Quantitative Trait Loci for Multiple Disease Resistance in Wild Barley

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ABSTRACT

Foliar diseases of barley (*Hordeum vulgare* L.) such as spot blotch [caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur], net type net blotch (NTNB; caused by *Pyrenophora teres* f. *teres* Drechs), Septoria speckled leaf blotch (SSLB; caused by *Septoria passerinii* Sacc.), leaf scald [caused by *Rhynchosporium secalis* (Oudem.) J. J. Davis], and powdery mildew (caused by *Blumeria graminis* f. sp. *hordei* Em. Marchal) can result in significant yield reductions in many production areas. The wild progenitor of cultivated barley, *Hordeum vulgare* subsp. *spontaneum* is well known as a rich source of disease resistance. To determine the location of *H. vulgare* subsp. *spontaneum*-derived alleles for disease resistance, we conducted quantitative trait locus (QTL) analysis of a recombinant inbred line (RIL) population derived from a cross between the resistant *H. vulgare* subsp. *spontaneum* accession OUH602 and the two-rowed malting cultivar Harrington. A total of 151 simple sequence repeats (SSR) markers were mapped into 11 linkage groups, covering 948 cM. Major QTLs for resistance to each disease were identified: one for spot blotch resistance on chromosome 1(7H); three for NTNB resistance on chromosomes 3(3H), 4(4H), and 5(1H); two for SSLB resistance on chromosomes 2(2H) and 6(6H); one for leaf scald resistance on chromosome 5(1H); and two for powdery mildew resistance on chromosomes 4(4H) and 5(1H). Resistance alleles for each QTL were contributed by OUH602, except those for NTNB and powdery mildew resistance on chromosome 5(1H) and chromosome 4(4H), respectively. The two QTLs identified for SSLB resistance are novel. All other QTLs mapped to regions where known resistance QTLs or major resistance genes have been reported. Our results indicate that most of the OUH602-derived loci are clustered in regions coincident with those described in cultivated barley. These resistance QTLs and their associated markers should be valuable for further exploitation of disease resistance variation in barley improvement.

CROP IMPROVEMENT relies on the ability to generate genetic variation and select for individuals with improved characteristics. Modern crop improvement efforts have relied heavily on the intensive use of favorable alleles present in cultivated germplasm collections, thereby contributing to the narrow genetic base of elite breeding germplasm (Matus and Hayes, 2002). In particular, six-rowed midwestern malting barleys have an especially narrow genetic base (Rasmusson and Phillips, 1997). As a consequence, genetic diversity in cultivated crops has been drastically reduced compared with their wild ancestors. The widespread cultivation of varieties with a narrow genetic base can increase vulnerability of crop plants to biotic and abiotic stresses whose unexpected occurrence is ever increasing because of global changes in environments and agricultural systems. In barley, only 40 to 56% of the alleles found in wild barley are present in elite breeding lines and cultivated barleys (Ellis et al., 2000; Matus and Hayes, 2002). Therefore, developing strategies to incorporate novel allelic variation into cultivated barley is an important activity.

Many diseases can severely affect the productivity and quality of barley in different growing regions of the world. In the USA, leaf scald, caused by the fungus *R. secalis*, is a serious disease in the Pacific Northwest, California and the Mid-Atlantic region where yield losses can reach 35 to 40% (Mathre, 1997). In the Midwest, net type net blotch (NTNB, caused by *P. teres*), spot blotch (caused by *C. sativus*), and Septoria speckled leaf blotch (SSLB, caused primarily by *S. passerinii*) are important foliar diseases of barley resulting in yield reductions from 5 to 35% each year (Steffenson et al., 1996; Toubia-Rahme and Steffenson, 2004). Leaf scald and spot blotch are also considered major barley diseases in Australia (Wallwork, 2000). Powdery mildew, caused by *B. graminis* f. sp. *hordei,* is a major disease around the world, especially in Europe (Jørgensen, 1994). In the USA, powdery mildew can sometimes cause significant loss on barley grown in the Mid-Atlantic region and also California.

The occurrence and severity of disease are affected by the genotypes of the host and pathogen and the environment. Most pathogens are highly variable and genetically diverse, thereby contributing to the development of more virulent pathotypes (Watson, 1970). The increase in frequency of virulent pathotypes of these pathogens has contributed to the demise of several barley cultivars and advanced breeding lines that previously were considered resistant to the diseases (Fetch and Steffenson, 1994; Steffenson and Webster, 1992; Turkington et al., 1999). Therefore, to achieve long-lasting resistance to these variable pathogens, diverse sources of resistance genes must be identified and deployed.

Plant breeders have explored germplasm collections and wild species as sources of favorable alleles for continued crop improvement. Studies have shown that the wild barley species *H. vulgare* subsp. *spontaneum* is a rich source of alleles for resistance to biotic and abiotic stresses, which are seldom found in the cultivated barley germplasm (Ellis et al., 2000; Fetch et al., 2003; Nevo, 1992; Williams, 2003). Accessions of *H. vulgare* subsp.

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Abbreviations: cM, centimorgan; NTNB, net type net blotch; QTL, quantitative trait locus; RIL, recombinant inbred line; SSLB, Septoria speckled leaf blotch; SSR, simple sequence repeats.

spontaneum have been identified that carry resistance to powdery mildew (Jahoor and Fischbeck, 1987a, 1987b), NTNB (Sato and Takeda, 1997), SSLB (Fetch et al., 2003; Metcalfe et al., 1977, 1978), and leaf scald (Genger et al., 2003).

Considerable effort has been devoted to identify and localize major disease resistance genes and QTLs from cultivated and wild barley. Currently, at least five QTLs for spot blotch resistance have been localized on chromosomes 1(7H), 2(2H), 3(3H), 5(1H) and 7(5H) (Bilgic et al., 2005; Steffenson et al., 1996). Loci conferring resistance to NTNB have been mapped to every barley chromosome, except 5(1H) (Richter et al., 1998; Steffenson et al., 1996). Recently, two SSLB resistance genes were mapped on chromosome 4(4H) and 5(1H) (Zhong et al., unpublished data; Toubia-Rahme et al., 2003), but no resistance QTL have been reported. Leaf scald and powdery mildew resistance QTLs have been mapped on all chromosomes (Garvin et al., 1997, 2000; Genger et al., 2003; Grønnerød et al., 2002; Backes et al., 2003; Falak et al., 1999; Heun, 1992; Saghai Maroof et al., 1994).

To identify resistance loci for the major diseases of spot blotch, NTNB, SSLB, leaf scald, and powdery mildew in *H. vulgare* subsp. *spontaneum*, we examined a recombinant inbred line (RIL) population developed from a cross between the *H. vulgare* subsp. *spontaneum* accession OUH602 and the two-rowed malting cultivar Harrington. Using this population, we developed a genetic map of the barley genome exclusively with SSR markers, identified QTLs conferring resistance to all of the diseases, compared the location of these QTLs with previously identified disease resistance loci in wild and cultivated barley, and reported marker-trait associations for marker-assisted selection.

MATERIALS AND METHODS

Plant Material

A RIL population was developed from a cross between *H. vulgare* subsp. *spontaneum* accession OUH602 and the barley cultivar Harrington. The accession OUH602 (provided by P.M. Hayes) originated from the Middle East, but the exact origin is unknown (Sato and Takeda, 1997). Harrington is the industry standard for two-rowed malting barley in North America. A preliminary study indicated that OUH602 was resistant to spot blotch, NTNB, SSLB, leaf scald, and powdery mildew (B. Steffenson, unpublished data). The RIL population was developed by single seed descent to the F_6 generation and consists of 104 lines. Seed for disease evaluations was bulk harvested from 12 F_7 plants derived from each F_6 plant.

DNA Marker Analysis

Genomic DNA for marker analysis was isolated from leaf samples from a single F_6 plant from each line as described by Mesfin et al. (1999). Previously mapped SSR markers (Becker and Heun, 1995; Liu et al., 1996; Ramsay et al., 2000; Thiel et al., 2003) and new markers developed by K. P. Smith (http://agronomy.coafes.umn.edu/barley; verified 15 July 2005) were initially screened on Harrington and OUH602. An informative set of markers that were polymorphic between the parents were then screened on the RIL population. PCR reactions were performed according to the procedures of Ramsay et al. (2000). Amplified products were separated on 5% (w/v) polyacrylamide gels and visualized by silver staining as described by Bassam et al. (1991). PCR products amplified by fluorescent-labeled primers were separated and detected using the IR^2 DNA analyzer (Global edition, LI-COR, Lincoln, NE, USA).

Linkage Map Development

Linkage map analysis in the OUH602/Harrington RIL population was performed by JoinMap (v 3.0; Van Ooijen and Voorrips, 2001). The linkage groups were initially separated into 21 groups on the basis of a LOD score of 5.0 calculated by the Kosambi function. The markers in the initial linkage groups were pooled into the known seven chromosome groups with reference to previously mapped SSRs (Karakousis et al., 2003; Ramsay et al., 2000; Thiel et al., 2003). Then, the markers in each group were regrouped on the basis of a LOD score of 2.0 or higher, resulting in a total of 11 linkage groups.

Plant Growth

Six seeds of each line were planted individually into six 8 × 8 cm plastic pots filled with Metro Mix 200 growing media (Scotts-Sierra Horticulture Products, Marysville, OH, USA). After planting, all pots were moistened and then moved to a cold room at 4°C for 7 d to break any seed dormancy. After the 4°C exposure period, pots were moved to a greenhouse, and plants were grown at approximately 22°C (range from 18–25°C) with a 16-h photoperiod under sodium lights. The plants were fertilized with a 20:20:20 (N:P:K) water soluble fertilizer (United Industries Corp., St. Louis, MO, USA) weekly at the recommended rate.

Experimental Design, Pathogen Inoculation, and Disease Evaluation

Two greenhouse experiments were conducted for each disease evaluation under the growth conditions described above. All experiments were arranged in a randomized complete block design with three replications per line. Each block included the RILs, the parents, and a set of controls that were susceptible or resistant to the respective pathogens. The parents and progeny from the OUH602/Harrington RIL population were evaluated for resistance to spot blotch, NTNB, SSLB, and leaf scald as seedlings by artificial inoculation in the greenhouse at the University of Minnesota in St. Paul. Powdery mildew resistance was evaluated on adult plants naturally infected in the greenhouse. The following pathogen isolates were used for disease evaluation: isolate ND85F of *Cochliobolus sativus* (spot blotch), isolate 30199013 of *Pyrenophora teres f. teres* (NTNB; from R. Dill-Macky, originated from Hallock, MN. USA, 1999), isolate SP97–15 of *Septoria passerinii* (SSLB), and isolate LA94–1A of *Rhynchosporium secalis* (leaf scald). All isolates except *P. teres* were from B.J. Steffenson. The pathogen isolates used represent common virulence spectra found in the Upper Midwest region of the USA.

Three plants per line were inoculated when their second leaf was fully expanded. Inoculations were performed according to Fetch et al. (2003). Disease reactions were assessed after specific incubation periods on the basis of published assessment guides for each respective pathogen (Fetch et al., 2003). Spot blotch reactions were rated 10 to 12 d after incubation by a 1-to-9 scale (Fetch and Steffenson, 1999). NTNB reactions were evaluated 18 d after inoculation by a 0-to-10 scale (Tekauz, 1985). Leaf scald and SSLB were evaluated 23 d after inoculation by a 0-to-4 and 0-to-5 scale, respectively (Jackson and Webster 1976; Toubia-Rahme and Steffenson 2004). Pow-

dery mildew was evaluated by the 0-to-4 scale of Mains and Dietz (1930). For each disease scale, the higher number indicates greater susceptibility. Analysis of variance for the phenotypic data was conducted by Statistix 8 (Analytical Software, Tallahassee, FL, USA).

QTL Analysis

Composite interval mapping (CIM; Zeng, 1994) was conducted by QTL Cartographer version 1.17 (Basten et al., 2003) and Windows QTL Cartographer 2.0 (Wang et al., 2004). For the traits with multiple QTL, multiple interval mapping was performed by Windows QTL Cartographer 2.0. Cofactors were chosen with a combination of forward and backward stepwise regression with a threshold p value of 0.05. To declare a QTL significant, experiment-wise significance levels and comparison-wise probabilities were established by running 1000 permutations for all traits at the significance level of $\alpha = 0.05$ (Churchill and Doerge, 1993).

RESULTS

SSR Marker Linkage Map

From a total of 395 SSR primer pairs surveyed for polymorphism between OUH602 and Harrington, 296 pairs were polymorphic. A total of 182 SSR primer pairs were used for genotyping the RIL population, of which 20 pairs amplified multiple loci. A total of 215 markers were generated from the 182 primer pairs, and 204 markers were used for linkage analysis after removing five ungrouped and six poorly amplified markers. Initially, the markers were grouped into 11 linkage groups, having a total map length of 948 cM. All of the previously mapped single-locus SSRs (Becker and Heun 1995; Karakousis et al., 2003, Liu et al., 1996; Ramsay et al., 2000; Thiel et al., 2003) were located to equivalent positions, except six: the previously mapped chromosome 2(2H) markers HvHOTR1 and EBmac0737 mapped to chromosome 1(7H) and 5(1H), respectively; the previously mapped chromosome 3(3H) markers GBM1069 and Bmag0841, and Bmag0306 mapped to chromosome 1(7H) and 4(4H), respectively; and the previously mapped chromosome 6(6H) marker GBM1068 mapped to chromosome 3(3H). Centromeric clustering of markers was apparent in all chromosomes. Thus, 53 markers in the clusters were removed to yield a minimum of 1-cM spacing between markers. The resulting map with 151 markers has 25, 25, 26, 18, 19, 18, and 20 markers mapped to chromosomes 1(7H) to 7(5H), respectively (Fig. 1). Markers in the centromeric region of chromosome 1(7H), Bmag0571.2, and the distal region of the long arm of chromosome 3(3H), Bmac0029 and GBM-1066b, showed strong segregation distortion toward Harrington and OUH602, respectively. Markers Bmac-0144.3 on chromosome 2(2H), GBM1013 and GBM1042 on chromosome 5(1H), and HvDHN7 on chromosome 7(5H) exhibited 17.3, 10.6, 9.62 and 9.62% heterozygotes, respectively. All other markers exhibited less than 5% heterozygotes and segregated 1:1 as expected.

Disease Response of Parents and RIL Lines

OUH602 exhibited very high levels of resistance to spot blotch, NTNB, SSLB, and leaf scald. In contrast, Harrington was highly susceptible to these four diseases. Both parents exhibited the same disease reaction (1.0) to powdery mildew (Table 1). There was significant variation among the RIL for all of the diseases evaluated. However, all disease phenotypes, with the exception of powdery mildew, showed significant interactions between the line and experiment (Table 1). Therefore, QTL analyses for each disease phenotype were conducted separately for each experiment.

Disease Resistance QTLs

Composite interval mapping analysis consistently detected a single QTL conferring resistance to spot blotch (*Resistance to Cochliobolus sativus Rcs* locus) on chromosome 1(7H) (Fig. 2A; Table 2). This chromosome 1(7H) QTL, designated *Rcs-7H-2–4*, mapped to the BIN 2 to 4 region and explained approximately 25–42% of the phenotype variation. The resistance allele was contributed by OUH602 (Table 2).

Three QTLs for resistance to NTNB (*Rpt* loci) were identified on chromosomes 3(3H), 4(4H), and 5(1H); however, only the locus on chromosome 4(4H) was consistently detected in the repeated experiments (Fig. 2B; Table 2). The chromosome 4(4H) QTL *Rpt-4H-5–7* mapped to the BIN 5 to 7 region and explained about 10 and 9% of the phenotypic variation. The resistance allele was contributed by OUH602. The QTL *Rpt-3H-4* mapped on chromosome 3(3H) in BIN 4. *Rpt-3H-4* explained about 12% of the phenotypic variation of the RIL lines, with the resistance allele contributed by OUH602. The QTL *Rpt-1H-5–6* mapped on chromosome 5(1H) in the BIN 5 to 6 region. This QTL explained about 10% of the phenotypic variation of the RIL lines, and the resistance allele was contributed by Harrington (Table 2).

Two QTLs conferring resistance to SSLB (*Rsp* loci) were consistently detected on chromosomes 2(2H) and 6(6H) (Fig. 2C; Table 2). The chromosome 2(2H) QTL *Rsp-2H-7–11* mapped to the BIN 7 to 11 region and explained about 36–42% of the phenotypic variation. The resistance allele was contributed by OUH602 (Table 2). The chromosome 6(6H) QTL *Rsp-6H-10–14* mapped to the BIN 10 to 14 region and explained about 8% of the phenotypic variation of the population. The resistance allele was contributed by OUH602 (Table 2).

A major QTL conferring resistance to leaf scald (*Rrs* loci) was consistently detected on chromosome 5(1H) (Fig. 2D; Table 2). The QTL *Rrs-1H-1–4* mapped to the BIN 1 to 4 region and explained approximately 87 and 34% of phenotypic variation. The resistance allele was contributed by OUH602.

Two major loci conferring resistance to powdery mildew (*Rbg* loci) were consistently identified on chromosomes 4(4H) and 5(1H) (Fig. 2E; Table 2). The chromosome 4(4H) QTL *Rbg-4H-5–7* mapped to the BIN 5 to 7 region and explained about 28 or 21% of the phenotypic variation in experiment 1 and 2, respectively. The resistance allele was contributed by Harrington (Table 2). The chromosome 5(1H) QTL *Rbg-1H-1–3* mapped to the BIN 1 to 3 region and explained between 22 and

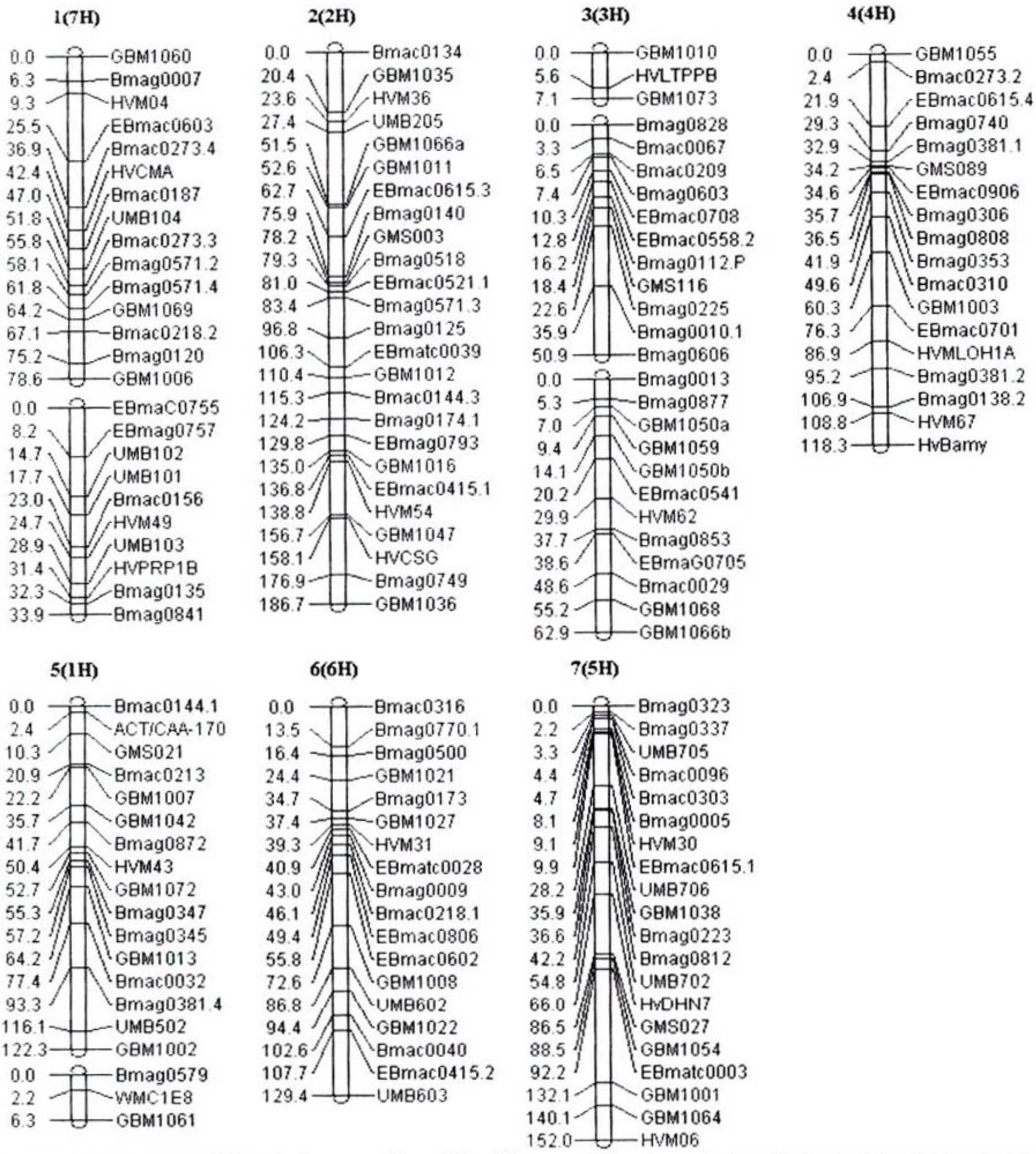

Fig. 1. Linkage map of the OUH602/Harrington recombinant inbred line population determined on the basis of simple sequence repeat (SSR) markers. Marker designations are given on the right side of each chromosome. Centimorgan distances were obtained from the Kosambi mapping function and are given on the left side of each linkage group.

24% of the phenotypic variation of the population. The resistance allele was contributed by OUH602 (Table 2).

DISCUSSION

Using a single *H. vulgare* subsp. *spontaneum* accession, we mapped QTLs for resistance to spot blotch, SSLB, leaf scald, NTNB, and powdery mildew. The wide cross used to conduct the mapping work allowed us to develop a comprehensive genetic map exclusively with SSR markers. Our results provide the opportunity to examine the genetic relationships between wild and cultivated barley for resistance to these diseases, identify novel QTLs for disease resistance, characterize the distribution of disease resistance loci in the barley genome, and identify marker-trait relationships for future marker-assisted breeding.

Disease Resistance QTL in Barley

Most of the resistance QTLs we identified in this study mapped to regions of the genome that were coincident with previously identified resistance QTLs or genes. We detected nine disease resistance QTLs, seven for which *H. vulgare* subsp. *spontaneum* carried the resistance allele. Five of these seven were in regions of the genome previously reported to contain resistance QTLs identified in populations created with barley cultivars or landraces. In total, of the nine detected QTLs in this study, seven were previously detected in similar

Table 1. Means, ranges, and variances of disease reactions for spot blotch (SB), net type net blotch (NTNB), Septoria speckled leaf blotch (SSLB), leaf scald (LS), and powdery mildew (PM) phenotypes of parents and lines from the *H. vulgare* subsp. *spontaneum* OUH602 and cultivated barley Harrington recombinant inbred line population.

				Population			*P* value	
Trait	Experiment	Harrington†	OUH602†	Mean†	Range†	Error MS‡	Line	Experiment × line§
SB	Exp. 1	7.7	1.0	4.5	1.3–7.7	1.792	<0.0001	
	Exp. 2	6.7	1.0	4.3	1.0–8.3	4.529	<0.0001	0.0057
NTNB	Exp. 1	5.3	0.0	2.0	0.0–8.0	3.887	<0.0001	
	Exp. 2	7.7	0.7	4.7	0.7–10.0	7.319	<0.0001	0.0105
SSLB	Exp. 1	5.0	0.0	0.9	0.0–5.0	0.231	<0.0001	
	Exp. 2	5.0	0.0	1.2	0.0–5.0	0.397	<0.0001	0.0188
LS	Exp. 1	3.7	0.0	2.4	0.0–4.0	0.551	<0.0001	
	Exp. 2	4.0	0.0	2.4	0.0–4.0	0.385	<0.0001	<0.0001
PM	Exp. 1	1.0	1.0	1.4	1.0–3.0	0.068	<0.0001	
	Exp. 2	1.0	1.0	1.4	1.0–3.0	0.089	<0.0001	0.9943

† Disease reaction readings for SB, NTNB, SSLB and LS were on the second leaf of seedlings and for powdery mildew on the leaves of adult plants. Disease reactions were evaluated according to the following scales: 1 to 9 for spot blotch (Fetch and Steffenson, 1999), 0 to 10 for NTNB (Tekauz, 1985), 0 to 5 for SSLB (Toubia-Rahme and Steffenson, 2004), 0 to 4 for leaf scald (Jackson and Webster, 1976), and 0 to 4 for powdery mildew (Mains and Dietz, 1930).

‡ Mean square for the experiment.

§ Interaction between the line and experiment (Genotype × Environment).

genomic regions. The spot blotch resistance QTL, *Rcs-7H-2–4*, is coincident with *Rcs5*, a previously mapped major gene conferring spot blotch seedling resistance in the Steptoe/Morex population (Steffenson et al., 1996). Three QTLs (*Rpt-3H-4*, *Rpt-4H-5–7*, *Rpt-1H-5–6*) for resistance to NTNB were detected on chromosomes 3(3H), 4(4H) and 5(1H), respectively, in our study. The chromosome 3(3H) and 4(4H) QTLs were located in similar regions as previously detected QTLs for NTNB resistance in the Steptoe/Morex population (Steffenson et al., 1996). *Rpt-1H-5–6* was coincident with a QTL identified using an Ethiopian landrace (Richter et al., 1998). The *Rrs-1H-1–4* QTL is in a region similar to several previously described leaf scald resistance loci (Garvin et al., 1997; 2000; Genger et al., 2003). Two QTLs, *Rbg-1H-1–3*, *Rbg-4H-5–7*, were coincident with the previously mapped *Mla* and *Mlg* powdery mildew resistance loci, respectively (Jahoor et al., 1990; Jørgensen, 1994). Thus, our data suggest that there is considerable coincidence in the location of disease resistance loci detected in this study compared with loci detected in other studies. It is not known if the coincident locations of disease resistance loci are due to alternate alleles or closely linked genes in a complex locus. Further genetic studies are required to resolve this question.

We identified two QTLs for SSLB resistance that mapped to locations of the barley genome not previously associated with SSLB resistance. Perhaps the reasons for the unique locations for SSLB resistance are (i) there have been comparatively few mapping studies conducted on SSLB relative to other diseases and (ii) SSLB resistance is extremely common in wild barley, possibly indicating a large pool of novel loci and alleles. For example, Fetch et al. (2003) found that 77 to 98% of accessions from Israel and Jordan were resistant to SSLB. The identification of the two SSLB QTLs in this study indicates that examining *H. vulgare* subsp. *spontaneum* germplasm can uncover novel QTLs.

Heterogeneous Distribution of Resistance Genes

One prominent feature revealed by mapping disease resistance loci is the clustering of genes along the barley chromosomes (Fig. 3; Williams, 2003). Numerous major genes and QTLs have been positioned on the short arm of chromosome 1(7HS), the centromeric region of chromosome 4(4H), and the short arm of chromosome 5(1H). Localization of resistance QTLs to the known gene clusters is also apparent in OUH602 (Fig. 3). OUH602-derived QTLs for NTNB resistance, and resistance to powdery mildew and scald were located on the centromeric region of chromosome 4(4H) and on the short arm of chromosome 5(1H), respectively. Except for two QTLs for SSLB resistance, all the QTLs detected in this study were located within resistance gene clusters on chromosome 1(7H), 3(3H), 4(4H), 5(1H), and 6(6H) (Fig. 3). Plant disease resistance genes evolve through the duplication of progenitor resistance genes and further expansion to create clustered gene families (Richter and Ronald, 2000). Therefore, localization of disease resistance QTLs to gene cluster regions is consistent with the current understanding of disease resistance gene evolution.

Small Numbers of QTLs Identified for Each Disease

No more than two major QTLs were detected for any one disease across experiments in this study. This is a rather small number given the number of resistance QTLs described for specific diseases from *H. vulgare* subsp. *spontaneum* in other studies. For example, multiple QTLs for resistance to leaf scald were found in a backcross population between cultivated barley and *H. vulgare* subsp. *spontaneum* (Abbott et al., 1992; Genger et al., 2003). The reason for the low number of resistance QTLs identified for each disease in this study is not known. The number of QTLs identified for complex traits is affected by many statistical and biological factors, including population size, quality of phenotypic data, analysis stringency, pathotypes, developmental stage of assessment, and genetic background (Young, 1996). In particular, the small population size used in this study could have affected marker order and thus the resulting QTL analysis. Several studies have shown that small population sizes result in low power for QTL

Fig. 2. Continued on next page.

detection (Melchinger et al., 1998; Utz et al., 2000). Further studies with larger populations, populations of different genetic backgrounds, phenotyping at different developmental stages, and the use of different pathotypes will be required to obtain a better understanding of the nature of disease resistance in OUH602.

OUH602 as a Source of Multiple Disease Resistance

Numerous studies indicate that *H. vulgare* subsp. *spontaneum* is a rich source of resistance to multiple diseases, like NTNB (Sato and Takeda, 1997), SSLB (Metcalfe et al., 1977, 1978), leaf scald (Genger et al., 2003), and powdery mildew (Thomas et al., 1995). However, little information is available about the nature of resistance of individual *H. vulgare* subsp. *spontaneum* accessions to multiple diseases. In this study, we showed the map locations of OUH602-derived resistance to five foliar pathogens. Most of the alleles from OUH602 had a major effect on resistance, explaining 25 to 87% of the phenotypic variation depending on the disease examined. Moreover, SSLB resistance alleles were unique

Table 2. Percentage phenotypic variation (R^2) and average effect of a gene substitution (α) for spot blotch (SB), net type net blotch (NTNB), Septoria speckled leaf blotch (SSLB), leaf scald (LS), and powdery mildew (PM) quantitative trait loci (QTLs) from composite interval mapping analysis for the *H. vulgare* subsp. *spontaneum* OUH602 and cultivated barley Harrington recombinant inbred line population.

Trait	Experiment	QTL†	Chromosome	Associated markers‡	Interval size§	BIN¶	LOD peak	α#	R^2††	Threshold‡‡
SB	Exp. 1	*Rcs-7H-2–4*	1 (7H)	HVM04-Bmac0273.4	28	2–4	12.11	−1.16	41.80	3.1
	Exp. 2	*Rcs-7H-2–4*	1 (7H)	HVM04-EBmac0603	16	2–4	6.00	−1.04	24.61	3.1
NTNB	Exp. 1	*Rpt-3H-4*	3 (3H)	Bmag0828-Bmac0067	3	4	4.22	−0.93	11.50	3.0
		Rpt-4H-5–7	4 (4H)	EBmac0906-Bmac0310	15	5–7	3.80	−0.89	10.33	
		Rpt-1H-5–6	5 (1H)	Bmag0872-HVM43	9	5–6	3.45	0.89	10.32	
	MM§§								34.56	
	Exp. 2	*Rpt-4H-5–7*	4 (4H)	Bmag0740-Bmag0306	6	5–7	3.37	−0.59	9.15	3.0
SSLB	Exp. 1	*Rsp-2H-7–11*	2 (2H)	EBmac0615.3-Bmag0125	34	7–11	10.34	−0.96	36.30	3.0
		Rsp-6H-10–14	6 (6H)	EBmac0415.2-UMB603	22	10–14	3.47	−0.47	8.52	
	MM								51.9	
	Exp. 2	*Rsp-2H-7–11*	2 (2H)	EBmac0615.3-GMS003	16	7–11	11.13	−1.03	42.30	3.0
		Rsp-6H-10–14	6 (6H)	UMB602-UMB603	43	10–14	3.06	−0.44	7.80	
	MM								61.06	
LS	Exp. 1	*Rrs-1H-1–4*	5 (1H)	ACT/CAA170-Bmac0213	19	1–4	46.22	−1.33	86.61	4.1
	Exp. 2	*Rrs-1H-1–4*	5 (1H)	Bmac0144.1-GMS021	10	1–4	11.39	−0.70	33.50	3.2
	MM								37.43	
PM	Exp. 1	*Rbg-4H-5–7*	4 (4H)	GMS089-Bmag0353	8	5–7	13.43	0.42	27.90	3.1
		Rbg-1H-1–3	5 (1H)	Bmac0144.1-Bmac0213	21	1–3	7.60	−0.39	23.73	
	MM								59.90	
	Exp. 2	*Rbg-4H-5–7*	4 (4H)	Bmag0740-Bmag0306	6	5–7	8.94	0.34	20.53	3.1
		Rbg-1H-1–3	5 (1H)	Bmac0144.1-Bmac0213	21	1–3	7.21	−0.36	22.41	
	MM								59.90	

† The QTLs were named after the conventional nomenclature: Resistant (*R*) to the disease (acronym of the scientific name of the pathogen causing the disease in lowercase) followed by chromosome name and BIN number.
‡ The nearest markers flanking the QTL LOD peaks.
§ Interval (cM) around the QTL with a LOD fall off of 1.0 from maximum.
¶ Estimated BIN based on the chromosome BIN location of barley markers (http://barleygenomics.wsu.edu/).
# Average effect of substituting Harrington allele with OUH602 allele.
†† Partial $R^2 \times 100$.
‡‡ LOD detection threshold at $p = 0.05$.
§§ Multiple regression model.

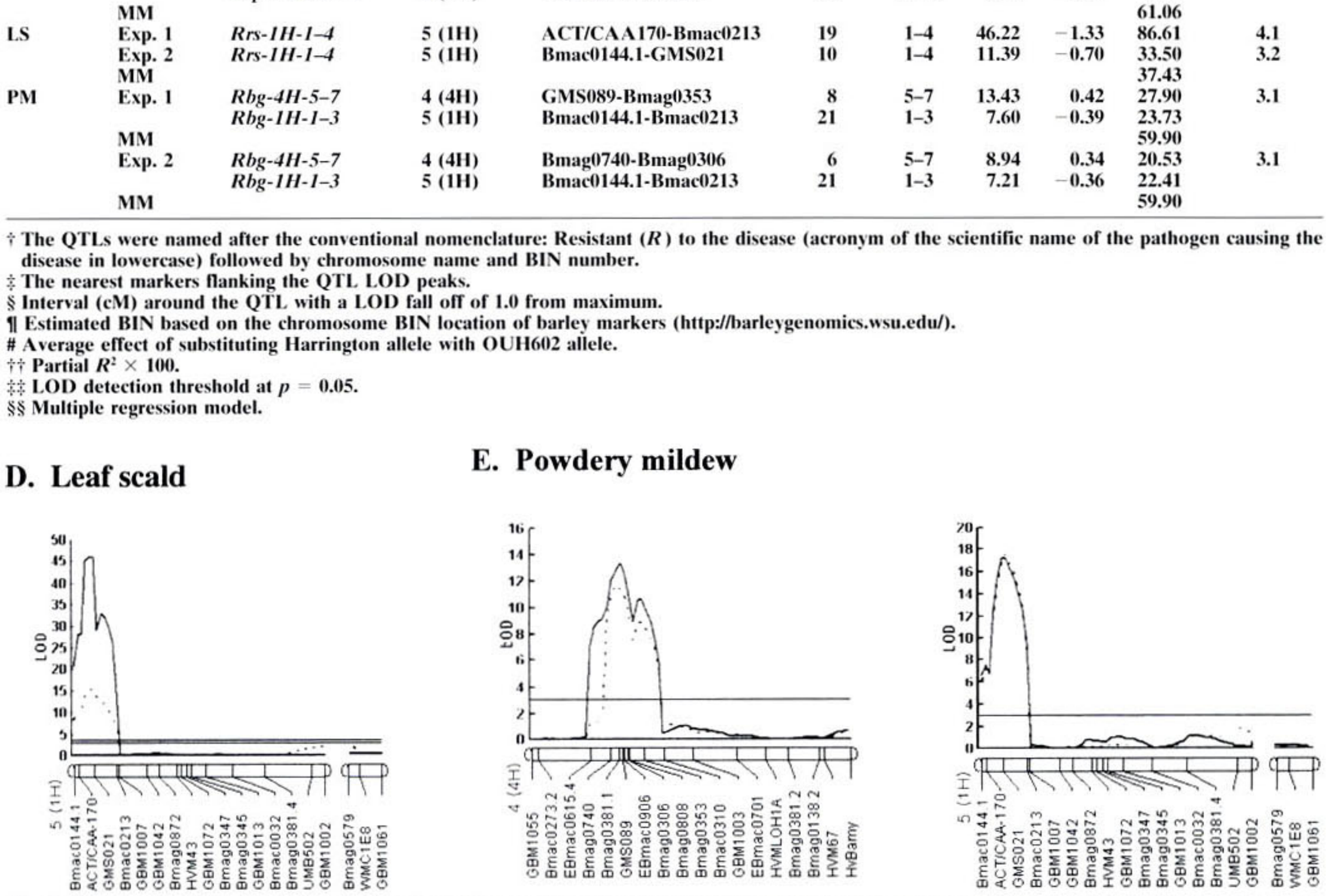

Fig. 2. Composite interval mapping LOD scans of linkage groups where quantitative trait loci were detected for spot blotch, net type net blotch (NTNB), Septoria speckled leaf blotch (SSLB), leaf scald, and powdery mildew in the OUH602/Harrington recombinant inbred line population. The horizontal lines indicate significance thresholds for each experiment, estimated from 1000 permutations of the data to maintain the experiment-wise Type I error rate below 5%. A. Spot blotch resistance on chromosome 1(7H). B. NTNB resistance on chromosomes 3(3H), 4(4H), and 5(1H). C. SSLB resistance on chromosome 2(2H) and 6(6H). D. Leaf scald resistance on chromosome 5(1H). E. Powdery mildew resistance on chromosomes 4(4H) and 5(1H). Solid line, experiment 1; broken line, experiment 2. The threshold values for each experiment are given in Table 2.

to OUH602. Importantly, they were linked closely to PCR-based SSR markers (Fig. 1; Table 2). In fact, the barley SSR map described here exhibits evenly spaced markers that cover a substantial proportion of the barley genome. Thus, this map will facilitate both marker-assisted selection for disease resistance QTLs and selection against OUH602 alleles at undesirable loci. U.S. malting barleys have inadequate levels of resistance to NTNB, SSLB, leaf scald, and powdery mildew. Therefore, incorporation of the resistance alleles from OUH-602 with the aid of the SSR-based genetic map will allow efficient improvement of disease resistance in cultivated barley.

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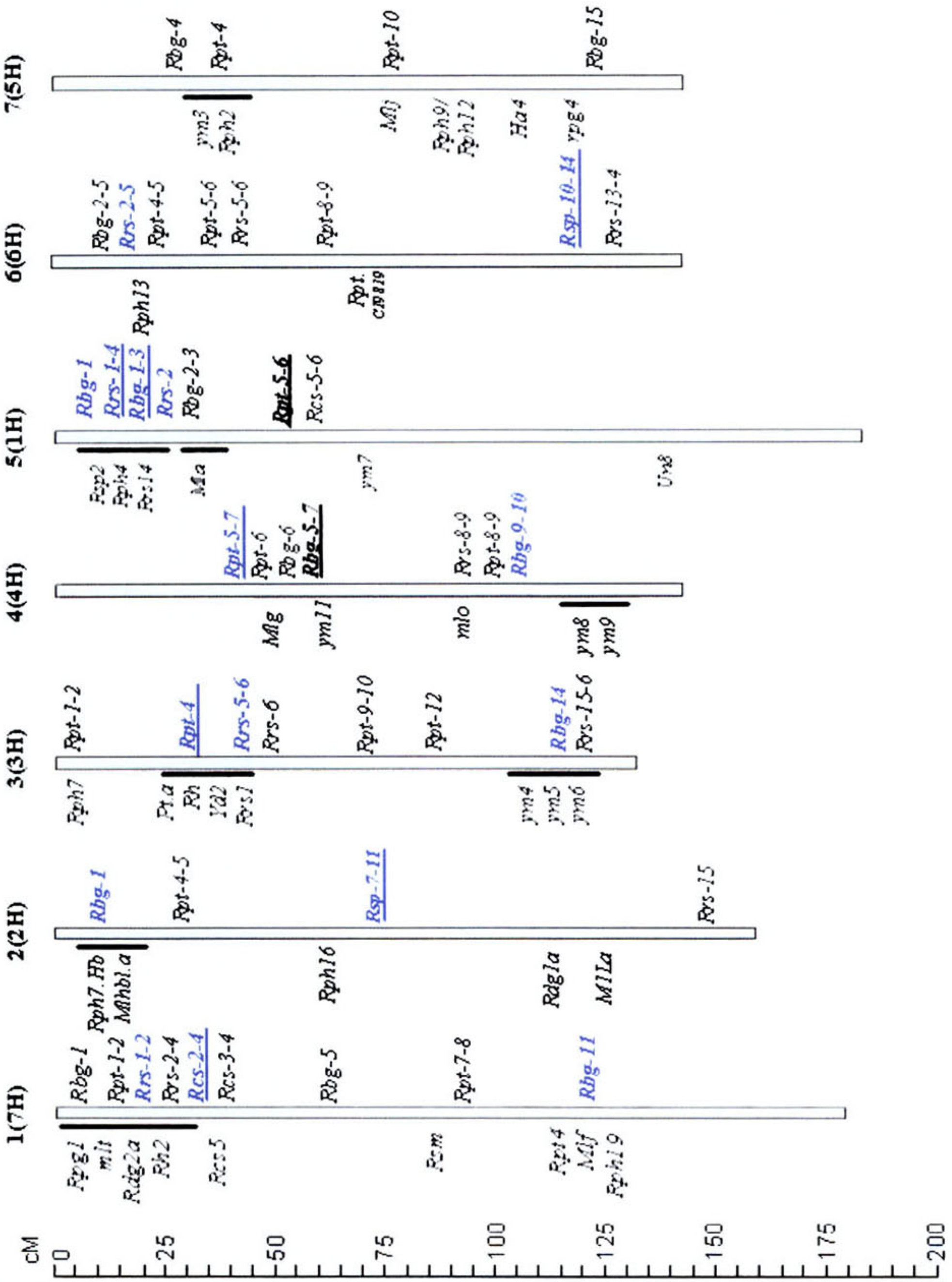

Fig. 3. Locations of major genes and quantitative trait loci (QTLs) for disease resistance in barley. Major disease resistance loci are indicated on the left side of each chromosome. Loci in the same genetic locations are marked with vertical lines. Resistance QTLs for spot blotch (*Rcs*), net type net blotch (*Rpt*), Septoria speckled leaf blotch (*Rsp*), leaf scald (*Rrs*) and powdery mildew (*Rbg*) are indicated on the right side of each chromosome with the estimated BIN location. QTLs identified in cultivated and *H. vulgare* subsp. *spontaneum* are labeled in black and blue, respectively. The underlined QTLs were identified in this study. Locations of the major disease resistance genes and QTLs were adopted from Williams (2003) on the U.S. Barley Genome Project internet site (http://www.barleyworld.org). Location of additional QTLs reported by Backes et al. (2003) and Genger et al. (2003) were estimated on the basis of the BIN information for the barley markers and genes available at http://barleygenomics.wsu.edu/; verified 15 July 2005.

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REFERENCES

Abbott, D.C., A.H.D. Brown, and J.J. Burdon. 1992. Genes for scald resistance from wild barley (*Hordeum vulgare* ssp. *spontaneum*) and their linkage to isozyme markers. Euphytica 61:225–231.

Backes, G., L.H. Madsen, H. Jaiser, J. Stougaard, M. Herz, V. Mohler, and A. Jahoor. 2003. Localization of genes for resistance against *Blumeria graminis* f. sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor. Appl. Genet. 106:353–363.

Bassam, B.J., G. Caetano-Anolles, and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80–83.

Basten, C.J., B.S. Weir, and Z.-B. Zeng. 2003. QTL Cartographer version 1.17. Bioinformatics Research Center. N.C. State University. Raleigh, NC, USA.

Becker, J., and M. Heun. 1995. Barley microsatellites: Allele variation and mapping. Plant Mol. Biol. 27:835–845.

Bilgic, H., B.J. Steffenson, and P. Hayes. 2005. Comprehensive genetic analyses reveal differential expression of spot blotch resistance in four populations of barley. Theor. Appl. Genet. (in press).

Churchill, G.A., and R.W. Doerge. 1993. Empirical threshold values for quantitative trait mapping. Genetics 138:963–971.

Ellis, R.P., B.P. Foster, D. Robinson, L.L. Handley, D.C. Gordon, J.R. Russell, and W. Powell. 2000. Wild barley: A source of genes for crop improvement in the 21st century? J. Exp. Bot. 51:9–17.

Falak, I., D.E. Falk, N.A. Tinker, and D.E. Mather. 1999. Resistance to powdery mildew in a doubled haploid barley population and its association with marker loci. Euphytica 107:185–192.

Fetch, T.G., and B.J. Steffenson. 1994. Identification of *Cochliobolus sativus* isolates expressing differential virulence on two-row barley genotypes from North Dakota. Can. J. Plant Path. 16:202–206.

Fetch, T.G., Jr., and B.J. Steffenson. 1999. Rating scales for assessing infection response of barley infected with *Cochliobolus sativus*. Plant Dis. 83:213–217.

Fetch, T.G., Jr., B.J. Steffenson, and E. Nevo. 2003. Diversity and sources of multiple disease resistance in *Hordeum spontaneum*. Plant Dis. 87:1439–1448.

Garvin, D.F., A.H.D. Brown, and J.J. Burdon. 1997. Inheritance and chromosome locations of scald-resistance genes derived from Iranian and Turkish wild barleys. Theor. Appl. Genet. 94:1086–1091.

Garvin, D.F., A.H.D. Brown, H. Raman, and B.J. Read. 2000. Genetic mapping of the barley *Rrs*14 scald resistance gene with RFLP, isozyme and seed storage protein markers. Plant Breed. 119:193–196.

Genger, R.K., K.J. Williams, H. Raman, B.J. Read, H. Wallwork, J.J. Burdon, and A.H.D. Brown. 2003. Leaf scald resistance genes in *Hordeum vulgare* and *Hordeum vulgare* ssp. *spontaneum*: Parallels between cultivated and wild barley. Aust. J. Agric. Res. 54:1335–1342.

Grønnerød, S., A.G. Marøy, J. MacKey, A. Tekauz, G.A. Penner, and A. Bjørnstad. 2002. Genetic analysis of resistance to barley scald (*Rhynchosporium secalis*) in the Ethiopian line 'Abyssinian' (CI668). Euphytica 126:235–250.

Heun, M. 1992. Mapping quantitative powdery mildew resistance of barley using a restriction fragment length polymorphism map. Genome 35:1019–1025.

Jackson, L.F., and R.K. Webster. 1976. Race differentiation, distribution and frequency of *Rhynchosporium secalis* in California. Phytopathology 66:719–725.

Jahoor, A., A. Ludwig, and G. Fischbeck. 1990. New genes for powdery mildew resistance in *Hordeum spontaneum* derived lines allelic or closely linked to the *Ml-p* locus. Barley Genet. Newslett. 19: 23–26.

Jahoor, A., and G. Fischbeck. 1987a. Genetical studies of resistance of powdery mildew in barley lines derived from *Hordeum spontaneum* collected from Israel. Plant Breed. 99:265–273.

Jahoor, A., and G. Fischbeck. 1987b. Sources of resistance to powdery mildew in barley lines derived from *Hordeum spontaneum* collected in Israel. Plant Breed. 99:274–281.

Jørgensen, J.H. 1994. Genetics of powdery mildew resistance in barley. Crit. Rev. Plant Sci. 13:97–119.

Karakousis, A., J.P. Gustafson, K.J. Chalmers, A.R. Barr, and P. Langridge. 2003. A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust. J. Agric. Res. 54:1173–1185.

Liu, Z.W., R.M. Biyashev, and M.A. Saghai Maroof. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor. Appl. Genet. 93:869–876.

Mains, E.B., and S.M. Dietz. 1930. Physiologic forms of barley mildew, *Erysiphe graminis hordei* Marchal. Phytopathology 20:229–239.

Mathre, D.E. 1997. Compendium of barley diseases. 2nd ed. American Phytopathological Society Press, St. Paul, MN.

Matus, I.A., and P.M. Hayes. 2002. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 45:1095–1106.

Melchinger, A.E., H.F. Utz, and C.C. Schoen. 1998. Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149:383–403.

Mesfin, A., R.C. Frohberg, and J.A. Anderson. 1999. RFLP markers associated with high grain protein and from *Triticum turgidum* L. var. *dicicoides* introgressed into hard red spring wheat. Crop Sci. 39:508–513.

Metcalfe, D.R., A.W. Chiko, J.W. Martens, and A. Tekauz. 1977. Reaction of barley from the Middle-East to Canadian pathogens. Can. J. Plant Sci. 57:995–999.

Metcalfe, D.R., A.W. Chiko, J.W. Martens, and A. Tekauz. 1978. Reaction of Ethiopian barleys to Canadian barley pathogens. Can. J. Plant Sci. 57:995–999.

Nevo, E. 1992. Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum* in the fertile crescent. p. 19–43. *In* P.R. Shewry (ed.) Barley genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, UK.

Ramsay, L., M. Macaulay, S. degli Ivanissevich, K. MacLean, L. Cardle, J. Fuller, K.J. Edwards, S. Tuvesson, M. Morgante, A. Massari, E. Maestri, N. Marmiroli, T. Sjakste, M. Ganal, W. Powell, and R. Waugh. 2000. A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005.

Rasmusson, D.C., and R.L. Phillips. 1997. Plant breeding progress and genetic diversity from de novo variation and elevated epistasis. Crop Sci. 37:303–310.

Richter, T.E., and P.C. Ronald. 2000. The evolution of disease resistance genes. Plant Mol. Biol. 42:195–204.

Richter, K., J. Schondelmaier, and C. Jung. 1998. Mapping of quantitative traits loci affecting *Drechslera teres* resistance in barley with molecular markers. Theor. Appl. Genet. 97:1225–1234.

Saghai Maroof, M.A., Q. Zhang, and R.M. Biyashev. 1994. Molecular marker analysis of powdery mildew resistance in barley. Theor. Appl. Genet. 88:733–740.

Sato, K., and K. Takeda. 1997. Net blotch resistance in wild species of *Hordeum*. Euphytica 95:179–185.

Steffenson, B.J., P.M. Hayes, and A. Kleinhofs. 1996. Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres f. teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor. Appl. Genet. 92:552–558.

Steffenson, B.J., and R.K. Webster. 1992. Quantitative resistance to *Pyrenophora teres* f. *teres* in barley. Phytopathology 82:407–411.

Tekauz, A. 1985. A numerical scale to classify reaction of barley to *Pyrenophora teres*. Can. J. Plant Pathol. 7:181–183.

Thiel, T., W. Michalek, R.K. Varshney, and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 106:411–422.

Thomas, W.T.B., W. Powell, R. Waugh, K.J. Chalmers, U.M. Barua, P. Jack, V. Lea, B.P. Forster, J.S. Swanston, R.P. Ellis, P.R. Hanson, and R.C.M. Lance. 1995. Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 91:1037–1047.

Toubia-Rahme, H., and B.J. Steffenson. 2004. Sources of resistance to Septoria speckled leaf blotch caused by *Septoria passerinii* in barley. Can. J. Plant Pathol. 26:358–364.

Toubia-Rahme, H., P.A. Johnston, R.A. Pickering, and B.J. Steffenson. 2003. Inheritance and chromosomal location of *Septoria passerinii* resistance introgressed from *Hordeum bulbosum* into *Hordeum vulgare*. Plant Breed. 122:405–409.

Turkington, T.K., P.A. Burnett, and K.G. Briggs. K. Xi, D.D. Orr, J.H. Helm, B.G. Rossnagel, W.G. Legge, and J.P Tewari. 1999. Proc. 16th American Barley Researchers Workshop, Scald of barley in Alberta: Disease resistance screening, breakdown and management.

Utz, H.F., A.E. Melchinger, and C.C. Schön. 2000. Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849.

Van Ooijen, J.W., and R.E. Voorrips. 2001. JoinMap 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands.

Wallwork, H. 2000. Cereal leaf and stem diseases. Grains Research and Development Croporation, Kingston, ACT.

Wang, S., C.J. Basten, P. Baffney, and Z.-B. Zeng. 2004. Windows QTL Cartographer 2.0. Bioinformatics Research Center, N.C. State University, Raleigh, NC.

Watson, I.A. 1970. Changes in virulence and population shifts in plant pathogens. Annu. Rev. Phytopathol. 8:209–230.

Williams, K.J. 2003. The molecular genetics of disease resistance in barley. Aust. J. Agric. Res. 54:1065–1079.

Young, N.D. 1996. QTL mapping and quantitative disease resistance in plants. Annu. Rev. Phytopathol. 34:479–501.

Zeng, Z. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457–1468.