New Leaf Rust Resistance Genes in Barley and Their Allelic and Linkage Relationships with Other *Rph* Genes

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ABSTRACT

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The inheritance of leaf rust resistance was investigated in four barley accessions (PI 531840, PI 531841, PI 531849, and PI 584760) that were resistant to isolates of *Puccinia hordei* with wide virulence combinations. Crosses were made between the resistant barley lines and barley lines with genes *Rph*1 to *Rph*12 (except for *Rph*8) to determine the allelic and linkage relationships with known *Rph* genes. F_2 populations were evaluated for leaf rust reaction at the seedling stage. An incompletely dominant gene was identified in accessions PI 531841 and PI 584760, and a completely dominant gene was identified in PI 531849. The resistance gene in PI 531841 is an allele at the *Rph*2 locus or a closely linked locus. This gene also is present in accession PI 531840. A linkage was detected between this gene and *Rph*5 with recombination fractions of 33.8 ± 3.8 and 17.0 ± 3.5%, respectively, in crosses of 'Magnif' with PI 531841 and PI 531840. The *Rph* genes in PI 531849 and PI 584760 were not allelic at any of the previously reported *Rph* loci. Locus symbols *Rph*13 and *Rph*14 are recommended for the leaf rust resistance loci in PI 531849 and PI 584760, respectively. A linkage was detected between *Rph*13 and *Rph*9 with a recombination fraction of 30.4 ± 4.5%.

Leaf rust of barley (*Hordeum vulgare* L.), caused by *Puccinia hordei* G. Otth, has been controlled primarily by the use of resistant cultivars. Changes in virulence in the *P. hordei* population, however, have rendered some leaf rust resistance genes (designated as *Rph* genes) ineffective in barley cultivars. Such has been the case for *Rph*3 in Europe (1), *Rph*7 in the southeastern United States (5,14), and *Rph*12 in Europe and Australia (1,2). The ineffectiveness of many *Rph* genes and the occurrence of *P. hordei* pathotypes with wide virulence warrant continued efforts in the search for new sources of resistance. Recent evaluations of cultivated barley for resistance to *P. hordei* indicated that sources of leaf rust resistance that possess genes with a broad resistance spectrum are very limited (8,9). Nevertheless, a few barley accessions possess resistance to *P. hordei* isolates with virulence combinations capable of overcoming all known leaf rust resistance genes (*Rph*1 to *Rph*12), suggesting that they likely possess resistance genes different from previously reported *Rph* genes. These accessions include PI 531840, PI 531841, PI 531849, and a single resistant plant selection from PI 531901, reaccessioned as PI 584760 in the USDA-ARS Small Grains Collection (Aberdeen, ID). The inheritance of resistance in PI 531849 was investigated previously by Jin and Steffenson (8). A dominant resistance gene was identified in this accession, which was not allelic to *Rph3* (8). In this report, we present the results of genetic studies of these new sources of resistance to *P. hordei*.

MATERIALS AND METHODS

Selection of resistant parents and crosses. Barley parental lines used in the crosses are given in Table 1. Accessions PI 531840, PI 531841, and PI 531849 were homogeneous for leaf rust reaction. Accession PI 584760 was a single plant selection from PI 531901, which was heterogeneous for leaf rust reaction and several morphological traits. These lines were crossed to the susceptible cultivar Bowman (PI 483237) to determine the number of genes conferring resistance. To test the allelic and linkage relationships with other *Rph* genes, the lines were intercrossed and crossed to lines with leaf rust resistance genes *Rph*1 to *Rph*12 (Table 1). F_1 plants were grown in a greenhouse to produce F_2 progeny. F_2 plants derived from a single F_1 plant were evaluated as a single F_2 population.

Evaluation of parents and progeny for leaf rust reaction. *P. hordei* isolates used in differentiating resistance genes and in evaluating F_2 progeny are given in Table 1. Isolate ND89-3 is virulent for all known *Rph* genes, except *Rph*3. It is one of the most widely virulent *P. hordei* pathotypes ever reported. Isolate BRS76-12 is virulent for *Rph*3. These two isolates allowed for the differentiation of resistances in the four barley accessions from *Rph*1 to *Rph*12 (Table 1). The selection of leaf rust isolates for evaluating progeny was based on parental infection types (ITs). Isolates avirulent to both parents were used to evaluate the segregating populations. Isolate ND8702 was used in most of the crosses because it is avirulent on all the new sources of resistance and on most of the *Rph* gene donors (Table 1). Isolate Aust220 was used in the progeny evaluation of crosses of lines with *Rph*1, *Rph*4, *Rph*10, and *Rph*11 because it is one of the few isolates that is avirulent for these genes (Table 1). Leaf rust isolates avirulent for *Rph*8 (from Egypt 4) were not available for this study; therefore, we were unable to evaluate the allelic and linkage relationships between *Rph*8 and the genes in the new sources of resistance.

Parental, F_1, and F_2 plants were grown in plastic pots (10 × 10 × 10 cm) filled with a potting mixture (3:1 peat moss/perlite) at 22 ± 3°C in a greenhouse. Twenty-five F_2 seedlings were grown in each pot. One-week-old seedlings (primary leaves fully expanded) were inoculated with urediniospores of *P. hordei* suspended in light-

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saturation by misting with ultrasonic humidifiers. Detailed inoculation and incubation procedures were reported previously (15). The ITs of parental, F_1 and F_2 plants to *P. hordei* infection were evaluated after an incubation period of 12 to 14 days in a greenhouse at 22 ± 3°C. ITs of 0, 0;, 1, 2, or combinations thereof were considered resistant (low IT), and ITs of 3, 4, or combinations thereof were considered susceptible (high IT), based on the rating scale of Levine and Cherewick (10). The number of F_2 plants evaluated varied from population to population depending on seed supply.

Data analyses. Many of the *Rph* genes are incompletely dominant. For the convenience of data analysis in this study, F_2 plants were categorized into only resistant and susceptible classes. The chi-square method was used to test the hypothesis of independent segregation in F_2 populations for the respective genes in each of the crosses. The exact probabilities were obtained by

probability = 1.0 – PROBCHI(chisq,df)

where PROBCHI is the chi-square probability function in SAS (Statistical Analysis System version 6.07, SAS Institute, Cary, NC) with chisq (calculated chi-square value) and df (degrees of freedom) as arguments of the function. Variance homogeneity among replicated F_2 populations of the same cross was tested, and data were pooled when the test statistic was not significant (data

TABLE 1. Seedling infection type (IT) of parental barley lines to *Puccinia hordei* isolates used in differentiating resistance genes and in evaluating F_2 populations

Accession or line	Recongnized *Rph* gene[a]	IT[b] to *Puccinia hordei* ND89-3	BRS76-12[c]	ND8702	Aust220[c]
Susceptible parental line					
Bowman (PI 483237)	None	3	3	3	4
New sources of resistance					
PI 531840	Unknown	0;,1	0;,1	0;	0;,1
PI 531841	Unknown	0;,1	1,2	0;	0;
PI 531849	Unknown	0;,1	0;	0;	0;
PI 584760	Unknown	0;,1	0;,1	0;	0;,1
Donors of the known *Rph* genes					
Sudan (CI 6489)	*Rph*1	4	4	4	0;,1
Peruvian (CI 935)	*Rph*2	3	3	2,1	2,1
Estate (CI 3410)	*Rph*3	1,0;	3	0;	0;,1
Gold (CI 1145)	*Rph*4	3	3	4	1,0;
Magnif (CI 13860)	*Rph*5	4	3	0;	4
Bolivia (CI 1257)	*Rph*6+*Rph*2	4	3	0;,1	0;,1
Cebada Capa (CI 6193)	*Rph*7	4	0;	0;	0;
Egypt 4 (CI 6481)	*Rph*8	4	3	3	3
Hor 2596 (CI 1243)	*Rph*9	3	0;,1	0;	0;,1
Clipper BC8	*Rph*10	3	3	3	1,0;
Clipper BC67	*Rph*11	3	3	3	2,1
Triumph (PI 290195)	*Rph*12	4	0;	0;	0;,1

[a] Gene designations of *Rph*10 and *Rph*11 were based on Feuerstein et al. (3), and *Rph*12 was based on Jin et al. (7).

[b] IT ratings were based on the scale of Levine and Cherewick (10). ITs of 0, 0;, 1, 2, or combinations were considered resistant (low IT), and ITs of 3, 4, or combinations were considered susceptible (high IT). When more than one IT was observed, the predominant type was listed first.

[c] Isolate BRS76-12 was provided by B. C. Clifford (IGER Welsh Plant Breeding Station, Aberystwyth, Wales), and isolate Aust220 was provided by R. G. Rees (Queensland Wheat Research Institute, Toowoomba, Australia).

two resistance genes was indicated.

RESULTS

Resistance gene in PI 531841. The ITs of parents and F_1 lines and segregation in F_2 populations to *P. hordei* are given in Table 2. F_2 plants segregated into a 3:1 (resistant/susceptible) ratio in the cross between PI 531841 and susceptible parent Bowman, indicating the presence of a single resistance gene. F_2 populations in most of the other crosses between PI 531841 and the *Rph* gene donors segregated in a 15:1 (resistant/susceptible) ratio, except for crosses with lines Peruvian (*Rph*2), Magnif (*Rph*5), Bolivia (*Rph*6+*Rph*2), and Clipper BC67 (*Rph*11). In the cross with Magnif, the number of susceptible F_2 plants was significantly lower than expected, indicating a linkage between the gene in PI 531841 and *Rph*5. The estimated recombination fraction was 33.8 ± 3.8%. In contrast, the number of susceptible plants in the cross between PI 531841 and Clipper BC67 was significantly higher than expected.

Susceptible F_2 plants were not observed in crosses of PI 531841 with Peruvian (*Rph*2) or Bolivia (*Rph*6+*Rph*2). The lack of segregation for susceptibility in these large F_2 populations (1,270 and 1,530 F_2 plants, respectively) strongly suggests an allelic relationship between the gene in PI 531841 and *Rph*2. The differential ITs of PI 531841 and Peruvian to several *P. hordei* isolates (Table 1) indicate that the gene in PI 531841 is different from that in Peruvian and perhaps Bolivia as well, although the camparison with the latter may be confounded by the presence of a second gene (*Rph*6). Thus, PI 531841 likely possesses a previously unidentified allele at the *Rph*2 locus. The possibility of a tight linkage relationship, however, cannot be excluded.

Resistance gene in PI 531840. Lines PI 531840 and PI 531841 exhibited similar reactions to the four isolates of *P. hordei* evaluated (Table 1). Segregation for susceptibility did not occur in an F_2 population of a cross between the two lines. Segregation also did not occur in the crosses of PI 531840 with Peruvian (*Rph*2) and Bolivia (*Rph*6+*Rph*2) (Table 2). Thus, PI 531840 probably possesses an allele at the *Rph*2 locus that may be the same as the *Rph*2 allele in PI 531841. A linkage also was observed in the cross of PI 531840 with Magnif (*Rph*5). The recombination fraction of 17.0 ± 3.5% found for this cross was much smaller than that observed in the cross between PI 531841 and Magnif.

Resistance gene in PI 531849. A dominant gene for leaf rust resistance, which segregated independently from *Rph*3, was previously identified in this accession (8). Segregation for resistance and susceptibility occurred in all crosses of PI 531849 with the *Rph* gene donors (Table 2); thus, the gene in PI 531849 is not allelic with any of the previously reported leaf resistance genes, although the allelic relationship with *Rph*8 is still unknown. A good fit to the expected 15:1 (resistant/susceptible) ratio was obtained in most of the F_2 populations of crosses, with the exception of those involving Bolivia and Hor 2596. A three-gene segregation pattern was observed in the cross with Bolivia. This segregation pattern was expected because Bolivia has two resistance genes (Table 1). The number of susceptible plants was significantly smaller than that expected for independent segregation in the cross between PI 531849 and Hor 2596 (donor of *Rph*9). This deviation suggested a linkage relationship between these two loci with an estimated recombination fraction of 30.4 ± 4.5%. A new locus designation, *Rph*13, is proposed for this dominant gene in PI 531849.

Resistance gene in PI 584760. Segregation of F_2 plants in the cross between PI 584760 and susceptible parent Bowman fit a ratio of 3:1 (resistant/susceptible), indicating that a single gene confers resistance in this accession. The F_1 ITs (ITs 1,2 to 2,1) and occurrence of resistant F_2 plants with higher ITs than the resistant parent indicated that the resistance gene in PI 584760 is incompletely dominant. F_2 plants in the crosses of PI 584760 with do-

nors of *Rph*1 to *Rph*13 segregated into the expected ratios in most cases. Significant deviations from the expected occurred in crosses of PI 584760 with Gold (donor of *Rph*4), Magnif (donor of *Rph*5), and Clipper BC8 (donor of *Rph*10). In these crosses, an excessive number of susceptible plants was observed. These deviations might be due to an interaction between the resistance genes or the possible involvement of suppressors under certain genotypic conditions. It also is possible that some of the progeny were misclassified; however, the ITs of most plants were clearly and easily distinguishable. The results indicate that the resistance gene in PI 584760 is not allelic with any of the previously reported *Rph* genes (the allelic relationship with *Rph*8 is unknown). The locus designation of *Rph*14 is proposed for the incompletely dominant *Rph* gene in PI 584760.

DISCUSSION

Currently, 12 leaf rust resistance gene loci, *Rph*1 to *Rph*12, have been described in barley, as presented in Table 1 (1,3,7,12). In a previous study, several new sources of leaf rust resistance were identified (9). The current study provided genetic evidence that the resistance genes in PI 531849 and PI 584760 are different from the previously reported *Rph* loci. The use of relatively complete allelism tests allowed us to identify two new resistance loci, *Rph*13 and *Rph*14. In tests with more than 90 *P. hordei* isolates from around the world, PI 531849 and PI 584760 were resistant to 52 and 96% of the isolates, respectively (B. J. Steffenson and T. G. Fetch, Jr., *unpublished data*). Thus, only *Rph*14 may be of value in barley improvement programs in breeding for leaf rust resistance.

The existence of a multiallelic series or a complex *Rph* locus in the barley genome may complicate the identification of leaf rust resistance genes. Thus, the assignment of new *Rph* loci based on differential reactions to various *P. hordei* isolates is unreliable. Considering the relatively small number of known *Rph* genes in barley, a complete set of allelism tests is highly recommended before any new *Rph* loci are designated. The number of allelism tests required to validate a new locus designation may be reduced once additional information on the chromosomal location of *Rph* loci is obtained.

TABLE 2. Infection type (IT) of F_1 lines and segregation in F_2 populations of resistant barley lines to *Puccinia hordei* evaluated at the seedling stage

Cross	Isolate	F_1 IT[a]	No. of F_2 plants		Ratio fit	χ^2	Probability ($>\chi^2$)
			Low IT	High IT			
Cross of PI 531841 with							
Bowman	ND8702	2,1	206	60	3:1	0.85	0.357
Sudan (*Rph*1)	Aust220	0;,1	336	19	15:1	0.49	0.485
Peruvian (*Rph*2)	ND8702	2,1	1,270	0	No seg.		
Estate (*Rph*3)	ND8702	0;,1	256	18	15:1	0.05	0.827
Gold (*Rph*4)	Aust220	0;,1	321	17	15:1	0.86	0.354
Magnif (*Rph*5)	ND8702	0;,1	645	19	15:1	13.01	<0.001
Bolivia (*Rph*6+*Rph*2)	ND8702	0;,1	1,530	0	No seg.		
Cebada Capa (*Rph*7)	ND8702	0;	274	18	15:1	<0.01	0.952
Hor 2596 (*Rph*9)	ND8702	0;,1	262	16	15:1	0.12	0.733
Clipper BC8 (*Rph*10)	Aust220	1,2	323	21	15:1	0.01	0.911
Clipper BC67 (*Rph*11)	Aust220	2,1	296	34	15:1	9.25	0.002
Triumph (*Rph*12)	ND8702	0;,1	266	21	15:1	0.56	0.455
Cross of PI 531840 with							
PI 531841 (*Rph*2)	ND8702	0;,1	315	0	No seg.		
Peruvian (*Rph*2)	ND8702	0;,1	266	0	No seg.		
Bolivia (*Rph*6+*Rph*2)	ND8702	0;	1,400	0	No seg.		
Magnif (*Rph*5)	ND8702	0;	828	6	15:1	43.54	<0.001
Cross of PI 531849 with							
Sudan (*Rph*1)	Aust220	0;	387	24	15:1	0.12	0.731
Peruvian (*Rph*2)	ND8702	0;,1	299	18	15:1	0.18	0.674
Gold (*Rph*4)	Aust220	0;	321	15	15:1	1.83	0.176
Magnif (*Rph*5)	ND8702	0;	721	37	15:1	2.42	0.120
Bolivia (*Rph*6+*Rph*2)	ND8702	0;,1	316	5	63:1	<0.01	0.994
Cebada Capa (*Rph*7)	ND8702	0;	338	16	15:1	1.81	0.179
Hor 2596 (*Rph*9)	ND8702	0;,1	977	17	15:1	34.96	<0.001
Clipper BC8 (*Rph*10)	Aust220	0;,1	358	18	15:1	1.92	0.166
Clipper BC67 (*Rph*11)	Aust220	0;	314	22	15:1	0.05	0.822
Triumph (*Rph*12)	ND8702	0;,1	417	24	15:1	0.49	0.483
PI 531841 (*Rph*2)	ND8702	0;,1	268	11	15:1	2.54	0.111
Cross of PI 584760 with							
Bowman	ND8702	...[b]	200	72	3:1	0.31	0.575
Sudan (*Rph*1)	Aust220	0;,1	368	20	15:1	0.80	0.373
Peruvian (*Rph*2)	ND8702	2,1	260	13	15:1	1.03	0.310
Estate (*Rph*3)	ND8702	0;,1	269	25	15:1	2.58	0.110
Gold (*Rph*4)	Aust220	0;,1	780	71	15:1	6.36	0.012
Magnif (*Rph*5)	ND8702	0;,1	596	106	15:1	93.83	<0.001
Bolivia (*Rph*6+*Rph*2)	ND8702	2,1	276	5	63:1	0.09	0.769
Cebada Capa (*Rph*7)	ND8702	0;	298	24	15:1	0.80	0.372
Hor 2596 (*Rph*9)	ND8702	1,2	277	27	15:1	3.59	0.058
Clipper BC8 (*Rph*10)	Aust220	2,1	283	52	15:1	49.01	<0.001
Clipper BC67 (*Rph*11)	Aust220	1,2	328	20	15:1	<0.01	0.956
Triumph (*Rph*12)	ND8702	2	263	16	15:1	0.13	0.722
PI 531841 (*Rph*2)	ND8702	0;1	269	12	15:1	1.88	0.170
PI 531849 (*Rph*13)	ND8702	0;,1	270	18	15:1	0.00	1.000

[a] The classification of ITs are given in Table 1.
[b] Data were not available.

Multiallelic series or complex loci for leaf rust resistance in barley have not been reported. Many different sources of *Rph2* have been identified in barley (12). These sources of *Rph2* vary greatly in reaction to different *P. hordei* isolates (Y. Jin and B. J. Steffenson, *unpublished data*), indicating that this may be a complex locus. PI 531841 and Peruvian also exhibit marked differences for IT to some isolates of *P. hordei* (Table 1). The absence of segregation found in the large F_2 populations led us to postulate the existence of different alleles at the *Rph2* locus; however, we cannot exclude the possibility of tight linkage. In several intensively investigated systems of multiallelic series and complex disease resistance loci, considerable efforts have been made to resolve the issue of an allelic relationship or the occurrence of closely linked loci (4,6,11, 13).

In this study, we detected a linkage between *Rph2* and *Rph5*. This linkage was further corroborated by the F_2 segregation of a cross between Bowman and Quinn (donor of *Rph5*+*Rph2*). A recombination fraction of 30.6 ± 3.7% was detected between these two loci based on a population size of 715 progeny. This recombination fraction is comparable to that found in the cross between PI 531841 and Magnif (33.8 ± 3.8%). A much smaller recombination fraction (17.0 ± 3.5%) was found between the *Rph2* and *Rph5* loci detected in the cross between PI 531840 and Magnif. The weighted average of recombination fractions from the three crosses was 26.4%. Other preliminary data indicate that *Rph5* might be linked to several other *Rph* loci (Y. Jin and B. J. Steffenson, *unpublished data*). We are currently investigating the chromosomal locations of these linked *Rph* genes in the barley genome with DNA markers.

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