated. Classes defined by QTL peak markers E35616 (6H), UMB301 (3H), bPb-8117 (2HL), bPb-1212 (2HS), and bPt-0005 (1H).

^b Class phenotypes: resistant (R), susceptible (S), or mixed (M).

^c Doubled-haploid lines that scored as R on one pathology test and S on the other are noted as R/S but were excluded from the calculation of the average IR for that genotypic class. —, not applicable.

lines either had the CI allele at the 6HS QTL or had CI alleles at both the 3H and 2HL QTLs. In addition, $\approx 1/16$ of the DH lines in the population were resistant due to one or more unidentified factors. According to the resultant genetic model, 5/8 of the DH lines are resistant due to two genotypes generated at three loci, and an additional 1/16 of the DH lines are resistant for unidentified reasons. Thus, the genetic model emerging from quantitative analysis plus analysis of genotypic classes differs from the genetic model suggested by qualitative genetic analysis. Both models fit the observed ratio of resistant and susceptible lines but only the more complex model also fits the QTL analysis and the analysis of genotypic classes.

Chromosome 6H QTL. An unnamed resistance locus from wild barley (*H. vulgare* subsp. *spontaneum*) has been mapped to chromosome 6H bins 10 to 14 (35). In cultivated barley, a different resistance locus, given the name *Rsp4*, has been mapped to chromosome 6H bins 3 to 4 by St. Pierre et al. (28). The resistance QTL on chromosome 6H identified in the present study is linked to DArT markers whose map locations, as shown on the consensus map of Wenzl et al. (33), overlap the DArT markers flanking *Rsp4*. Therefore, recombination mapping suggests that the QTL identified in the present study and the *Rsp4* locus map very close to each other and may actually be the same locus, though perhaps carrying different resistance alleles. A more accurate assessment of map locations for *Rsp4* and the Leger $\times$ CIho9831 QTL would require a much larger mapping population.

St. Pierre et al. (28) reported that their SSLB resistant parental line, Sep2-72, derived its *Rsp4* resistance gene from the line Pc-84. Pc-84 is a six-rowed breeding line developed at CIMMYT (GRIN accession PI 584764, NSGC accession 5543) and, based on pedigree, derives its SSLB resistance from a series of sister lines, Gloria-sib/Copal-sib (14,31). In contrast, CIho9831 is a two-rowed accession. Although it remains to be determined whether the 6H QTL identified in the present study is or is not the same locus as *Rsp4*, the level of resistance in the two lines is comparable because, in parallel testing, CIho9831 and Pc-84

scored IRs of 0 to 1 and 1, respectively (31). Further allelic studies are required to address this question, which will have implications for the utilization and perhaps pyramiding of these two resistance genes within barley breeding programs.

Disease resistance genes and resistance gene analogs are often organized into clusters, and it is possible that *Rsp4* and the QTL identified in the present study are tightly linked within such a cluster. Evidence supporting the presence of a cluster includes the fact that many of the DArT markers mapping to this region have homology to disease resistance genes (Table 4) and that the same regions (bins 3 to 4) are known to carry other resistance genes for scald (27) and net blotch (28). Further, based on mutual tight linkage to SSR marker BMAG500, these two SSLB resistance loci are tightly linked to leaf rust resistance locus *Rph13* and resistance QTLs for powdery mildew (*Rbg-2-5*), leaf scald (*Rrs-2-5*), and net type net blotch (*Rpt-4-5*) (35).

Chromosome 3H QTL. The SSLB resistance gene *Rsp1* has been mapped to chromosome 3HS, distal to the DArT markers bPb-9945 and bPb-6978 (18). From this, the position of *Rsp1* on the DArT consensus map can be estimated as 31.8 cM. By comparison, eight DArT markers within the support interval of the QTL identified in the present study are located at map positions of 1.0 to 10.2 cM on the DArT consensus map. Thus, the CIho9831 QTL identified in the present study appears to be a unique resistance locus well separated from *Rsp1*. This new SSLB resistance gene is located in a region of DArT markers with homology to several cereal rust resistance genes (Table 4), including *Rph7*, as well as a net-type net blotch resistance QTL (*Rpt-1-2*) (35). It has potential for breeding barley for SSLB resistance.

Chromosome 2H QTLs. Two additional QTLs were detected on chromosome 2H at peak markers that map at 42.5 cM on 2HS and at 123 to 133 cM on 2HL on the DArT consensus map (33). These map positions are well separated from that of an SSLB resistance QTL (*Rsp-7-11*) identified in wild barley (*H. vulgare* subsp. *spontaneum*), which mapped to chromosome 2H and peaked distal to the SSR marker BMAG0518 (72.5 cM on

TABLE 4. Resistance genes and analogs linked to Septoria speckled leaf blotch resistance quantitative trait loci (QTLs)

Chr. ^a	Present map (cM) ^b	Consensus map (cM) ^b	Marker	Homologies identified by BLAST (% base pair match)	Accession number of match
6H	30.0	24.0	BMAG500	...	...
6H	...	26.5	bPb-8398	Barley serine/threonine kinase-like protein (76)	DQ469713.1
6H	...	26.5	bPb-8398	Pseudogene of barley stem rust resistance gene <i>Rpg1</i> (70)	AF509765.1
6H	...	26.5	bPb-8492	Wheat calmodulin (71)	U48691.1
6H	...	26.5	bPb-8492	Wheat leaf rust resistance gene <i>Lr1</i> (69)	EF567062.1
6H	...	26.5	bPb-8492	Wheat malate dehydrogenase (68)	EF109239.1
6H	23.0	26.5	bPb-7165	<u>Near peak marker for 6H QTL^c</u>	...
6H	23.0	26.5	bPb-7165	Wheat BAC contig with homology to <i>Hv-pg4</i> , HGA-like, and glycosyl-transferase genes [the same genes are part of <i>Rph-7</i> locus at 3HS QTL (70)]	FN564434.1
6H	...	28.4	cMWG652A	Barley catalase 2 (99)	AF021939
6H	...	28.8	bPb-6659	Duplicate of bPb-8398	...
6H	...	30.8	ksuA3b	Position of <i>Rsp4</i> from comparative mapping	...
6H	...	33.7	bPb-9749	<i>Triticum monococcum</i> peroxidase 6 (97)	AY857760.1
6H	...	38.0	bPb-9807	Barley NBS-LRR1 disease resistance protein-like protein (79) ^d	FJ807681.1
6H	...	38.0	bPb-9807	Barley resistance gene analog S-112 (79)	AJ507088.1
3H	...	4.8	bPb-3865	Maize receptor-like cytoplasmic kinase (76)	NM_001152713.1
3H	...	6.0	bPb-7937a	Castor bean hydrolase (88)	XM_002531912.1
3H	...	6.0	bPt-1350	Oat receptor kinase gene cluster (80); wheat leaf rust resistance kinase (<i>Lrk10</i> , <i>Lrk14</i> , <i>Lrk19</i> , others) (80+)	AY083680.1; U51330.1; others
3H	...	10.2	bPb-1137	<u>Near peak marker for 3H QTL^c</u>	...
3H	...	10.2	bPb-1137	Contig for barley leaf rust resistance <i>Rph7</i> locus (92)	AF521177.1
3H	...	...	bPb-7965	Complement of bPb-1137	...
3H	...	...	bPb-7965	Contig for barley leaf rust resistance <i>Rph7</i> locus (95)	AF521177.1
3H	14.1	...	UMB301	Wheat Ark and Lrk resistance genes (80)	AF085169
3H	...	30.7	CDO395	Linked to <i>Rsp1</i> by comparative mapping ^e	...

^a Chromosome.

^b cM = centimorgans.

^c Underlined notation of the position of the peak at the major 6H and 3H QTLs.

^d NBS-LRR = nucleotide-binding site leucine-rich repeat.

^e Lee and Neate (18,19); Wenzl (33).

consensus map) (35). Yu et al. (34) reported a QTL on 2HL between the DArT markers bPb-0541 and bPb-5755. The peak marker for the current QTL on 2HL is bPb-8117 and co-maps with bPb-0541 on the consensus map. Therefore, only the QTL on 2HS is believed to be unique. The SSLB resistance QTL identified in the present study at position 42.5 cM on 2HS may well be part of a resistance gene cluster because leaf stripe resistance locus *Dg2* is located at 45.8 cM on the barley DArT consensus map (33). Bogacki et al. (3) report that SSLB, scald, and powdery mildew resistance QTLs co-locate with RFLP marker CDO370 (at 77.4 cM) and with HvPtr16, a defense response gene with homology to rice CBS domain-containing protein. This latter SSLB QTL was not detected in the present population, nor did we detect any effect of the *Vrs1* locus, which maps to the 92.6-cM position and is a major locus controlling row number and often having a significant influence on other traits.

Chromosome 1H QTL. The present study detected a minor QTL in the SS at peak marker bPt-0005. Lee and Neate (18) have mapped two SSLB resistance loci to the same 1HS chromosome. *Rsp2* is estimated to be 6 cM distal from *Rsp3* and is tightly linked to bPb-1348. *Rsp3* is flanked by bPb-3622 and bPb-6482 in the populations studied by Lee and Neate (18). However, all four DArT markers, and others linked to *Rsp3*, co-map to the same location at 7.2 cM on the DArT consensus map (33). This is consistent with the earlier mapping and allelism testing of Zhong et al. (36) which failed to detect a recombinant between *Rsp2* and *Rsp3* among 458 F_2 progeny. Therefore, the QTL on 1H detected in the present study would appear to correspond with one or both of the known SSLB resistance loci *Rsp2* and *Rsp3*. Yu et al. (34) also found a major QTL at this location on their DArT map. *Rsp2* has been shown to be part of a cluster which includes resistance loci for stripe rust (*Rps4*) (36), leaf rust (*Rph4*), and leaf scald (*Rrs14*), and resistance QTLs for powdery mildew (*Rbg-1* and *Rbg-1-3*) and leaf scald (*Rrs-1-4* and *Rrs-2*) (35) and for covered smut (*Ruh.q*) (12) and Russian wheat aphid (25). This QTL was only detected in the SS of the present mapping population and suggests that the two Clho9831 paternal genotypes carry two different SSLB-susceptibility alleles at this resistance locus.

Cereal orthologs. Through nonhost infection studies of barley with the wheat leaf rust fungus *Puccinia triticina* (24), Neu et al. identified a locus (HvNR-F6) that codes for a serine-threonine kinase with high homology to the rice *Xa21* disease resistance gene. Mapping placed HvNR-F6, tightly linked to ksuA3D, in a region of barley chromosome 6H which is collinear with the region of rice chromosome 11 carrying *Xa21*, and the two loci are tentatively identified as orthologs. The 6H QTL detected in the present study is also tightly linked to ksuA3D, and may be part of an orthologous cluster between barley and rice.

Duplicated resistance loci and resistance gene clusters. The DArT marker most closely linked to the peak of the 6H QTL has homology to genes which have homologs at the barley leaf rust resistance *Rph7* locus. It also co-maps with DArT markers having very high homology to the barley stem rust resistance gene *Rpg1* and the wheat leaf rust resistance gene *Lr1* (Table 3). The DArT marker most closely linked to the 3H QTL has very high homology to the contig spanning the barley leaf rust resistance *Rph7* locus. In addition, the 6H QTL is near the two tightly linked and homologous RFLP marker loci ksuA3B and ksuA3D, which are most likely duplicated loci with homology to the ksuA3 probe. This probe also has homology to locus ksuA3C, which maps ≈ 20 cM from the 3H QTL detected in the current study. Because the peak markers for both QTLs have homology to the same *Rph7*-associated genes, and because both are associated with loci homologous to the same RFLP probe, it is possible that these two QTLs are related through gene duplication. In addition, both are flanked by tightly linked markers which have homology to known disease resistance-type genes. Thus, both the 6H and 3H QTLs locate to clusters of disease resistance genes. The

observation of clusters is consistent with the results of others. For example, Lee and Neate (19) identified a RAPD fragment, OPBA12_{314C}, as tightly linked to the *Rsp3* locus on 1HS and used that fragment to develop a highly diagnostic STS marker. The sequence of the RAPD fragment OPBA12_{314C} has 97% homology to a receptor-like kinase ARK1AS gene in barley, and such receptor-like kinase genes are associated with disease resistance in plants.

The present study has determined that resistance to SSLB in the Leger $\times$ Clho9831 DH population is conferred primarily either by having the CI allele at a major QTL on 6HS or by having the CI allele at both of two QTLs on 3H and 2HL. QTLs were also detected on 2HS and 1H. A variety of evidence suggests that at least the 6HS, 3H, and 2HS QTLs are part of resistance gene clusters and that the 6HS and 3H QTLs may be related through duplication. The 6HS QTL is identical to or closely linked to *Rsp4* and the 1H QTL to *Rsp2* or *Rsp3*. The 2HL QTL corresponds to a previously reported QTL. The 3H and 2HS QTLs appear to be unique and offer new opportunities for pyramiding resistance genes through marker-assisted breeding for resistance to *S. passerinii*.

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