Molecular Mapping and Marker-Assisted Selection of Genes for Septoria Speckled Leaf Blotch Resistance in Barley

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ABSTRACT

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Septoria speckled leaf blotch (SSLB), caused by *Septoria passerinii*, has emerged as one of the most important foliar diseases of barley in the Upper Midwest region of the United States. To map and tag genes for SSLB resistance, we developed two populations derived from the resistant accessions CIho 4780 and CIho 10644 and the susceptible malting cv. Foster. Segregation analysis of F₂ plants or F_{2,3} families from the Foster/CIho 4780 and Foster/CIho 10644 populations revealed that a single dominant gene conferred resistance at the seedling stage. Bulk segregant analysis identified an amplified fragment length polymorphism

marker, E-ACT/M-CAA-170, that co-segregated with the SSLB resistance gene *Rsp2* in the Foster/CIho 4780 F₂ population. Southern hybridization analysis with DNA from the wheat/barley addition lines localized E-ACT/M-CAA-170 on the short arm of the barley chromosome 5(1H). Restriction fragment length polymorphism analysis with DNA clones previously mapped to the short arm of chromosome 5(1H) placed *Rsp2* at a position flanked by the markers Act8 and ksuD14. A sequence-characterized amplified region (SCAR) marker (E-ACT/M-CAA-170a) was developed that co-segregated with not only *Rsp2* in the Foster/CIho 4780 population but also resistance gene *Rsp3* in the Foster/CIho 10644 population. This result indicates that *Rsp3* is closely linked to *Rsp2* on the short arm of chromosome 5(1H). The utility of SCAR marker E-ACT/M-CAA-170a for selecting *Rsp2* in two different breeding populations was validated.

Septoria speckled leaf blotch (SSLB) is a fungal disease of barley (*Hordeum vulgare* L.) that is caused by two different pathogens, *Septoria passerinii* Sacc. and *Stagonospora avenae* Bisset f. sp. *triticea* T. Johnson (teleomorph: *Phaeosphaeria avenaria* (G. F. Weber) O. Eriksson f. sp. *triticea* T. Johnson) (24). In the Upper Midwest region of the United States, *Septoria passerinii* is the most common SSLB pathogen, although *Stagonospora avenae* f. sp. *triticea* also frequently is isolated from SSLB-infected barley tissue (21). The SSLB pathogens overwinter in infected barley stubble. With the wide adoption of minimum tillage practices, inoculum levels have increased significantly in fields throughout the region. This, coupled with unusually wet weather conditions, has contributed to the increased frequency and severity of SSLB epidemics. Indeed, over the past decade, SSLB has become one of the most important foliar diseases of barley in the Upper Midwest. Yield losses up to 38% have been reported in barley infected with *Septoria passerinii* (42). In addition to markedly reducing yield, SSLB also detrimentally affects grain quality by reducing kernel size and the amount of malt extract (24).

Deployment of resistant cultivars is the most economic and environmentally sound means of preventing SSLB epidemics. Unfortunately, all of the major malting and feed barley cultivars in the Upper Midwest region are highly susceptible to SSLB (41). Many sources of resistance to *S. passerinii* have been identified in both cultivated and wild barley (*H. vulgare* subsp. *spontaneum* and *H. bulbosum*) (3,9,13,23,27,28,34,41,43), but few studies

have investigated the genetics of resistance. In the early 1960s, the first genetic studies on SSLB resistance were completed in barley (3,34). Two complementary recessive genes were found to confer resistance to *S. passerinii* in barley cv. Feebar (CIho 7260) (3), and three different dominant resistance genes (originally designated *Sep*, *Sep*₂, and *Sep*₃ and now designated *Rsp1*, *Rsp2*, and *Rsp3*) were identified in accessions CIho 4249-2, CIho 4780, and CIho 10644, respectively (34). *Rsp2* in CIho 4780 and *Rsp3* in CIho 10644 are linked with a recombination frequency of 3.9 ± 1.3% (34). Recently, a single dominant gene and two quantitative trait loci (QTL) for *S. passerinii* resistance were identified from a *H. vulgare* × *H. bulbosum* recombinant (41) and a *H. vulgare* subsp. *spontaneum* × *H. vulgare* population (47), respectively. The dominant gene originally derived from the *H. bulbosum* parent was positioned on the long arm of chromosome 4(4H) by genomic and fluorescent in situ hybridizations and by Southern hybridization. The two major effect QTL were derived from *H. vulgare* subsp. *spontaneum* and mapped to chromosomes 2(2H) and 6(6H). The chromosomal locations of other described SSLB resistance genes are not known.

The mapping of resistance genes and identification of molecular markers linked to them can facilitate the development of disease-resistant cultivars in barley breeding programs employing marker-assisted selection (MAS). Moreover, this information contributes to the evolving database on the chromosomal position of resistance genes in possible gene-rich regions of the barley genome (36,46). Numerous molecular markers (restriction fragment length polymorphism [RFLP], random amplified polymorphic DNA, amplified fragment length polymorphism [AFLP], simple sequence repeats, and sequence tagged site) have been developed for barley and have greatly facilitated mapping of many resistance genes (1,11,12,14,32,37,48). The objectives of

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this study were to map the SSLB resistance genes *Rsp2* and *Rsp3*, develop polymerase chain reaction (PCR)-based markers for their selection, and validate the use of these markers for selection of SSLB resistance in breeding populations. A preliminary report of this work was published previously (38).

MATERIALS AND METHODS

Plant materials and disease evaluations. In a previous study, CIho 4780 and CIho 10644 were found to possess a high level of resistance to SSLB at both the seedling and adult plant stages (43). Thus, these two accessions were selected for further study of the genetics of SSLB resistance and for mapping. Both resistance sources were used as the pollen parent in crosses with the susceptible malting cv. Foster (PI 592758). In all, 113 F_2 individuals and $F_{2:3}$ families from the cross Foster/CIho 4780 and 132 $F_{2:3}$ families from the cross Foster/CIho 10644 were used for the seedling disease evaluation in the greenhouse and subsequent genetic mapping analysis. The F_2 individuals were derived from a single F_1 plant of each cross. These F_2 plants were selfed to produce $F_{2:3}$ families. For evaluations in the $F_{2:3}$ generation, 20 to 25 seedling plants per family were phenotyped for their reaction to *S. passerini* in the greenhouse. To clarify the allelic and linkage relationships between *Rsp2* and *Rsp3*, F_2 progeny were developed from the cross CIho 4780/CIho 10644 and evaluated at the seedling stage to *S. passerini* in the greenhouse.

Plant growth conditions, inoculum preparation, and inoculation methods for the seedling resistance evaluations were as described by Toubia-Rahme and Steffenson (43). Parental, F_2 , and $F_{2:3}$ plants were grown in plastic pots (10 by 10 cm) filled with a commercial potting mixture (peat moss:perlite, 3:1 ratio) (#1 Sunshine Mix, Fisons Horticulture, Inc., Vancouver, Canada). All seedlings were grown in a greenhouse at $20 \pm 3^\circ\text{C}$ with 14 h of supplemental lighting. Seedlings were inoculated with isolate ND97-15 of *S. passerinii* 10 to 12 days after planting, when the second leaves were fully expanded. The 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 mistings from ultrasonic humidifiers. The first 40 h of incubation were in darkness, followed by a photoperiod of 5 h (150 to $270 \mu\text{E m}^{-2} \text{s}^{-1}$) for each of the next 2 days. Plants were allowed to dry off slowly before being transferred to the greenhouse under the same conditions previously described. Infection responses (IRs) were assessed on the second leaves of seedlings 20 days after inoculation using a 0-to-5 rating scale (43) where 0, 1, and 2 were considered indicative of resistance and 3, 4, and 5 were considered indicative of susceptibility. The barley cvs. Bowman (PI 483237) and Betzes (PI 129430) were used as susceptible checks and Atlas (PI 539108) as a resistant check in all experiments.

Bulked segregant analysis with AFLP markers. DNA was extracted from F_2 individuals of the Foster/CIho 4780 and $F_{2:3}$ families of the Foster/CIho 10644 populations using the methods of Riede and Anderson (35). For bulked segregant analysis (BSA) (30) with the Foster/CIho 4780 cross, aliquots of DNA from eight F_2 individuals exhibiting resistance were combined for the resistant bulk, and aliquots of DNA from eight F_2 individuals exhibiting susceptibility were combined for the susceptible bulk. The parents and the two bulks were subjected to AFLP analysis (45) according to methods described previously (48). Briefly, template DNA ($0.25 \mu\text{g}$) from the parents and bulks was digested using the restriction enzymes *EcoRI* and *MseI*, according to the manufacturer's protocol (New England Biolabs Inc., Beverly, MA). The adapter ligation, preamplification, and selective amplification were carried out using an AFLP kit (Gibco BRL, Grand Island, NY). Selective amplification products were separated in a de-

naturing polyacrylamide gel (7 M urea, 0.5 Tris-borate EDTA, 6% Long Ranger Gel Solution [BMA, Rochland, ME], 0.05% TEMED, and 0.05% ammonium persulfate), and AFLP bands were detected using the DNA silver-staining system (Promega Corp., Madison, WI). The AFLPs detected between both the parents and bulks were tested in the entire F_2 population for linkage analysis. An AFLP fragment (E-ACT/M-CAA-170) showing cosegregation with the resistance gene in F_2 progeny of the Foster/CIho 4780 population was reamplified using the method of Men and Gresshoff (25) and cloned using the TA Cloning Kit (Invitrogen, Carlsbad, CA). The DNA insert in the clone was sequenced by the Advanced Genetics Analysis Center, University of Minnesota, St. Paul, MN.

RFLP analysis. To identify the chromosomal location of the target gene, the cloned AFLP marker E-ACT/M-CAA-170 was used as a probe to hybridize with membranes containing digested DNA from the wheat/barley addition lines developed by Islam (17). The parents (Foster and CIho 4780) and their derived F_2 progeny also were subjected to RFLP analysis with probe E-ACT/M-CAA-170. Southern analysis with the wheat/barley addition lines revealed a chromosome 5(1H) position for marker E-ACT/M-CAA-170. To confirm this initial finding and to construct a molecular linkage map of the region containing the resistance gene, we selected DNA clones that generated RFLP markers on the short arm of chromosome 5(1H). Five restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, and *Hind*III) were used to digest DNA samples according to the manufacturer's protocol (New England Biolabs Inc.). Southern blotting, probe labeling, hybridization, and membrane washing and stripping procedures were carried out as described by Riede and Anderson (35). RFLP clones were kindly provided by A. Kleinhofs, (Washington State University, Pullman, WA) and A. Graner (Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany).

Development of sequence-characterized amplified region markers. To convert the AFLP marker E-ACT/M-CAA-170 into a sequence-characterized amplified region (SCAR) marker, oligonucleotide primers (5'-CATGTCACGCACTTATCAAGC-3' and 5'-CCACACACGATTGCAACTTAG-3') were designed based on sequence information and used for PCR amplification with genomic DNA of the parents (Foster and CIho 4780) and their progeny (i.e., the Foster/CIho 4780 F_2 plants). Primers were synthesized by Life Technology Inc. (Grand Island, NY). PCR reactions were performed as described above for reamplification of the excised AFLP bands. The PCR products were separated in a sequencing gel and detected with the DNA silver-staining system (Promega Corp.) as used for AFLP analysis (48).

Data analysis and linkage construction. The χ^2 method was used to test the goodness of fit of observed ratios to theoretically expected ratios in all populations using SAS (Statistical Analysis System, Cary, NC). AFLP and RFLP markers detected between the two parents and also between the two bulks subsequently were evaluated in 113 F_2 plants from the Foster/CIho 4780 cross for segregation analysis using the χ^2 test ($\alpha = 0.05$). Linkage analysis was performed using the computer program MAPMAKER, version 2.0 (22). The linkage maps were constructed based on a log of the likelihood ratio (logarithm of odds) threshold of 3.0, and the Kosambi mapping function (20) was used to convert recombination frequency to map distance in centiMorgans.

MAS. For MAS, we generated two breeding populations from the crosses Sep1-29/M110 and Sep1-44/M96-46. Sep1-29 and Sep1-44 are resistant selections derived from the Foster/CIho 4780 population and M110 and M96-46 are elite breeding lines from the University of Minnesota barley breeding program, susceptible to SSLB. The F_2 plants from each population were planted in cones (3.8 cm in diameter and 21 cm in depth) in a greenhouse and tagged for identification. DNA was isolated from each of 192 individual plants per population using a method modified from Edwards et al. (6) in which 2- to 3-cm leaf samples

were extracted in 500 µl of buffer in deep-well microtiter plates. PCR was carried out using the primer set for the SCAR marker (described above). Each plant was scored as carrying the CIho 4780 allele (MAS-*Rsp2*) or not (MAS-*rsp2*). The genotypes of selected plants were verified in the F_{2.3} generation for the Sep1-29/M110 population. Randomly selected (RAND) plants not subjected to MAS were included in this study as a control to compare with MAS. F_{4.6} lines from each of the three selection classes (MAS-*Rsp2*, MAS-*rsp2*, and RAND) of each population were evaluated for SSLB resistance in the greenhouse using the methods described above. Four seeds of each F_{4.6} line were evaluated.

RESULTS

Genetics of resistance to SSLB. In all, 113 F₂ individuals from the Foster/CIho 4780 cross were evaluated for their reaction to *S. passerinii* at the seedling stage. The number of resistant (IRs of 0, 1, or 2) to susceptible (IRs of 3, 4 or 5) plants in the population approximated a 3:1 ratio (Table 1), indicating that a single dominant gene confers resistance to *S. passerinii* in CIho 4780. This result was confirmed from the evaluation of F_{2.3} families, which segregated in an approximate 1:2:1 ratio for homozygous resistant:segregating:homozygous susceptible families (Table 1). For the cross between Foster and CIho 10644, 132 F_{2.3} families were evaluated for their reaction to *S. passerinii*. The number of homozygous resistant:segregating:homozygous susceptible families fit a 1:2:1 ratio (Table 1), indicating single gene control of SSLB resistance in CIho 10644. In most of the segregating F_{2.3} families from the Foster/CIho 10644 population, the number of resistant plants exceeded the number of susceptible plants by approximately 3:1, indicating dominant gene action (data not shown). The reaction of Foster/CIho 10644 F₁ plants also indicated dominant to partially dominant gene action. No susceptible plants were observed in 458 F₂ progeny from the cross CIho 4780/CIho 10644, suggesting that *Rsp2* and *Rsp3* in CIho 4780 and CIho 10644, respectively, are either allelic or very tightly linked.

Molecular mapping. BSA with AFLP identified four polymorphic bands between Foster and CIho 4780 as well as between

the two bulks. Two of the bands were not linked to the resistance gene in CIho 4780 when the entire F₂ population was screened (data not shown). The other two AFLP bands, amplified with primer pairs E-ACT/M-CAA and E-AGG/M-CAC, respectively, were present only in resistant F₂ individuals and absent in susceptible F₂ individuals (Fig. 1A and B), indicating that they cosegregated with the resistance gene. The band designated E-ACT/M-CAA-170, with a size of approximately 170 bp, was cloned and used as a probe in RFLP analysis of the two parents and the entire Foster/CIho 4780 F₂ population. Three and four hybridization bands were observed in Foster and CIho 4780, respectively, when the restriction enzyme *EcoRI* was used for DNA digestion. The unique band present in CIho 4780 appeared only in resistant F₂ plants and not in the susceptible F₂ plants (Fig. 2), indicating its close linkage to the resistance gene in CIho 4780.

To identify the chromosomal location of this marker, the E-ACT/M-CAA-170 clone was used as a probe to hybridize with filters made from *EcoRV*-digested DNA of the wheat (Chinese Spring)-barley (Betzes) addition lines. Hybridization bands were observed only in the barley parent Betzes and in the wheat-barley addition line carrying a pair of the short arms of barley chromosome 5(1H) (Fig. 3). This indicated that E-ACT/M-CAA-170 is located on the short arm of chromosome 5(1H).

Fig. 2. Southern hybridization of the cloned amplified fragment length polymorphism (AFLP) marker E-ACT/M-CAA-170 to *EcoRI*-digested DNA of Foster, CIho 4780, and their F₂ progeny (a subset is shown here). The hybridization band (at arrow) unique to the resistant parent CIho 4780 is present in resistant F₂ individuals but absent in susceptible individuals.

TABLE 1. Segregation for seedling resistance to *Septoria passerini* in the F₂ or F_{2.3} generations of two barley populations

Cross	Generation	Resistant ^a	Segregating ^b	Susceptible ^c	Expected ratio	χ ²	P
Foster/CIho 4780	F ₂	87	NA	26	3:1	0.24	0.62
Foster/CIho 4780	F _{2.3}	24	63	26	1:2:1	1.57	0.46
Foster/CIho 10644	F _{2.3}	32	73	27	1:2:1	1.86	0.39

^a Number of resistant F₂ plants or homozygous resistant F_{2.3} families.
^b Number of segregating F_{2.3} families; NA = not applicable to the F₂ generation.
^c Number of susceptible F₂ plants or homozygous susceptible F_{2.3} families.

Fig. 1. Identification of an amplified fragment length polymorphism (AFLP) marker linked to resistance gene *Rsp2* in CIho 4780 by bulk segregant analysis. **A**, AFLP bands were amplified using primer pair E-ACT and M-CAA and detected using the DNA silver-staining system. The AFLP marker E-ACT/M-CAA-170 (at arrow) was present only in the resistant parent CIho 4780 and the resistant bulk. **B**, When marker E-ACT/M-CAA-170 was tested on F₂ progeny from the Foster/CIho 4780 population, only resistant individuals contained the distinguishing band. A subset of the F₂ population is shown.

Further RFLP analysis using RFLP clones previously mapped to the short arm of chromosome 5(1H) identified three markers (Act8, *ksuD14-1*, and *ksuD14-2*) which were polymorphic between the parents Foster and CIho 4780. Analysis of the entire F_2 population with these RFLP markers revealed their linkage with

Fig. 3. Southern hybridization of the cloned amplified fragment length polymorphism (AFLP) marker E-ACT/M-CAA-170 to *EcoRV*-digested DNA of the wheat-barley addition lines. The hybridization bands are present only in barley parent Betzes and the addition line with the short arm of barley chromosome 5(1H).

Fig. 4. Partial linkage map of the short arm of chromosome 5(1H) showing the position of the Septoria speckled leaf blotch resistance gene *Rps2*; the two co-segregating amplified fragment length polymorphism (AFLP) markers E-ACT/M-CAA-170 and E-AGG/M-CAC-198; and three anchoring restriction fragment length polymorphism (RFLP) markers Act8, *ksuD14-1*, and *ksuD14-2*. The telomere is oriented toward the top in the figure.

Fig. 5. Identification of a sequence-characterized amplified region (SCAR) marker E-ACT/M-CAA-170a (at arrow) linked to the Septoria speckled leaf blotch resistance gene *Rps2* in CIho 4780. E-ACT/M-CAA-170a, amplified by the primers 5'-CATGTCACGCACTTATCAAGC-3' and 5'-CCACACACGATTGCAACTTAG-3', is present in the resistant parent CIho 4780 and resistant F_2 progeny but absent in the susceptible parent Foster and susceptible F_2 progeny.

the resistance gene *Rsp2* in CIho 4780. The map position of these RFLP markers in relation to *Rsp2* on the short arm of chromosome 5(1H) is shown in Figure 4.

Development of SCAR marker. A pair of primers were designed based on the sequence information of E-ACT/M-CAA-170 and were used in PCR with DNA of the parental barley lines as templates. One band was detected in Foster, whereas two bands (one of which was the same as found in Foster) were observed in CIho 4780 when the PCR products were separated in denatured sequencing gels (Fig. 5). Analysis of the 113 F_2 plants from the Foster/CIho 4780 cross showed that the PCR band E-ACT/M-CAA-170a was unique to CIho 4780 and only present in resistant individuals. This result indicates that E-ACT/M-CAA-170a co-segregates with the resistance gene *Rsp2* in the Foster/CIho 4780 population.

The same primers also were used in the Foster/CIho 10644 population. Two PCR bands were detected in CIho 10644—one being the same size as that found in Foster and the other of the same size as E-ACT/M-CAA-170a. In the Foster/CIho 10644 population, all homozygous resistant and segregating $F_{2,3}$ families exhibited the same banding pattern as CIho 10644, whereas the homozygous susceptible $F_{2,3}$ families had the same banding pattern as Foster (Fig. 6). This result indicated that E-ACT/M-CAA-170a also is linked to the resistance gene *Rsp3* in CIho 10644.

MAS. To evaluate the effectiveness of MAS in breeding for SSLB resistance, the two primers used to amplify the SCAR marker E-ACT/M-CAA-170a were tested in two breeding populations produced from crosses Sep1-44/M96-46 and Sep1-29/M110. In the Sep1-44/M96-46 population, the primers produced a polymorphic band in both the resistant and susceptible parents (plus a shared band) and, thus, the SCAR marker could be scored as a co-dominant marker. Among the 191 F_2 plants tested, 4.2% (8/191) yielded no PCR amplification, 28.3% (54/191) carried the CIho 4780 allele, 27.2% (52/191) carried the M96-46 allele, and 40.3% (77/191) carried both the CIho 4780 and M96-46 alleles (i.e., were heterozygous). In the Sep1-29/M110 cross, only the band that was unique to the resistant parent was scorable; therefore, the SCAR marker was scored as a dominant marker. In this population, 192 F_2 plants were tested, and 8.3% (16/192) yielded no PCR amplification, 66.7% (128/192) carried the CIho 4780 allele, and 25.0% (48/192) carried the M110 allele. All lines carrying the CIho 4780 allele were further confirmed by testing 8 to 10 progeny from each $F_{2,3}$ family to determine the F_2 genotype (i.e., whether they were homozygous resistant or heterozygous). Plants homozygous for the CIho 4780 allele (MAS-*Rsp2*) and the non-CIho 4780 allele (MAS-*rsp2*) were advanced to an $F_{4,5}$ generation. The RAND plants also were advanced by single-seed descent to an $F_{4,5}$ generation. Disease evaluation of seedling plants at an $F_{4,5}$ generation showed that 90.6% (29/32) and 100% (19/19) of the lines genotypically selected as MAS-*Rsp2* in the F_2 generation were scored as resistant for populations Sep1-29/M110 and Sep1-44/M96-46, respectively (Table 2). Similarly, 93.3% (14/15) and 100% (14/14) of the lines genotypically selected as MAS-*rsp2* in the F_2 generation were scored as susceptible for the two populations. The RAND lines showed an approximate 1:1 resistant:susceptible ratio for both populations as would be expected in an $F_{4,5}$ generation in the absence of selection.

DISCUSSION

Molecular mapping analysis clearly demonstrated that the resistance gene *Rsp2* in CIho 4780 is located on the short arm of chromosome 5(1H) in barley. This conclusion was made on the basis of three lines of evidence. First, the cloned AFLP marker E-ACT/M-CAA-170, which co-segregated with the gene *Rsp2* in the Foster/CIho 4780 F₂ population, hybridized only to the wheat-barley addition line carrying the short arm of barley chromosome 5(1H) (Fig. 3). Second, when the E-ACT/M-CAA-170 clone was sequenced, the insert (minus the two AFLP primers E-CAT and M-CAA) generated a sequence of 125 bp. BLAST analysis against the GenBank database revealed that the sequence is similar (with 94% identity) to barley hordein genes (10) and also wheat genes for low molecular weight glutenin (33). The hordein genes of *Hor1*, *Hor2*, and *Hor5* are located on the short arm of chromosome 5(1H) (19) and comprise a diverse gene family, which shows high polymorphism among barley cultivars. The high similarity found strongly suggests that E-ACT/M-CAA-170 may belong to one of the hordein gene families on chromosome 5(1H). Third, two RFLP markers (Act8 and ksuD14) mapping to the short arm of chromosome 5(1H) showed linkage with *Rsp2* and established the orientation of the map (Fig. 4).

The failure to detect recombinants between E-ACT/M-CAA-170 and resistance in both mapping populations indicates a very close linkage between the molecular marker and respective genes (i.e., *Rsp2* and *Rsp3*). It also indicates a strong linkage between the two genes themselves. This aspect was confirmed in an allelism test where, in 458 F₂ progeny of the cross CIho 4780/CIho 10644, no susceptible individuals were found. These data indicate that *Rsp2* in CIho 4780 and *Rsp3* in CIho 10644 are allelic or tightly linked and establish chromosome 5(1H) as the location for *Rsp3*. Our results agree with those of Rasmusson and Rogers (34), who found that *Rsp2* was closely linked with *Rsp3* at a recombination frequency of $3.9 \pm 1.3\%$. Analysis of larger segregating populations may reveal recombinations among the closely linked molecular markers and resistance genes; however, the data presented herein were sufficient for establishing the chromosomal location of the resistance genes and identifying closely linked molecular markers for MAS.

The region around the hordein gene family on chromosome 5(1H) where *Rsp2* and *Rsp3* were mapped appears to be a gene-rich region. Many qualitative and quantitative resistance genes to diseases and insects have been mapped in the same general region, including *Rph4* for resistance to leaf rust (5), *Rps4* (44) and several QTL for resistance to stripe rust (4,39,40), *Mla* (15,18,37) and three QTL for resistance to powdery mildew (2,8,39), *Rrs14* for resistance to leaf scald (11), *Ruh.q* for resistance to covered smut (14), and one QTL for resistance to the Russian wheat aphid (31). Thus, the short arm of chromosome 5(1H) in barley appears to be a rich region for disease resistance genes and one worthy of further investigation. Multilocus clusters of resistance genes with different specificities to the same pathogen and to different pathogens have been reported in a range of plant species, including barley (7,16,29).

Only a few studies have been published on the genetics of SSLB resistance in barley (3,26,34,41,47). In all of the early

studies (3,26,34), the chromosomal positions of the resistance genes were not determined. Recently, a single dominant gene was mapped in a recombinant line developed from an *H. vulgare* × *H. bulbosum* cross by genomic and fluorescent in situ hybridizations and by Southern hybridization (41). Additionally, two QTL for *S. passerinii* resistance were identified in an *H. vulgare* subsp. *spontaneum* × *H. vulgare* population (47). The resistance alleles for these major effect QTL were contributed by wild barley and were mapped on chromosomes 2(2H) and 6(6H) (47). In the present study, *Rsp2* and *Rsp3* were mapped to the short arm of chromosome 5(1H), a region where no other SSLB resistance genes are known.

Implementing MAS in segregating F₂ populations was very effective for introducing the *Rsp2* gene into breeding lines. Although the simple inheritance of SSLB resistance can be observed in greenhouse seedling assays, MAS for resistance offers several advantages. One advantage is that, in at least some breeding populations, it should be possible to score the SCAR marker as co-dominant, thereby eliminating the need for progeny testing to identify lines that are fixed for *Rsp2*. Another clear advantage of MAS is that it does not rely on establishing optimal environmental conditions for disease expression. It is sometimes difficult to obtain reliable infection and disease phenotyping for SSLB. This is particularly true for the field, where multiple inoculations and mist irrigation often are required to obtain adequate amounts of infection. Because *Rsp2* and *Rsp3* are equally effective at both the seedling and adult plant stages (43), in the past we have employed the more reliable greenhouse evaluation to screen for SSLB resistance. Finally, as other loci for SSLB resistance are mapped, it will be possible to pyramid these resistance alleles by MAS. It is likely that, using current field or greenhouse screening

TABLE 2. Disease reaction of F_{4,6} lines from two breeding populations subjected to marker assisted selection in the F₂ generation using the sequence-characterized amplified region (SCAR) marker E-ACT/M-M-CAA-170a that co-segregates with the Septoria speckled leaf blotch (SSLB) resistance gene *Rsp2*

Population, selection class ^a	Total evaluated ^b	Resistant ^c	Susceptible ^d
Sep1-29/M110			
MAS- <i>Rsp2</i>	32	29	3
MAS- <i>rsp2</i>	15	1	14
RAND	16	8	8
Sep1-44/M96-46			
MAS- <i>Rsp2</i>	19	19	0
MAS- <i>rsp2</i>	14	0	14
RAND	16	7	9

^a Sep1-29 and Sep1-44 are resistant to SSLB and carry *Rsp2*, whereas M110 and M96-46 are susceptible. The lines in the marker-assisted selection (MAS)-*Rsp2* class are homozygous for the CIho 4780 allele based on the SCAR marker E-ACT/M-M-CAA-170a, whereas those in the MAS-*rsp2* class are homozygous for the alternative allele. The randomly selected (RAND) class includes progeny not subjected to MAS.

^b Total number of F_{4,6} lines evaluated.

^c Number of resistant F_{4,6} lines based on disease phenotype.

^d Number of susceptible F_{4,6} lines based on disease phenotype. A line was scored as susceptible if any of the four plants in the pot exhibited a susceptible reaction.

Fig. 6. Amplification of E-ACT/M-CAA-170a using the primers 5'-CATGTACGCACTTATCAAGC-3' and 5'-CCACACAGATTGCAACTTAG-3' in the resistant barley accession CIho 10644 carrying the Septoria speckled leaf blotch resistance gene *Rsp3*. This band is present in the homozygous resistant and segregating F_{2,3} families and absent in the homozygous susceptible F_{2,3} families derived from the Foster/CIho 10644 cross. A subset of F_{2,3} families is shown.

methods, it would be difficult to identify lines that carry more than one resistance gene. Pyramiding of multiple resistance genes through MAS may result in more durable or higher levels of resistance.

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