Molecular Mapping of *Rsp1*, *Rsp2*, and *Rsp3* Genes Conferring Resistance to Septoria Speckled Leaf Blotch in Barley

S. H. Lee and S. M. Neate

Department of Plant Pathology, North Dakota State University, Fargo 58105.

Accepted for publication 18 August 2006.

ABSTRACT

Lee, S. H., and Neate, S. M. 2007. Molecular mapping of *Rsp1*, *Rsp2*, and *Rsp3* genes conferring resistance to Septoria speckled leaf blotch in barley. *Phytopathology* 97:155-161.

Septoria speckled leaf blotch (SSLB) caused by *Septoria passerinii* is a common disease in barley. SSLB resistance genes *Rsp1*, *Rsp2*, and *Rsp3* have previously been identified in the United States Department of Agriculture National Small Grains collection accessions CIho 14300, CIho 4780, and CIho 10644, respectively. Populations of 100 to 120 F_2 individuals were evaluated for SSLB resistance in the greenhouse. Inheritance was evaluated in $F_{2:3}$ -derived families in the field. Partial molecular maps for three *Rsp* genes were constructed on F_2 and $F_{2:3}$ families derived from crosses between Robust and the resistant accessions CIho 14300, CIho 4780, and CIho 10644. The resistant locus *Rsp1* was mapped to the short arm of chromosome 3H with two flanking diversity arrays technology (DArT) markers, bPb-6978 (8.9 cM) and bPb-9945 (16.3 cM), and two random amplified polymorphic DNA (RAPD)

markers, OPC2_{441R} (3.0 cM) and UBC285_{158R} (4.3 cM). The genes *Rsp2* and *Rsp3* were positioned on the short arm of barley chromosome 1H with two restriction fragment length polymorphism (RFLP), six DArT, and three RAPD markers. An RFLP marker, MWG938, and an RAPD marker, OPAH5_{545C}, were tightly associated with *Rsp2* at a distance of 0 cM. Five DArT markers spanning the short arm of 1H surrounded *Rsp3* at a distance of 2.3 and 5.8 cM, while two RAPD markers—OPBA12_{314C} (2.4 cM) in coupling and OPB17_{451R} (3.5 cM) in repulsion—flanked *Rsp3*. Molecular marker data associated with *Rsp2* and *Rsp3* indicated that the two genes are closely linked on chromosome 1HS. A total of 17 of 154 simple sequence repeats (SSRs) tested were associated with *Rsp* genes on chromosome 1H and 3H, and they were also integrated into genetic linkage maps of the three F_2 Robust populations. Knowledge about the map position of *Rsp* genes on barley chromosomes will be useful for breeding for SSLB resistance in barley and eventual gene cloning.

S. passerinii causes Septoria speckled leaf blotch (SSLB) in barley (*Hordeum vulgare*) and has become one of the most serious diseases of barley in the Upper Midwest region of the United States. The disease is considered to be a major factor in causing low yields and reducing kernel weights (8). The use of genetic resistance to SSLB is the most efficient and cost-effective means of preventing SSLB epidemics. SSLB resistance has been identified in different barley accessions. The cvs. Atlas and Feebar, and United States Department of Agriculture (USDA) National Small Grains Collection accession CIho 4439 were identified in Canada as resistant to SSLB (2,19,21). Seedling resistance to SSLB was reported in the United States in three six-rowed barley accessions: CIho 4780 and CIho 14300, which are rough-awned varieties of Northeast China origin; and CIho 10644, which is a semi-rough awned selection from the cross Feebar and Kindred (27). To date, three single, incompletely dominant SSLB resistance loci designated *Rsp1*, *Rsp2*, and *Rsp3* have been identified in CIho 14300, CIho 4780, and CIho 10644, respectively (27).

Molecular markers can be used to track specific loci in segregating populations and to monitor the entire genome. Several molecular techniques such as restriction fragment length polymorphisms (RFLP) (1), random amplified polymorphic DNA (RAPD) (40), amplified fragment length polymorphisms (AFLP) (37), and microsatellites or simple sequence repeats (SSR) (26) have been widely used in barley for mapping studies. A barley consensus map was constructed mainly on the basis of RFLP

markers and used to identify markers closely linked to traits of interest (15). To date, approximately 700 barley SSR markers have been developed (18,22,26), and 242 SSR markers have been integrated into a linkage map of the doubled haploid mapping population 'Lina'/*Hordeum spontaneum* 'Canada Park' (26). More recently, diversity arrays technology (DArT) has become available to enable whole-genome profiling of barley without the need for sequence information (39). This technique is based on microarray hybridization that detects absence or presence of individual fragments in genomic representations (10). Currently, an integrated linkage map assembled from seven individual barley maps comprising 1,990 DArT and 869 SSR/RFLP markers can be used to effectively create a medium-density genetic map of barley, and is available online from Triticarte Pty. Ltd., New South Wales, Australia.

Identification and mapping of molecular markers for *Rsp* genes in the barley genome facilitate development of marker assisted selection (MAS) for developing SSLB-resistant cultivars and has the potential to create gene combinations for improved SSLB control. Recently, the chromosomal location of *Rsp2* was mapped on chromosome 1H (33). However, in a recent paper, Toubia-Rahme et al. (36) placed *Rsp2* on the short arm of chromosome 3H. The map location of *Rsp1* has not been described and the close linkage of *Rsp2* and *Rsp3* reported by Rasmusson and Rogers (27) has not been confirmed with molecular markers. Information on the chromosomal location of the three genes *Rsp1*, *Rsp2*, and *Rsp3* will be valuable for MAS in barley breeding for SSLB resistance.

In the present study, we (i) report on the inheritance of SSLB resistance in six populations at the seedling and adult stages, and (ii) provide partial molecular genetic maps for all three *Rsp* genes.

Corresponding author: S. M. Neate; E-mail address: Stephen.Neate@ndsu.edu

DOI: 10.1094/PHYTO-97-2-0155

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MATERIALS AND METHODS

Plant materials. To develop six segregating populations, susceptible cvs. Robust and Foster were crossed with resistant accession CIho 14300, containing the resistance gene *Rsp1*; CIho 4780, containing *Rsp2*; and CIho 10644, containing *Rsp3*. The three resistant accessions were obtained from J. Franckowiak at North Dakota State University, Fargo. Robust (28) was released by the University of Minnesota (St. Paul) barley breeding program and its pedigree is Morex/Manker. Foster (9) was released by the North Dakota State University (Fargo) breeding program and its pedigree is Robust/6/Glenn/4/Nordic//Dickson/Trophy/3/Azure/5/Glenn/Karl. In each case the F_1 plants were selfed to obtain between 100 and 120 F_2 plants. To determine the inheritance of resistance to SSLB at the adult stage in the field, $F_{2,3}$ families were developed in the greenhouse from the Robust F_2 populations. For the allelism test between *Rsp2* and *Rsp3* genes, crosses were made between CIho 4780 (*Rsp2*) and CIho 10644 (*Rsp3*).

Disease assessment in greenhouse. Inoculation procedures for the SSLB assessment in the greenhouse were according to the techniques of Toubia-Rahme et al. (34) with minor modifications to growth chamber incubation temperature, atomizer pressure, and plant growth stage for inoculation. The isolate ND97-15 of *S. passerinii*, obtained from B. Steffenson, University of Minnesota, St. Paul, was used for the inoculation test in the greenhouse. To prepare inoculum, isolates were grown on yeast malt agar in petri dishes at 23°C in a 12 h alternating day/night cycle under cool-white fluorescent light. When pycnidia formed and sporulation occurred after 4 to 5 days of incubation, spores were harvested by flooding the surface of the plates with deionized water and then scraping the agar surface with a rubber policeman. The spore suspension was homogenized for 30 s in a blender and then filtered through four layers of cheesecloth. To prepare inoculum, the spore suspension was adjusted to 5×10^5 pycnidiospores ml^{-1} and Tween 20 was added to the suspension at 100 μl -liter $^{-1}$. Parents and F_2 progeny were assessed at the seedling stage in the greenhouse for disease reaction to *S. passerinii* isolates. Four plants of each susceptible and resistant parent in every population were included as a reference to standard SSLB reaction. The plants were grown in a 10-cm-diameter pot filled with commercial potting mixture #1 Sunshine Mix (Fisons Horticulture Inc., Vancouver, B.C., Canada) and maintained in a 20°C greenhouse. Two weeks after planting, plants were inoculated at the three-leaf stage with the pycnidiospore suspension (5×10^5 pycnidiospores ml^{-1}) with an atomizer (100 kPa). Each plant was sprayed with approximately 0.4 ml of aqueous pycnidiospore suspension of a mixture of three isolates. The inoculated plants were immediately transferred to a misting chamber and incubated for 40 h in the dark at 21°C. Plants were then incubated with 5 h light (150 to 270 $\mu\text{E mol m}^{-2} \text{s}^{-1}$)/19 h dark at 21°C for 2 days. Plants were dried slowly and returned to the greenhouse. The first disease severity was assessed 20 days after inoculation and the second disease score was done 5 days after the first reading. The disease scale for *S. passerinii* was adapted from Rosielle's *S. tritici* scale based on the percent coverage of pycnidia and necrosis. The disease symptom scale was 0 to 5: 0, immune; 1, highly resistant (HR); 2, resistant (R); 3, intermediate (I); 4, susceptible (S); and 5, very susceptible (VS) (29).

Disease assessment in the field. In order to evaluate adult stage resistance to SSLB, five parents and $F_{2,3}$ families (20 individuals) from three crosses (Robust/CIho 14300, Robust/CIho 4780, and Robust/CIho 10644) were grown in the field at two locations, Langdon and Osnabrock, ND. The two sites differ in microclimate with Osnabrock being lower lying and experiencing more fog and dew. The Langdon site was sown adjacent to a shelter belt of trees which reduced wind and increased humidity in the crop. The experiment was inoculated with a mixture of

ND97-15, ND03338, and ND03190 *S. passerinii* isolates. The isolates ND03338 and ND03190 were isolated from plants naturally infected with SSLB at Langdon, ND, in 2003. The trials were inoculated with a pycnidiospore suspension of 5×10^5 pycnidiospores ml^{-1} , and 75 liter ha^{-1} of the spore solution was applied with a CO_2 pressured hand sprayer equipped with 8001VS nozzles. Inoculation was at the jointing stage (GS 31-32) (42), and was performed at dusk to utilize natural dew formation in order to maintain optimum humidity conditions for disease establishment. Experimental units (20 plots per replicate) were single rows 1.4 m long in an augmented block design with two replicates at each site. Disease symptoms were assessed on leaf number three to five of 10 to 15 randomly selected plants per replicate. The first disease assessment was performed at flag leaf extension stage of development (GS 41-43) and the last at the dough stage of development (GS 85-87), where a score of 0, 1, or 2 was regarded as resistant, and 3, 4, or 5 as susceptible (29).

DNA extraction and bulk preparation. From each parent and progeny at the three-leaf stage (GS 13), the first leaf was collected and placed in 15-ml tubes at -80°C . For $F_{2,3}$ families planted at Langdon, leaf tissue was collected from approximately 10 randomly selected individuals. Leaf samples were transported on dry ice and stored at -80°C . Leaves were ground to a fine powder in liquid nitrogen with a micropestle and genomic DNA was extracted by a modified micro-cetyltrimethylammoniumbromide method (24). DNA was quantified with a UV spectrophotometer and diluted to a final concentration of 25 ng/ μl for further molecular marker analysis.

Two different DNA bulks were prepared based on the principle of bulked-segregant analysis (20). Both bulks were composed of equal volumes of standardized DNA (25 ng/ μl) from five resistant or susceptible individuals separately in each F_2 population (Robust/CIho 14300, Robust/CIho 4780, Robust/CIho 10644, Foster/CIho 14300, Foster/CIho 4780, and Foster/CIho 10644). Microsatellite primer pairs and RAPD primers were used to survey polymorphic fragments between susceptible and resistant accessions in the F_2 bulks.

RAPD and SSR analysis. A total of 480 10-mer RAPD primers (200 from the University of British Columbia, Vancouver, Canada, and 280 from Operon Technologies, Alameda, CA) were used to screen polymorphism between the two different DNA bulks from resistant and susceptible F_2 plants, and between the resistant and susceptible parents. RAPD primers that showed a specific polymorphism between a resistant and susceptible bulk were selected to determine the genetic linkage between SSLB resistance genes and markers. RAPD analyses were performed in a volume of 25 μl (1 $\times$ PCR buffer, 2.0 mM MgCl_2 , 0.2 mM of each nucleotide (dNTP), 1 U of *Taq* polymerase, 0.4 μM of primers, and 25 ng of template DNA) overlaid with 30 μl of mineral oil. Amplifications were carried out in an MJ Research thermocycler (Watertown, MA) programmed for one cycle at 94°C for 4 min, followed by 44 cycles of 1 min at 94°C , 1 min at 35°C , and 1 min at 72°C , and then a final extension step at 75°C for 5 min before cooling to 4°C . Polymerase chain reaction (PCR)-amplified products were separated by electrophoresis in 1.5% agarose gels at 4 v/cm in 1 $\times$ Tris-borate-EDTA (TBE) buffer (100 mM Tris-HCl, 100 mM boric acid, 2 mM EDTA, pH 8.3) or in 6% polyacrylamide gels run in 0.5 $\times$ TBE buffer at 350 v constant power for 1.5 h with a high-throughput sequencing gel system (C.B.S Scientific Company, Inc., Del Mar, CA). Gels were stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$). DNA fragments were visualized with UV light and photographed.

A total of 154 SSR primer pairs developed from Ramsay et al. (26) were obtained from L. Dahleen (USDA-Agricultural Research Station, Fargo, ND), and other necessary SSR primer pairs were constructed from sequences provided in the GrainGenes database. Additional SSR primers were constructed from data in Ramsey et al. (26) and from unpublished data of

K. Chalmers (University of Adelaide, Australia). To select informative SSR markers, two susceptible parents (Robust and Foster) and three resistant parents containing *Rsp1*, *Rsp2*, and *Rsp3* were first screened for polymorphisms in SSRs. SSRs showing polymorphisms between resistant and susceptible parents were tested on the two F₂ DNA bulks to evaluate the association with the parental polymorphism. If polymorphisms were observed between the DNA bulks, then resistant and susceptible F₂ individuals were finally tested with the SSRs. SSRs were amplified and examined for polymorphisms using the PCR protocols obtained from the GrainGenes database available online from the USDA or as described by Liu et al. (18). Each PCR mixture (20 µl) contained 0.2 mM of each dNTP, 2.0 mM of MgCl₂, 1× PCR buffer, 1 U of *Taq* polymerase, and 0.3 µM of forward and reverse primers. Fifty nanograms of each template PCR mixture was placed in a thermocycler (MJ Research). PCR products were separated on a gel containing 6% polyacrylamide and 0.5× TBE buffer at 350 v constant power for 1.5 h with a high-throughput sequencing gel system (C.B.S Scientific Company, Inc.). DNA banding patterns were visualized under UV light.

DArT and RFLP analysis. For more precise mapping of *Rsp* genes on barley chromosomes, DArT for whole-genome profiling of barley was used for 92 F₂ progeny of two populations, Robust/CIho 14300 (*Rsp1*) and Robust/CIho 10644 (*Rsp3*). For DArT assays, 500 to 1,000 ng of DNA resuspended in Tris-EDTA at a concentration of 50 to 100 ng was prepared in microtiter plates. Parents and controls were included in the last four wells of the microtiter plates. DArT analysis was conducted by Triticarte (Yarralumla, Australia), using Barley *Pst*I(*Bst*NI) version 2.0. DArT markers were scored as 1 (fragment present or abundant in the genomic representation of the sample), 0 (fragment absent or rare in the genomic representation of the sample), or × (marker could not be scored for the sample). Only the markers with *P* value (analysis of variance [ANOVA]-based measure of marker quality) above 75 were included for the genotyping. Markers with a call rate (the number of effective scores divided by the number of samples assayed) above 85% were used in DArT analysis for *Rsp* genes.

From barley genetic maps in the GrainGenes database, sequence-tagged site (STS) markers were selected that were located near DArT markers that showed close genetic association with the *Rsp* gene. Primer pairs were constructed from sequences provided in the GrainGenes database. The amplification of DNA was conducted in a 25-µl reaction mixture as described for the RAPD reaction, but with 10 pmol of each reverse or forward STS primer. PCR conditions were one cycle of 5 min at 94°C, 30 to 37 cycles of 30 s at 94°C, 1 min at optimum annealing temperature (temperatures used were 50, 53, 55, 58, 60, or 62°C), 1.5 min at 72°C, and then a final step of 10 min at 72°C. The amplified

products with 4 µl of 6× loading buffer were separated by electrophoresis using 1.5% agarose gel in 0.5× TBE buffer, and visualized under UV light. The markers showing polymorphisms between parents and two bulks were used for segregation analysis in F₂ progeny.

Linkage analysis and genetic mapping. The mode of inheritance of the resistance was determined by chi-square analysis of phenotypic data. The phenotypic segregation ratios in six F₂ populations and three F_{2:3} families were determined for SSLB resistance. Both RAPD and microsatellite marker data of F₂ populations were tested for fit to the expected F₂ or F_{2:3} segregation ratio. Linkage relationships between molecular markers and SSLB resistance genes were analyzed using MAPMAKER software (14), DOS version 3.0. Recombination frequencies were converted into approximate map distances (centimorgans) by using the Kosambi mapping function (11). Linkage maps were constructed on the basis of a minimum logarithm of odds threshold of 3.0 and maximum Kosambi distance of 50 cM.

RESULTS

Differential SSLB reactions between two susceptible cultivars (Robust and Foster) and three resistant accessions (CIho 14300, containing *Rsp1*; CIho 4780, containing *Rsp2*; and CIho 10644, containing *Rsp3*) were observed 20 days after inoculation in a greenhouse. In every inoculation test, the resistant and susceptible parents were included as checks. A goodness-of-fit of 3:1 of the number of resistant to susceptible F₂ plants was observed in all six F₂ populations (Table 1). The genotypic ratio based on the disease reactions of the corresponding three Robust F_{2:3} families all fit a 1:2:1 ratio, and this segregation ratio confirms that a single dominant gene (*Rsp1*, *Rsp2*, or *Rsp3*) confers the seedling stage resistance to SSLB in all three resistant accessions (Table 1). The presence of a single dominant gene (*Rsp1*, *Rsp2*, or *Rsp3*) in the three resistant accessions found here agrees with the early report (26) that single incomplete dominant genes conferred SSLB resistance at the seedling stage in greenhouse tests of F₂ and F₃ populations Traill/CIho 14300, Traill/CIho 4780, and Traill/CIho 10644.

In field experiments to determine SSLB resistance at the adult stage, high levels of SSLB infection were observed at both Langdon and Osnabrock, ND. Disease rating was 5 in the susceptible check Robust and 0 to 1 in three resistant checks (CIho 14300, CIho 4780, and CIho 10644) in both field locations, except that one of six replications of Robust had a rating of 2 and three of six replications of CIho 14300 at Osnabrock had a rating of 2 to 3. The greenhouse and field phenotypic data differed slightly. In the F₃ population for *Rsp1* (Robust/CIho 14300), one heterozygous resistant progeny at Osnabrock was recorded as homozygous resistant. Seven heterozygous progeny in the

TABLE 1. Segregation analysis for Septoria speckled leaf blotch resistance genes *Rsp1*, *Rsp2*, and *Rsp3* in six F₂ populations derived from two susceptible cultivars (Robust and Foster) and three resistant accessions (CIho 14300, CIho 4780, and CIho 1044) and two F₃ populations from crosses of Robust/CIho 14300, Robust/CIho 4780, and Robust/CIho 10644 at the seedling stage in the greenhouse in Fargo, ND, in 2004

Cross Female (S)/Male (R)	Gene	Generation	Observed number	Expected ratio	χ^2	<i>P</i> ^b
			R- ^a :rr	R-:rr		
Foster/CIho 14300	<i>Rsp1</i>	F ₂	93:25	3:1	0.92	0.34
Robust/CIho 14300		F ₃	74:29	3:1	0.55	0.46
Robust/CIho 14300		F ₂	18:51:21	1:2:1	1.80	0.41
Foster/CIho 4780	<i>Rsp2</i>	F ₂	93:27	3:1	0.40	0.53
Robust/CIho 4780		F ₂	85:23	3:1	0.79	0.37
Robust/CIho 4780		F ₃	27:48:25	1:2:1	0.24	0.89
Foster/CIho 10644	<i>Rsp3</i>	F ₂	82:32	3:1	0.57	0.45
Robust/CIho 10644		F ₂	83:32	3:1	0.49	0.48
Robust/CIho 10644		F ₃	21:49:30	1:2:1	1.66	0.44

^a R-, homozygous or heterozygous resistant; RR, homozygous resistant; Rr, heterozygous; and rr, homozygous susceptible.

^b *P* < 0.05 = significant deviation from the expected segregation ratio.

greenhouse were recorded as susceptible at Langdon, and four heterozygous progeny were recorded as susceptible at Osnabrock. In the F_3 population derived from Robust/Ciho 4780 and Robust/Ciho 10644, disease escape in susceptible progeny was the main factor that caused conflicting phenotypic data between the greenhouse and field, especially in the Osnabrock experiment. Seven susceptible progeny were rated as heterozygous, and four segregating progeny were rated as homozygous resistant (infection responses = 0 to 1) (Tables 1 and 2). The segregation ratio of three Robust F_3 populations in both field locations (Langdon and Osnabrock, ND), however, was not significantly different from a 1:2:1 ratio, confirming that a single major gene controls SSLB resistance in all three resistant lines. The field data also indicated that SSLB resistance conferred by a single dominant resistant *Rsp* gene was consistently effective at conferring good adult stage resistance in all three resistant accessions (Table 2).

Initial screening of 154 SSR primer pairs previously mapped in seven barley chromosomes identified a total of 80 SSR loci that were polymorphic between the susceptible and the three resistant parents: 41 (26.6%) for *Rsp1*, 23 (14.9%) for *Rsp2*, and 16 (10.4%) for *Rsp3*. These SSR markers were then tested for polymorphisms between two F_2 DNA bulks and 100 to 120 individual F_2 plants, but no SSRs were found to be strongly associated with *Rsp* genes. After determining chromosome locations of *Rsp* genes using DArT, 11 SSRs on chromosome 1H and 6 SSRs on chromosome 3H were analyzed for their linkage associations with *Rsp* genes. Among seven SSRs (Bmac0067, Bmag0006, Bmac0209, Bmag0010c, Bmag0225, Bmag0877, and Bmag0013) associated with *Rsp1*, Bmac0067 showed the closest linkage with *Rsp1* (23 cM). Seven SSRs for *Rsp2* (Bmac0032, HVM20, Bmag0211, Bmag0347, Bmac0154, Bmag0228a, and Bmag0382), and three for *Rsp3* (Bmac0090, Bmag0228a, and HVHVA1) on chromosome 1H were analyzed for their linkage relationships with *Rsp2* and *Rsp3*. The two nearest SSR markers, Bmac0032 and HVM20 from *Rsp2*, were found at a distance of 33 and 39 cM, respectively. The closest SSR marker for *Rsp3* was Bmac0090 (28 cM).

DArT was conducted on the F_2 progeny of Robust/Ciho 14300 (*Rsp1*) and Robust/Ciho 10644 (*Rsp3*) to determine the map location of *Rsp* genes in the barley genome. The mapping population for *Rsp2* was not included for DArT analysis because of the previously reported close linkage relationship between *Rsp2* and *Rsp3* (27). Genotyping data from DArT analysis provided 256 useful DArT markers for *Rsp1* and 377 useful DArT markers for *Rsp3*. The markers were analyzed for linkage relationships with the two *Rsp* genes. *Rsp1* was mapped on the short arm of chromosome 3H at a distance of 8.9 and 16.3 cM distal from DArT markers bPb-6978 and bPb-9945. Five DArT markers (bPb-1348, bPb-9881, bPb-1165, bPb-3622, and bPb-6482) showed close genetic association with the *Rsp3* locus, and linkage analysis revealed that the markers are located on the short arm of chromosome 1H. As a result, *Rsp3* was positioned on chromosome 1HS 2.3 to 5.8 cM from the five DArT markers (Fig. 1). To determine the precise map location of *Rsp2*, seven STS

markers converted from RFLPs previously mapped to chromosome 1HS of barley were screened to detect polymorphism between the two parents and also between the two F_2 DNA bulks. A polymorphic STS marker, MWG938, was found to be located at the telomeric region of chromosome 1HS. Linkage analysis of all 107 F_2 plants in the Robust/Ciho 4780 population showed no recombination between MWG938 and the *Rsp2* gene (Fig. 1).

To identify more closely associated molecular markers, RAPD analysis was performed on susceptible and resistant parents and bulks from three populations, Robust/Ciho 14300, Robust/Ciho 4780, and Robust/Ciho 10644 (16). Five RAPD markers were identified for *Rsp* genes. RAPD markers OPC2_{441R} (3.0 cM) and UBC285_{158R} (4.3 cM) were linked in repulsion to *Rsp1*, RAPD marker OPAH5_{545C} (0 cM) was linked in coupling to *Rsp2*, and RAPD marker OPBA12_{314C} (2.4 cM) was linked in coupling and OPB17_{451R} (3.5 cM) was linked in repulsion to *Rsp3* (16). These RAPD markers were integrated into the linkage maps of *Rsp* genes (Fig. 1).

The genetic linkage maps of chromosome 1H and 3H containing *Rsp1*, *Rsp2*, and *Rsp3* loci were constructed with 33 markers in Robust/Ciho 14300, Robust/Ciho 4780, and Robust/Ciho 10644 populations, including 13 DArT markers, 1 RFLP, 5 RAPDs, and 14 SSRs. The total genetic distance is 110 cM for *Rsp1*/*Rsp2* and 80 cM for *Rsp3* (data for the whole chromosome 1H and 3H linkage maps are not shown).

DISCUSSION

Genetic inheritance of SSLB resistance in three resistant accessions, Ciho 14300 (Ciho 4249-2), Ciho 4780, and Ciho

Fig. 1. Partial genetic linkage maps of Septoria speckled leaf blotch (SSLB) resistance genes *Rsp1*, *Rsp2*, and *Rsp3* on chromosomes 1HS and 3HS of barley. The positions and the genetic distances in centimorgans between SSLB resistance genes (*Rsp1*, *Rsp2*, and *Rsp3*) and molecular markers were calculated in MAPMAKER (Whitehead Inst. for Biomedical Res., Cambridge, MA) at a $\log_{10}$ odds ratio score of 3. Markers linked to *Rsp* genes were mapped using three populations derived from crosses Robust/Ciho 14300 (*Rsp1*), Robust/Ciho 4780 (*Rsp2*), and Robust/Ciho 10644 (*Rsp3*).

TABLE 2. Segregation analysis of *Rsp* genes in three $F_{2,3}$ families derived from susceptible cv. Robust and three resistant accessions (Ciho 14300, Ciho 4780, and Ciho 10644) using plants at the adult stage in the field in 2004

Cross	Location	Observed number			Expected ratio	χ^2	<i>P</i> ^a
		<i>RspRsp</i>	<i>Rsprsp</i>	<i>rsprsp</i>			
Robust/Ciho 14300	Langdon ^b	18	44	28	1:2:1	2.27	0.32
	Osnabrock ^b	19	46	25		0.84	0.66
Robust/Ciho 4780	Langdon	25	49	26	1:2:1	0.06	0.97
	Osnabrock	31	51	18		3.42	0.18
Robust/Ciho 10644	Langdon	25	53	27	1:2:1	0.09	0.96
	Osnabrock	21	59	25		1.91	0.39

^a $P < 0.05$ = significant deviation from the expected segregation ratio.

^b Field location in North Dakota.

10644, were previously studied in Traill F₂ populations at the seedling stage (27). Rasmusson and Rogers (27) reported incomplete dominance in all three *Rsp* genes in the three resistant accessions at the seedling stage in the greenhouse. In our F₂ populations, SSLB responses of heterozygous resistance (*Rsp/rsp*) were not distinguishable from parental checks containing these genes. Homozygote and heterozygote were only clearly separated in SSLB screening of F₃ populations. In a recent study, Toubia-Rahme and Steffenson (35) described the SSLB responses of 78 barley accessions at seedling and adult-plant stages in the greenhouse and field. They confirmed the resistance of CIho 4780 and CIho 10644 in the seedling stage in the greenhouse and in the adult stage in the field, and showed that CIho 4249-2, another putative source of *Rsp1*, was moderately resistant at the adult stage in the field, but susceptible in the seedling stage in the greenhouse. These results differ from the results of Rasmusson and Rogers (27) and this study.

In this study, we found that all three resistant accessions have good resistance to *S. passerinii* at both seedling and adult-plant stages in the greenhouse and in the field. Adult plant resistance in CIho 14300 was only a little lower than adult plant resistance of CIho 4780 and CIho 10644. Among 24 plots of resistant CIho 14300 in both field trials, three showed some disease development (infection response = 2). No disease was found on the other resistant accessions CIho 4780 (*Rsp2*) and CIho 10644 (*Rsp3*). Disease seen in the field in a few homozygous and heterozygous resistant F₃ progeny may be an indication of a decreased level of adult plant resistance in CIho 14300 (Table 2). However, from the field screening of SSLB, highly significant correlations were found between the SSLB infection response of seedlings in the greenhouse and adult plants in the field. Segregation ratios of 3:1 in six F₂ populations and 1:2:1 in three Robust F₃ populations were found. These results strongly indicate that a single dominant gene located in barley chromosome 1HS and 3HS confers resistance to *S. passerinii* at both seedling and adult stages.

The variability of SSLB reaction in CIho 14300 found by different researchers (36) may be due to the use of different seed stocks or experimental conditions. CIho 4249-2, which also has the alternate accession number CIho 14300, is a reselection from CIho 4249 (PI64878) and may be heterozygous. The resistant parent CIho 14300 used in this study was originally received from the USDA National Small Grains collection in 1993 and the line was reselected three times to ensure seed purity, based on SSLB reactions in the greenhouse (J. Franckowiak, North Dakota State University, Fargo, *personal communication*).

Many sources of resistance to *S. passerinii* have been reported in different *Hordeum* accessions (17,4,35). Several genetic studies of SSLB resistance have been conducted in older accessions of barley including Feebar (2), Atlas (21), and CIho 4439 (19). Despite the abundant resources for SSLB resistance, most of the resistant loci have not been genetically mapped using molecular markers in barley chromosomes. In previous studies, the *H. bulbosum* introgressions of SSLB resistance were mapped on chromosome 4HL and 7HS (36), and a putative map location of *Rsp2* was positioned on chromosome 1H (33). In different reports, research from the same laboratory placed *Rsp2* on chromosomes 3H (36) and 5H (44). Chromosome 3HS, where *Rsp1* was mapped in this study, contains many disease resistance genes for leaf rust (43), loose smut (23), stem rust (30), *Barley mild mosaic virus* (7), and *Barley yellow dwarf virus resistance* (25). The marker MWG938, used in this study, showed that *Rsp2* is located on the telomeric region of chromosome 1HS in a similar region to *Mla* (powdery mildew) (32), *Rph4* (leaf rust) (3), and *Rrs14* (scald) loci (5). Several DArT markers surrounding the *Rsp3* locus clearly demonstrate that this gene is located on chromosome 1HS, and is closely linked to *Rsp2* (Fig. 2). The resistant locus of barley *Yellow mosaic virus* complex, *ym7*, (6) is positioned near *Rsp3*. In recent studies, quantitative trait loci for

SSLB resistance on chromosomes 1HS and 2HL were identified in the ND16092/ZAU7 recombinant inbred line population using DArT (G. Yu, North Dakota State University, *personal communication*). The two QTL for SSLB resistance identified by Yu are located on the short arm of chromosome 1H in a similar position to *Rsp2*. However, it is not clear whether the QTL is related to the *Rsp2* gene. More than half of the mapped disease-resistant genes identified arise in clusters for a wide variety of pathogens (41). For example, the cluster at the *Mla* locus contains multiple classes of genes associated with plant defense mechanisms, and 15 of the 32 predicted genes are related to plant defense responses (38).

Rasmusson and Rogers (27) suggested that the distance between loci *Rsp2* and *Rsp3* was 3.87 ± 1.29 cM in their F₃ segregating populations and that *Rsp1* is inherited independently from other linked *Rsp* genes. In this study, the relative map positions of the two linked *Rsp* genes were estimated by comparing the linkage maps of *Rsp2* and *Rsp3* with the Triticarte integrated linkage map for barley. As we did not undertake DArT analysis on *Rsp2*, we were not able to determine if the DArT markers for *Rsp3* were also common to *Rsp2*. However the Triticarte integrated linkage map shows bPb-1348 and MWG938 at the same position on chromosome 1HS. In our DArT analysis bPb-1348 was linked to *Rsp3* and in our STS analysis MWG938 was linked to *Rsp2*. Using this information we estimated that *Rsp2* is approximately 6 cM distal from *Rsp3* on the short arm of barley chromosome 1H. This estimated genetic distance between *Rsp2* and *Rsp3* is in close agreement with the previous result by Rasmusson and Rogers (27). The limited number of markers for *Rsp2*, however, makes it difficult to accurately analyze the linkage distance between *Rsp2* and *Rsp3*.

SSR markers have been demonstrated to be powerful tools for genetic studies and for barley breeding in several studies (18,31). However, in this study, we found that SSRs are not an efficient marker system for the genetic study of *Rsp* genes. For all *Rsp* genes, an average of 17.3% of the 154 SSR markers tested show polymorphisms between parents, but most of the polymorphic SSRs tested are clustered near the centromere region of the chromosomes. Several recent studies report that the physical mapping of the seven barley chromosomes indicates a severe suppression of recombination around the centromeres leading to

Fig. 2. Estimation of the relative linkage relationships of *Rsp2* and *Rsp3* Septoria speckled leaf blotch resistance genes based on the comparison of three linkage maps on the short arm of chromosome 1H. **A**, Partial Triticarte integrated linkage map of 1HS from seven populations. This map was assembled from seven individual maps using PhenoMap (GeneFlow Inc., Alexandria, VA): Barque-73/CPI1284-48, Clipper/Sahara, Dayton/ZP2, Foster/CI4196, Franklin/TX9425, Steptoe/Morex, and Yerong/Franklin; **B**, Robust/CIho 4780; and **C**, Robust/CIho 1064.

extensive clustering of markers in genetic linkage maps (12,13). Thus, large gaps are still present on the barley linkage map based on SSRs because of this clustering around the centromeres. Due to the limitations of SSR markers, DaRT was applied for the study of mapping *Rsp* genes. In contrast to SSRs, DaRT markers provide good coverage and distribution on all barley chromosomes (39). From the genotyping data of *Rsp3*, 11 DaRT markers linked to *Rsp3* were found covering the total genetic distance of 80 cM on chromosome 1H.

Information on chromosome location and molecular markers linked to SSLB resistance loci is important in breeding for SSLB resistance. MAS can allow breeders to bypass laborious and costly phenotypic screening and greatly enhance the opportunity for gene pyramiding in improved cultivars, especially in this pathosystem where different SSLB resistances cannot be separated phenotypically. *Rsp* genes have been identified for more than 40 years, but chromosomal location and molecular markers linked to resistance genes have not been available. Molecular linkage maps of *Rsp* genes in barley chromosomes provided in this study can be utilized for fine mapping and positional cloning of *Rsp* genes. Moreover, the knowledge concerning molecular mapping of *Rsp1*, *Rsp2*, and *Rsp3* genes can be useful in the creation of gene combinations for more durable resistance to SSLB in barley.

ACKNOWLEDGMENTS

We thank M. Y. Sun and P. Gross for support during the establishment of field trials; R. Horsley for discussions prior to the research; B. Steffenson for the gift of an isolate of *S. passerinii*; J. Franckowiak for supplying resistant and susceptible parent accessions; L. Dahleen for the gift of SSR primers; and K. Chalmers for unpublished SSR primer sequences.

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