Sources and Genetics of Crown Rust Resistance in Barley

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ABSTRACT

Crown rust, caused by Puccinia coronata var. hordei, is a new disease threat to barley (Hordeum vulgare). The first serious outbreak of the disease was reported in south central Nebraska in 1991 (13), although the rust was likely present in the Great Plains region before this time (17). Crown rust has been reported on barley in Minnesota, Nebraska, North Dakota, South Dakota, and Wisconsin in the United States and Manitoba and Saskatchewan in Canada (11). The primary telial hosts for the rust include cultivated barley, foxtail barley (H. jubatum), quackgrass (Elyttrigia repens), slender wheatgrass (Elymus trachycaulus), and western wheatgrass (Pascopyrum smithii) (9,11). Common buckthorn (Rhamnus cathartica) is the alternate host (10,11).

Since the initial outbreak in 1991, this disease has been reported every year on barley in the Upper Midwest region of the United States, although the incidence and severity may vary. In some years, the rust may be found in only trace amounts, usually near the alternate host, but in other years, the rust may be more widespread across the region, suggesting significant secondary spread from the primary inoculum source of infected buckthorn. Rust severities as high as 60% were observed on barley growing near common buckthorn in the central part of the Red River Valley region of Minnesota and North Dakota (15). Losses due to crown rust have not been established for barley. However, the potential for severe yield loss does exist because this rust can infect the crop early in the season (middle May to early June) from local inoculum sources (i.e., aeciospores from buckthorn growing adjacent to barley). Preliminary studies revealed that P. coronata var. hordei possesses virulence on all of the widely grown barley cultivars in the Upper Midwest region (12). The objective of this study was to investigate the sources and genetics of crown rust resistance in barley.

MATERIALS AND METHODS

Seedling evaluations. To identify possible sources of resistance to P. coronata var. hordei, a geographically diverse sample of barley germ plasm (526 accessions total) from the USDA National Small Grains Collection (NSGC) was evaluated at the seedling stage. These accessions were arbitrarily selected from different countries to achieve the greatest geographic diversity possible and were provided by H. Bockleman, curator of the NSGC in Aberdeen, Idaho. Susceptible checks included cvs. Foster (PI 592758), Aim (Clho 3737), and Steptoe (Clho 15229). The resistant check was Hor2596 (Clho 1243). These checks were previously characterized for their reaction to crown rust in an earlier experiment. For the seedling screening test, three to five seeds of each accession were planted in a plastic cone (3.8-cm diameter and 21 cm depth) filled with a peat moss/perlite (3:1) potting mix (#1 Sunshine Mix from Fisons Horticulture, Inc., Vancouver, Canada). Plants were grown in a greenhouse with a daily average temperature range of 20 to 24°C and a 14- to 16-h photoperiod.

A single aecidial isolate (ND91-36) of P. coronata var. hordei was used in all rust evaluations. This isolate was originally collected from barley in Nebraska in 1991 and possesses a high level of virulence on many barley cultivars. Plants were inoculated 1 week after planting when the primary leaves were fully expanded. Urediniospores, suspended in a lightweight mineral oil (3 mg of urediniospores per 0.5 ml of oil), were applied with an atomizer at a rate of 10 µg of urediniospores per plant. Plants were then placed in chambers maintained near saturation by intermittent mistings from ultrasonic humidifiers (19). The plants were incubated at 20 to 21°C for 16 h in the dark and then they were
allowed to dry off slowly for several hours before being placed in a greenhouse at 20 to 24°C with a 14- to 16-h photoperiod.

Crown rust infection types (ITs) were assessed 14 days post-inoculation. A 0-to-4 qualitative scale was used for assessing ITs on barley. This scale was adapted from the one developed for the wheat stem rust pathosystem by Stakman et al. (18), where 0 = no visible infection; 1 = minute uredinia, often surrounded by necrosis; 2 = small uredinia surrounded by extensive chlorosis; 3 = medium-sized uredinia with or without chlorosis; and 4 = large uredinia with or without chlorosis. ITs of 0, 0; 1, 1; or 2 were considered indicative of a resistant host response, whereas ITs of 3 and 4 were considered indicative of a susceptible host response. Accessions exhibiting crown rust resistance in the initial screening test were reevaluated in a second experiment using the same methods described previously.

Adult plant evaluations. Accessions exhibiting a high level of crown rust resistance at the seedling stage were subsequently evaluated at the adult plant stage in the greenhouse. Three to five seeds of each accession (including susceptible and resistant checks) were sown in clay pots (15 cm in diameter) filled with the peat moss/perlite potting mix. Three planting dates, 5 days apart, were made to obtain plants at a similar growth stage for inoculation. A completely randomized design was used with three replicates. Plants were inoculated at the heading stage following the same protocols as described for the seedling tests, except that the rate of inoculum application was approximately 60 µg of spores per plant. Infection responses on the leaves of adult plants were assessed 20 days after inoculation following a scale modified from Stubbs et al. (20) for other cereal rusts, where 0 = immune, no visible infection; R = resistant, necrotic areas with or without small uredinia; MR = moderately resistant, small uredinia surrounded by necrotic areas; MS = moderately susceptible, medium-sized uredinia without necrosis, but some possible chlorosis; and S = susceptible, large uredinia without necrosis or chlorosis. The selected resistant accessions and checks also were planted in a field nursery at Casselton, ND, in 1993 to 1995. Entries were sown in 1.5-m row plots (5 g of seed per plot) arranged in a randomized complete block design with three replicates. The field site was located near a windbreak where common buckthorn plants were abundant; thus, crown rust infections were initiated byaeciospores from the alternate host.

Crosses and progeny evaluation. Hor2596 carries Rph9, a gene conferring resistance to the leaf rust pathogen P. hordei (1, 6). It also was found to carry a high level of resistance to P. coronata var. hordei at the seedling stage in a preliminary study and was therefore used as the resistant check. Moreover, Hor2596 proved highly resistant at both the seedling and adult plant stages in subsequent experiments. To study the inheritance of crown rust resistance, Hor2596 was crossed with susceptible cv. Bowman (PI 483237) and two susceptible genetic stocks carrying multiple recessive (MR) and multiple dominant (MD) morphological markers (21). F2 populations of the three crosses (238 to 267 progeny) were evaluated for segregation to isolate ND91-36 of P. coronata var. hordei at the seedling stage using methods previously described. One hundred thirty-two randomly selected F2 families (30 seeds per family) derived from the Bowman × Hor2596 cross were evaluated for crown rust reaction to verify results obtained in the F2 generation. A subsample (20 to 25 seeds) of each selected F2 family from the Bowman × Hor2596 cross was also evaluated for reaction to isolate ND8702 of P. hordei at the seedling stage to investigate the possible genetic relationship between resistance to crown rust and resistance to leaf rust. Leaf rust evaluations were done according to the methods of Steffenson et al. (19).

In an attempt to associate the crown rust resistance gene with a specific barley chromosome, each F2 plant from the crosses MR × Hor2596 and MD × Hor2596 was scored for crown rust reaction (resistant/susceptible) and for presence/absence of the morphological traits. The morphological markers in these genetic stocks were previously mapped to one of the seven chromosomes in barley (5). Thus, the detection of linkage between a morphological marker and the gene of interest will allow one to assign the latter to a chromosome. This inexpensive method for associating loci to a specific chromosome has been successfully used in other studies (3, 8).

### RESULTS

Of the 526 barley accessions screened at the seedling stage, only 10 (1.9% of the total) were resistant (i.e., exhibited ITs 0 to 1) to crown rust (Table 1). All other barley accessions were moderately susceptible to susceptible, exhibiting ITs 3 to 4. Complete IT data for the screened germ plasm are available from the authors upon request. Three of the resistant accessions were from Ethiopia, and the remaining ones originated from Afghanistan, Belgium, India, Italy, Japan, Turkey, and the United States. The 10 accessions exhibiting resistance at the seedling stage were also evaluated at the adult plant stage in the greenhouse, and all exhibited resistant or moderately resistant responses (Table 1). Attempts to obtain crown rust infection data on adult plants in the field were

<table>
<thead>
<tr>
<th>Accession</th>
<th>Origin</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Adult response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clio 6258</td>
<td>India</td>
<td>0; 1</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 6460</td>
<td>Belgium</td>
<td>0; 0; 0</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 6577</td>
<td>Afghanistan</td>
<td>0; 0; 0</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 7513</td>
<td>Italy</td>
<td>0; 1</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 7351</td>
<td>Japan</td>
<td>0; 0; 1</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 8230</td>
<td>Turkey</td>
<td>0; 0; 0</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 13742</td>
<td>Ethiopia</td>
<td>0; 0; 0</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>Clio 14304</td>
<td>USA</td>
<td>0; 0; 0</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td>PI 356467</td>
<td>Ethiopia</td>
<td>0; 0; 0</td>
<td>R-MR</td>
<td></td>
</tr>
<tr>
<td>PI 356543</td>
<td>Ethiopia</td>
<td>0; 0; 0</td>
<td>R-MR</td>
<td></td>
</tr>
<tr>
<td>Hor2596 (Clio 1243)</td>
<td>Ethiopia</td>
<td>0; 0; 0</td>
<td>R-MR</td>
<td></td>
</tr>
<tr>
<td>Aim (Clio 3737)</td>
<td>Egypt</td>
<td>4; 4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Steptoe (Clio 15227)</td>
<td>WA, USA</td>
<td>3; 3</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Foster (PI 592758)</td>
<td>ND, USA</td>
<td>3; 3</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Bowman (PI 383237)</td>
<td>ND, USA</td>
<td>-; -</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

* A 0-to-4 qualitative scale was used for assessing ITs on barley. This scale was adapted from the one developed for the wheat stem rust pathosystem by Stakman et al. (18), where 0 = no visible infection; 1 = minute uredinia, often surrounded by necrosis; 2 = small uredinia surrounded by extensive chlorosis; 3 = medium-sized uredinia with or without chlorosis; and 4 = large uredinia with or without chlorosis.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Number of F2 plants</th>
<th>Cross</th>
<th>Number of F2 plants</th>
<th>Chi²</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowman × Hor2596</td>
<td>1,2</td>
<td>196</td>
<td>65</td>
<td>&lt;0.01</td>
<td>3:1</td>
</tr>
<tr>
<td>MR × Hor2596</td>
<td>2</td>
<td>207</td>
<td>60</td>
<td>0.91</td>
<td>3:1</td>
</tr>
<tr>
<td>MD × Hor2596</td>
<td>2</td>
<td>181</td>
<td>57</td>
<td>0.24</td>
<td>3:1</td>
</tr>
</tbody>
</table>

* F2 plants exhibiting infection types of 0, 0; 1, 1, and 2 were classified as resistant (R), whereas those plants exhibiting infection types (ITs) of 3 and 4 were classified as susceptible (S).
* MR denotes Wolfe’s multiple recessive marker stock (21).
* Not tested.
* MD denotes Wolfe’s multiple dominant marker stock (21).
unsatisfactory. Natural infection did occur on the susceptible checks at the tillering stage in each of the three field seasons; however, excessive precipitation during the growing season led to the development of severe Septoria speckled leaf blotch infections (caused by *Septoria passerinii* and *Stagonospora avenae* f. sp. *triticea*) and confounded the crown rust infection responses.

Three F₂ populations were evaluated for segregation to crown rust at the seedling stage. A close fit to a 3:1 ratio of resistant/susceptible plants was observed in all three populations (Table 2) and is consistent with the segregation of a single resistance gene. In the Bowman × Hor2596 population, intermediate ITs of 1 to 2, similar to those observed on F₁ plants (Table 2), were common among F₂ plants, suggesting that the resistance gene in Hor2596 is incompletely dominant. One hundred thirty-two randomly selected F₂:3 families were evaluated for crown rust reaction at the seedling stage. Segregation for homozygous resistant, segregating, and homozygous susceptible F₂:3 families closely fit a 1:2:1 ratio, thereby confirming the results of the F₂ generation that a single

gene controls crown rust resistance in Hor2596 (Table 3). This is the first crown rust resistance gene described for barley. The locus symbol * Rpc1*, denoting reaction to *P. coronata* 1, and allele symbol * Rpc1.a* are recommended for designating the crown rust resistance gene in Hor2596. Evaluation of Bowman/Hor2596 F₂:3 families for leaf rust reaction indicated that * Rpc1* segregated independently from *Rph9* (Table 3).

Twenty morphological traits were scored in the F₂ populations of crosses MR × Hor2596 and MD × Hor2596. No close linkages were detected between the morphological markers and the crown rust resistance gene in Hor2596. Two morphological markers, * alm* (albino lemma) and *Pvc* (purple veined lemma), showed distant linkages with the crown rust resistance gene (Table 4), with recombination percentages of 36.2 ± 3.8% and 40.9 ± 5.4%, respectively. The linkage with the * alm* locus on chromosome 3HS was considered more positive because the corresponding marker segregated as expected. *Pvc* and seven other morphological markers showed a significant deviation from an expected single gene ratio (Table 4, *χ²* column). These deviations probably resulted from the incorrect scoring of the morphological phenotypes.

**DISCUSSION**

Crown rust of barley appears to be well established in the Upper Midwest region of the United States and represents a new disease threat to the crop. Early season infections initiated by aeciospores from local buckthorn plants could result in severe epidemics of barley crown rust in the future. In addition to providing an early and local inoculum source, buckthorn is also important as the host where *P. coronata* var. * hordei* can undergo sexual hybridization. Sexual recombination may lead to the production of new pathogen races with increased virulence and adaptation toward telial hosts. *P. coronata* var. * hordei* has a very wide host range that includes many wild gramineous species, rye, wild relatives of wheat, and even some cultivated wheat accessions (9,11,14). It is therefore, a potentially dangerous pathogen of barley, wheat, rye, and several important forage grasses. To detect possible shifts in the pathogen population, pathogenicity and virulence assessments of *P. coronata* var. * hordei* should be conducted.
every year on a differential host set consisting of selected small grain cereal species and other major gramineous host species.

The deployment of resistant cultivars is the only economically viable option for the control of crown rust in barley. In this study, we identified 10 sources of crown rust resistance after screening 526 barley accessions from around the world at the seedling stage (Table 1). Although the frequency of resistance was low (1.9%), the resistant accessions came from diverse geographic origins that included North Africa (Ethiopia), northern Europe (Belgium), southern Europe (Italy), East Asia (Japan), South Asia (India), central Asia (Afghanistan), West Asia (Turkey), and North America (United States). Three of the ten resistant accessions and the resistant check Hor2596 originated from Ethiopia. This suggests that Ethiopia may be a rich source of crown rust resistance in barley. Allelism tests are planned to elucidate the relationships among the crown rust resistance genes in these sources.

In this study, we failed to obtain reliable data on the infection responses of selected resistant accessions in the field due to severe Septoria speckled leaf blotch infections. We expect that the resistant infection responses observed on the selected accessions at the adult plant stage in the greenhouse (Table 1) would also hold in the field, but this must be validated in another field experiment where other confounding diseases can be controlled. Interestingly, most of the Septoria speckled leaf blotch infections observed on plants in the field nursery started within crown rust uredinia. This suggests that crown rust uredinia may provide an important infection avenue for the Septoria speckled leaf blotch pathogens.

Inheritance studies with three different crosses clearly showed that crown rust resistance in Hor2596 is controlled by a single (incompletely dominant) gene (Tables 2 and 3). Incorporating crown rust resistance in barley improvement programs should be easy because of the simple inheritance of the trait and effective screening at the seedling stage. Utilization of Hor2596 will also enhance leaf rust resistance in barley breeding programs because Rph9 is effective against many pathotypes of P. hordei in the Upper Midwest region (4). When Hor2596 was found to be resistant to crown rust, it was thought that Rph9 might be involved because crown rust is also considered a leaf rust. However, genetic analysis of F_{2:3} families showed that crown rust resistance conferred by Rpc1 segregated independently from Rph9 (Table 3), which was previously mapped to chromosome 5H (1).

An attempt to associate the crown rust resistance gene Rpc1 with one of the seven barley chromosomes by analyzing linkage data with previously mapped morphological markers was unsuccessful; no close linkages were detected betweenRpc1 and the 20 morphological markers in genetic stocks MR and MD (Table 4). Although this technique of assigning genes to chromosomes has been successfully employed in other studies (3,8), the sparse genome coverage of the 20 morphological markers makes the identification of close linkages a relatively rare event. Molecular markers offer a more powerful and efficient means to precisely position genes on the barley genome (2,7). Of the different marker types available, microsatellites or simple sequence repeats are probably the most convenient to use because they are numerous, polymerase chain reaction-based, highly polymorphic, and robust, i.e., they often map to the same location across different barley crosses. An investigation is underway to map Rpc1 in barley using microsatellite markers.

ACKNOWLEDGMENTS

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LITERATURE CITED

20. Stubbs, R. W., Prescott, J. M., Saari, E. E., and Dubin, H. J. 1986. Characterization of F2:3 families showed that crown rust resistance conferred by Rpc1 segregated independently from Rph9 (Table 3), which was previously mapped to chromosome 5H (1).

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