Molecular Mapping of the Crown Rust Resistance Gene *Rpc1* in Barley

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ABSTRACT

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Crown rust of barley, caused by *Puccinia coronata* var. *hordei*, occurs sporadically and sometimes may cause yield and quality reductions in the Great Plains region of the United States and Canada. The incompletely dominant resistance allele *Rpc1* confers resistance to *P. coronata* in barley. Two generations, F_2 and $F_{2:3}$, developed from a cross between the resistant line Hor2596 (CIho 1243) and the susceptible line Bowman (PI 483237), were used in this study. Bulked segregant analysis combined with random amplified polymorphic DNA (RAPD) primers were used to identify molecular markers linked to *Rpc1*. DNA genotypes produced by 500 RAPD primers, 200 microsatellites (SSRs), and 71 restriction fragment length polymorphism (RFLP) probes were applied to map *Rpc1*. Of these, 15 RAPD primers identified polymorphisms between resistant and susceptible bulks, and 62 SSR markers and 32 RFLP markers identified polymorphisms between the resistant and susceptible parents. The polymorphic markers were applied to 97 F_2 individuals and $F_{2:3}$ families. These markers identified 112 polymorphisms and were used for primary linkage mapping to *Rpc1* using Map Manager QT. Two RFLP and five SSR markers spanning the centromere on chromosome 3H and one RAPD marker (OPO08-700) were linked with *Rpc1* and, thus, used to construct a 30-centimorgan (cM) linkage map containing the *Rpc1* locus. The genetic distance between *Rpc1* and the closest marker, RAPD OPO08-700, was 2.5 cM. The linked markers will be useful for incorporating this crown rust resistance gene into barley breeding lines.

Crown rust, caused by *Puccinia coronata* var. *hordei* Jin & Steff (19), is a relatively new disease of barley (*Hordeum vulgare* L.) in the Great Plains region of the United States. (23). The first serious outbreak of this disease occurred in 1991 (16). Since that time, crown rust has been observed on barley every year in varying levels of incidence and severity. Yield losses due to *P. coronata* var. *hordei* have not been established for barley, but have the potential to be significant because the pathogen can infect the crop early in the season and increase to severity levels as high as 60% (17). Deployment of resistant cultivars is the only economically viable option for the control of this disease. Unfortunately, all currently grown barley cultivars in the region are susceptible to the disease. To identify sources of crown rust resistance, Jin and Steffenson screened a geographically diverse collection of barley germ plasm (526 accessions) for *P. coronata* var. *hordei* reaction at the seedling stage (17). Only 10 accessions (1.9% of the total screened) were found to be resistant. One of these accessions, Hor2596 (CIho 1243), exhibited a high level of resistance at both the seedling and adult plant stage and was used in subsequent genetic studies. A single incompletely dominant gene, designated *Rpc1*, was found to confer crown rust resistance in Hor2596 (17).

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Identification of markers linked to *Rpc1* will facilitate marker-assisted selection of the gene in breeding populations as more resistance genes are identified. Such markers also may help to resolve the chromosomal position of the gene. Polymerase chain reaction (PCR)-based markers, such as random amplified polymorphic DNA (RAPD) (38,39), can reduce the cost of identifying genetic markers and allow large-scale genotyping of individuals at any locus. The choice of marker system for a particular application depends on the type of genomic information required and its ability to detect polymorphism in a given population. The use of anchoring markers such as restriction fragment length polymorphisms (RFLPs) and microsatellites (SSRs) with known chromosomal locations can be used in conjunction with RAPD markers to position the latter and their linked target genes to a linkage group.

Bulked segregant analysis (BSA) can be used to identify markers linked to a gene of interest (26). This process is highly efficient because it detects only a small percentage of polymorphisms in F_2 individual progeny tests. BSA has been used to identify RAPD markers linked to genes controlling resistance to many pathogens, including leaf scald (4), leaf rust (31), and common root rot and spot blotch (22) in barley; downy mildew in corn (3); and common rust bean (14). The use of BSA in combination with PCR-based molecular markers has proven to be a very powerful technique for identifying markers tightly linked to, or cosegregating with, genes underlying monogenic traits (2,9,28,34,36).

The objective of this research was to determine the chromosomal location of the crown rust resistance gene *Rpc1* from Hor2596. We used BSA to identify RAPD markers linked with *Rpc1*. RFLP and SSR markers with known chromosomal locations then were used to map these RAPD markers and the resistance gene to a specific chromosome region of barley.

MATERIALS AND METHODS

Plant material and disease screening. The resistant barley accession Hor2596 (CIho 1243) and susceptible cv. Bowman (PI 483237) were crossed to produce F_2 and $F_{2:3}$ populations (17,18). Hor2596 carries an incompletely dominant gene *Rpc1*, that confers a low infection type (IT) of 0;,1 to *P. coronata* var. *hordei* (17). Bowman is fully susceptible to crown rust and exhibits an IT of 3,4. In all, 261 F_2 progeny were tested for crown rust resistance when the primary leaves were fully expanded (17). Plants were harvested individually to produce F_2-derived F_3 families. In all, 15 to 20 seedlings of each $F_{2:3}$ family were grown for crown rust evaluation. $F_{2:3}$ families were evaluated at two different times to determine the genotypes for crown rust reaction. The sowing, growing, inoculation, and disease assessment protocols were as described by Jin and Steffenson (17).

DNA extraction and RAPD analysis. Leaf tissues of 97 randomly selected F_2 plants were collected for DNA extraction and molecular marker assays. DNA was extracted from lyophilized leaf tissue of the parents and F_2 progeny using the methods of Kleinhofs et al. (20). Initial RAPD analysis was conducted on the parents and bulks of homozygous resistant (infection type 0;,1) or susceptible (infection type 3 to 4) F_2 plants. Bulks were constructed by examining the crown rust reaction of $F_{2:3}$ families and selecting F_2 plants that were homozygous for resistance or susceptibility. Equal amounts of DNA from six homozygous F_2 plants were mixed to create each bulk sample. A total of 500 10-base random primers (Operon Technologies, Alameda, CA) were screened against the two parents and the susceptible and resistant bulks, following the method of Horvath et al. (15). Primers that amplified potential polymorphisms between parents and bulks were retested and those that gave reliable differences were tested against the 97 individual F_2 plants.

RFLPs and SSRs. For RFLP analysis, 8 µg of DNA from each line was digested separately with *Eco*RI, *Bam*HI, or *Hin*dIII, then separated in 0.8% agarose gels and Southern-blotted to Hybond N^+ filters (Amersham Biosciences, Piscataway, NJ). Seventy-one probes from the North American Barley Genome Project (20) were labeled with ^{32}P and hybridized and detected as described by Dahleen (10). In all, 216 SSRs (33) were tested for polymorphisms between the two parents. SSR loci were amplified in a 20-µl reaction mixture containing 50 ng of template DNA, 0.3 µM each primer, 200 µM each dNTP, 2 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase, and 1× PCR buffer supplied with the enzyme. Temperature cycling conditions were as described by Ramsay et al. (33). Amplified products were separated in 4% SFR agarose (Amresco, Solon, OH) gels stained with ethidium bromide and visualized using UV light.

Statistical analysis and mapping of *Rpc1*. This population had been phenotyped previously for disease reaction (17). Observed and expected segregation ratios of resistant and susceptible plants in the 97-line F_2 and $F_{2:3}$ populations were compared by χ^2 tests. Mapping data were obtained by visual scoring of gels (RAPDs and SSRs) and autoradiograms (RFLPs). Segregating markers were scored for the presence or absence of amplified bands from dominant markers. Co-dominant marker segregation was coded according to alleles present, following Map Manager QT (25) data file coding. Markers defining polymorphisms that fit the expected 3:1 or 1:2:1 Mendelian ratio at $P = 0.05$ were used for mapping the *Rpc1* locus. Linkage between the markers and *Rpc1* was analyzed using Map Manager QT (25). Map distances in centimorgans (cM) were calculated from recombination frequencies using Kosambi's mapping function (21).

RESULTS

This report describes mapping of the crown rust resistance gene *Rpc1* in barley line Hor2596 using a combination of BSA and genetic mapping with anchoring RFLP and SSR markers. Segregation in the 97 lines fit a 3:1 ratio of resistant to susceptible phenotypes in the F_2 and a 1:2:1 ratio of homozygous resistant/segregating/homozygous susceptible phenotypes in the $F_{2:3}$ (Table 1), confirming that the subset of lines represented the original population of 261 F_2 plants (17).

Initial screening of 71 RFLP clones identified 32 loci that were polymorphic between the parents. These loci were mapped on the 97 F_2 lines but no strong linkages with *Rpc1* were detected; therefore, BSA was conducted by testing 500 RAPD primers on the parents and crown rust resistant and susceptible bulks. Only 15 (3% of those tested) primers generated 18 polymorphic markers. When a polymorphic RAPD marker was identified, the primer was tested against all 97 F_2 progeny to score the segregating bands and determine if they were linked to *Rpc1*. A 700-bp RAPD amplified by primer OPO08 subsequently was found to be associated with *Rpc1* (Fig. 1). Additionally, 62 SSR loci segregated in the population using SFR agarose, which could resolve as small as a 2- to 4-bp size difference.

Data from the 18 RAPD, 32 RFLP, and 62 SSR markers were used for mapping *Rpc1*. Pairwise recombination between markers was calculated using Map Manager. Two RFLPs (ABG460 and ABG377), one RAPD (OPO08-700), and five SSRs showed close genetic association (2.6 to 21.1% recombination) with *Rpc1* (Table 2). Linkage was established at log of likelihood scores of 14 to 3. The order of the RFLP and SSR markers fit those in published maps (20,33). The map position of *Rpc1* was 2.5 cM (2.6% recombination) distal to RAPD marker OPO08-700 and within a region of 30.4 cM defined by all the markers that spanned the centromere of chromosome 3H (Fig. 2; Table 2). The mapped RFLPs and SSRs placed *Rpc1* near the centromere of chromosome 3H.

DISCUSSION

BSA is an efficient method to identify molecular markers associated with simply inherited traits, without creating a complete linkage map. This method has been used with RAPD markers to identify seven markers in barley associated with resistance to *Pyrenophora teres* f. *maculata* (27). Also, BSA was used to identify a RAPD marker associated with leaf scald resistance that mapped to chromosome 3H in the Vada/B87 barley population (35). BSA was used in barley for tagging and mapping both leaf (5,6) and stem (7) rust resistance genes.

The mode of inheritance of *Rpc1* in barley has been studied in three F_2 populations (17), including the Hor2596 × Bowman population which we used in this study. The other two populations were derived from crosses of Hor2596 to Wolfe's multiple dominant (MD) and multiple recessive (MR) marker stocks. This allowed Jin and Steffenson to identify a loose association be-

TABLE 1. Segregation for crown rust resistance and susceptibility in F_2 and $F_{2:3}$ progeny from a cross between Bowman and Hor2596[a]

Generation	Ratio fit	Number of plants			χ^2	Probability (>χ^2)
		Resistant	Segregating	Susceptible		
F_2	3:1	74	…	23	0.086	0.7694
F_3	1:2:1	25	52	20	1.021	0.6003

[a] This is a subset of the population tested by Jin and Steffenson (17).

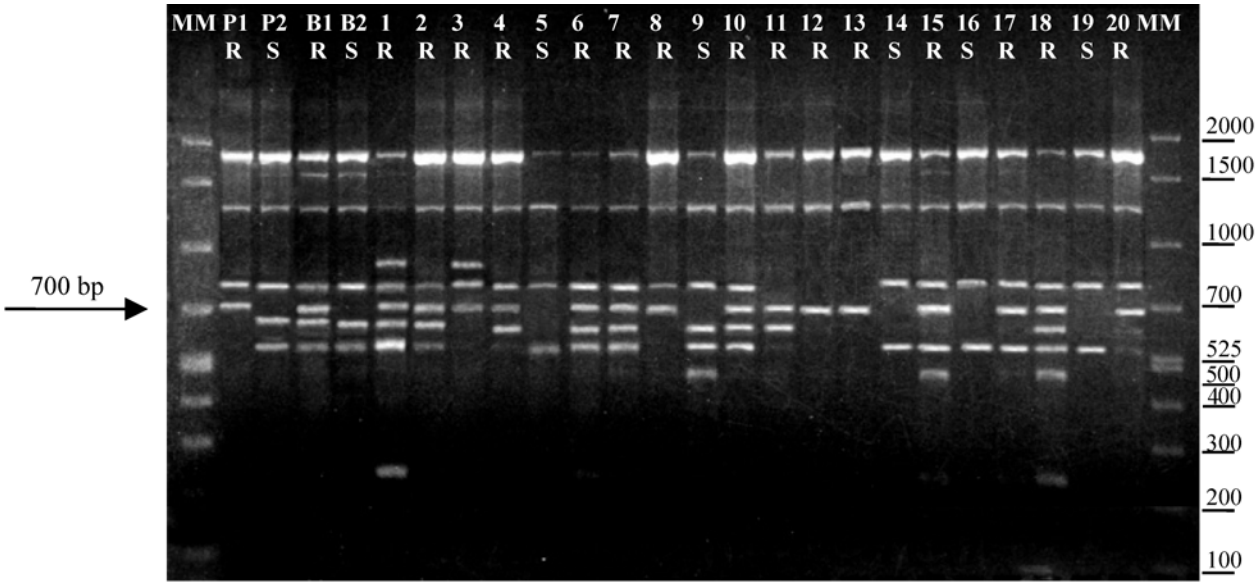

Fig. 1. Random amplified polymorphic DNA marker Operon O08 obtained by bulked segregant analysis. P1, Hor2596; P2, Bowman; B1, resistant bulk; B2 susceptible bulk; lanes 1 to 20, F_2 individuals. The first and last lanes MM are BioMarker EXT standard DNA (BioVentures, Inc). The arrow indicates the polymorphic 700-bp fragment that was linked to the crown rust resistance gene *Rpc1*.

TABLE 2. Summary of loci linked to *Rpc1*, the gene for resistance to crown rust in barley

Marker	Recombination frequency (% ± SE[a])	Log of likelihood
ABG460	20.99 ± 4.77	3.3
Bmac0209	13.82 ± 3.41	7.2
Bmag0136	11.39 ± 2.98	9.2
Bmag0006	06.02 ± 2.02	14.2
OPO08-700	02.59 ± 1.23	13.2
HVM033	14.24 ± 3.48	6.6
Bmag0225	15.44 ± 3.85	5.7
ABG377	21.07 ± 4.98	3.1

[a] SE = standard error.

tween *Rpc1* and the morphological marker *alm* for albino lemma, on the short arm of chromosome 3HS (17). Because few morphological markers segregated in prior studies, these markers were of limited use for mapping. The morphological markers in these MR and MD stocks are of less use in breeding, because most of the traits are not desirable in commercial cultivars. Traits such as albino lemma usually are associated with reduced yield, because the reduced chlorophyll content provides less photosynthate for the developing seed.

Use of the numerous molecular markers developed for barley greatly facilitated the mapping of *Rpc1* to a specific chromosome region. Although the *Rpc1*-linked RAPD marker (OPO08-700) was identified using BSA, RFLP and SSR markers were required to determine the chromosome position of *Rpc1*, because the RAPD marker was not polymorphic in other mapping populations (data not shown). When the RAPD band was cloned and used as an RFLP probe, multiple copies were observed with no polymorphisms between genotypes. Our data with the RFLP and SSR markers clearly demonstrated that *Rpc1* is located on the short arm of chromosome 3H in the same region as *alm*. Many resistance genes have been mapped to the same region of chromosome 3H using genetic markers (i.e., *Rph5*, *Rph6*, and *Rph7*) for leaf rust resistance (8,12,24,41), *Run6* for loose smut resistance (29), *Rp1-D* for stem rust (34), *rym5* and *rym4* for *Barley mild mosaic virus* resistance (11,13), and *Ryd2* for resistance to *Barley yellow dwarf virus* (32). It is possible that these genes, conferring resistance to a wide variety of pathogens, arose through gene duplications and subsequent mutations. An example of a resistance

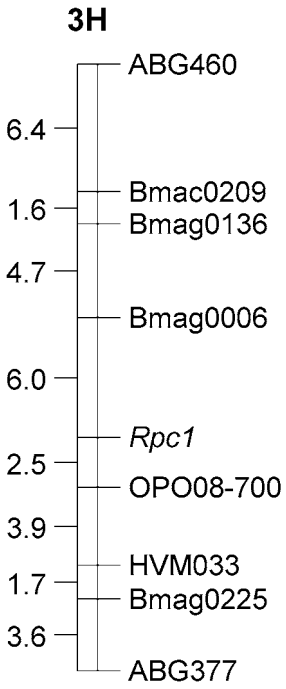

Fig. 2. Region of chromosome 3H of barley containing molecular markers linked to the *Rpc1* locus, based on Bowman × Hor2596 F_2 and $F_{2:3}$ populations. Marker identifications are provided on the right side of the map and genetic distance in Kosambi centimorgans are on the left.

gene cluster spans the *Mla* (powdery mildew) locus for resistance to the obligate fungal biotroph *Blumeria graminis* f. sp. *hordei* (37). This 261-kb region includes 32 predicted genes, 15 of which are associated with plant defense responses and 6 which are involved with response to powdery mildew infection.

The use of BSA with RAPD markers plus RFLP and SSR marker analysis can facilitate the identification of markers linked more closely to the trait of interest. Most importantly, it provides information on chromosome location without the expense of creating a full genetic map. Armed with the chromosomal locations of resistance genes, plant breeders can make more informed

decisions about combining genes which may be linked in coupling or repulsion (40). The markers linked to *Rpc1* can be utilized for fine mapping and positional cloning of the gene (1) and will extend the barley function map for pathogen resistance. This is the first report placing a gene for resistance to *Puccinia coronata* on the barley molecular marker map.

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