Genes Governing Resistance to *Puccinia hordei* in Thirteen Spring Barley Accessions

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Accepted for publication 28 June 2000.

ABSTRACT

Brooks, W. S., Griffey, C. A., Steffenson, B. J., and Vivar, H. E. 2000. Genes governing resistance to *Puccinia hordei* in thirteen spring barley accessions. Phytopathology 90:1131-1136.

Leaf rust, caused by *Puccinia hordei,* is an important disease of barley in many parts of the world. In the eastern United States, this disease was effectively controlled for over 20 years through the deployment of cultivars carrying the resistance gene *Rph*7. Isolates of *P. hordei* with virulence for *Rph*7 appeared in this region in the early 1990s rendering barley cultivars with this gene vulnerable to leaf rust infection. From a preliminary evaluation test, 13 accessions from diverse geographic locations possessed resistance to *P. hordei* isolate VA90-34, which has virulence for genes *Rph*1, 2, 4, 6, 7, 8, and 11. Each of these 13 accessions was crossed with susceptible cvs. Moore or Larker to characterize gene number and gene action for resistance to *P. hordei*. Additionally, the 13 accessions were intercrossed and crossed to host differential lines possessing genes *Rph*3, *Rph*5, and *Rph*9 to determine allelic relationships of resistance genes. Seedlings of F_1, F_2, and BC_1F_1 populations were evaluated in the greenhouse for their reaction to *P. hordei* isolate VA90-34. Leaf rust resistance in six of the accessions including Collo sib, CR270.3.2, Deir Alla 105, Giza 119, Gloria, and Lenka is governed by a single dominant gene located at or near the *Rph*3 locus. All accessions for which the gene *Rph*3 was postulated to govern leaf rust resistance, except for Deir Alla 105, likely possess an allele different than *Rph*3.c found in Estate based on the differential reaction to isolates of *P. hordei*. The resistance gene in Grit and Donan is located at or near the *Rph*9 locus. Alleles at both the *Rph*3 and *Rph*9 loci confer resistance in Femina and Dorina. In addition to *Rph*3, Caroline and CR366.13.2 likely possess a second unknown recessive gene for leaf rust resistance. Resistance in Carre 180 is governed by a recessive gene that is different from all other genes considered in this study. Identification of both known and unique genes conferring leaf rust resistance in the barley germplasm included in this study provides breeding programs with the knowledge and opportunity to assess currently used sources of leaf rust resistance and to incorporate new sources of resistance into their programs.

Leaf rust, caused by the fungus *Puccinia hordei* G. Otth, is one of the most destructive diseases of barley (*Hordeum vulgare* L.) in many areas of the world (22). The disease can markedly reduce both grain yield and seed quality of the crop. Local epidemics of leaf rust have been reported in Australia and Europe resulting in significant yield losses (6). In Australia, yield losses as large as 31% were reported by Cotterill et al. (7) in commercial barley fields with moderate leaf rust infection in 1990. King and Polley (16) reported yield differences ranging from 17 to 31% in Europe from trials of fungicide-treated versus nontreated plots of barley infected with leaf rust. In Virginia, average yield loss due to leaf rust was estimated to be between 6 and 16% under severe epidemics in 1990 and 1991 for barley genotypes varying in reaction to race 30 (12).

Development and use of resistant cultivars is the most economical and environmentally safe method to control *P. hordei*. Sixteen major resistance genes *(Rph*1 to *Rph*16) have been identified in barley (5,10,13,14,20,21). Of these 16 genes, only *Rph*3, 7, and 9 have been deployed in commercial cultivars worldwide. Virulence for *Rph*3 and *Rph*9 has been identified in Europe, South America, and the Middle East. Virulence for *Rph*9.z (formerly designated *Rph*12) has been identified in Europe and Australia. Prior to 1990, virulence to *Rph*7 was known only in Israel (11) and Morocco (19). In the early 1990's, virulence for *Rph*7 was identified in California, Pennsylvania, and Virginia (12,26) and more recently in South America (2). The prevalence of pathotypes with virulence for the major genes for resistance to *P. hordei* is of great concern to plant breeders and pathologists, because some of these genes, including *Rph*3, 7, and 9, were considered the most effective and have been used widely in barley breeding programs. A few barley accessions possessing genes conferring resistance to *P. hordei* isolates capable of overcoming all previously reported *Rph* genes have been identified (13–15,27). However, resistance conferred by major genes has frequently failed to provide long-term disease control and deployment of single major genes in cultivars grown over a broad area potentially can lead to serious epidemics.

Information on genetic variability for resistance to leaf rust among accessions of *H. vulgare* can facilitate efficient exploitation of such resistance and lead to the development of barley cultivars with broad-based resistance. In order for breeders to efficiently use the available sources of leaf rust resistance, it is necessary to determine the inheritance and genetic relationship of resistance genes. The objectives of this study were to determine (i) the inheritance of resistance in 13 spring barley accessions to isolates of *P. hordei* possessing virulence to *Rph*7, and (ii) the allelic relationships between resistance genes in these accessions.

MATERIALS AND METHODS

Resistance sources, plant populations, and pathogen isolates. Thirty-one spring barley accessions, originating from diverse locations and obtained from the International Center for Agricultural Research in Dry Areas (ICARDA; Aleppo, Syria) and the International Maize and Wheat Improvement Center (CIMMYT;

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Publication no. P-2000-0724-02R

Mexico, D.F.) Barley Program, and host differential lines possessing leaf rust resistance genes *Rph*1 to R*ph*11 were initially evaluated for resistance to four isolates of *P. hordei*. Three of the isolates have virulence/avirulence patterns that have not been observed in the *P. hordei* population of North America. Isolate ND89-3 originated in Morocco (15) and isolates BRS76-12 and BRS90-40 originated in the United Kingdom (15). Isolate VA90-34 was collected in a breeding nursery in Blacksburg, Virginia in November 1990. Differential reaction of barley genotypes to these four isolates allowed for an initial characterization of the 31 accessions for putative resistance genes. Thirteen accessions were selected for the current study on the basis of diverse origin, resistance to isolate VA90-34, and reaction to other differential races of leaf rust (Table 1).

P. hordei isolate VA90-34 (virulence for *Rph*1, 2, 4, 6, 7, 8, and 11) was used in the current genetic studies because it is avirulent for the resistance genes in the 13 sources, and the race group (race 30) of isolate VA90-34 is prevalent in Virginia and the mid-Atlantic region of North America (12,26). Inheritance of resistance in each accession, including gene number and action, was obtained from analysis of segregation patterns of progeny derived from crosses of the 13 resistant accessions with susceptible parent cvs. Moore and Larker (Table 2). Cv. Moore was used as the universal susceptible parent in most crosses, and cv. Larker was used as an alternative susceptible parent when spikes of cv. Moore were not available for crossing. F_1 seed from these crosses were used to produce F_2 populations and to develop BC_1F_1 (cv. Moore or Larker × F_1) populations. Genetic populations were developed to investigate allelic relationships among the resistance genes. Resistant accessions were intercrossed and crossed to four host differential lines in all possible combinations, excluding reciprocal crosses (Tables 3 and 4). Resistant parents were crossed only to differential lines possessing resistance to isolate VA90-34. These differential lines were Estate (PI 57700) with *Rph*3.c, Magnif (CIho 13806) with *Rph*5.e, Hor 2596 (CIho 1243) with *Rph*9.i, and Triumph (PI 268180) with *Rph*9.z (formally designated *Rph*12). F_1 seeds from these crosses were used to produce F_2 populations.

TABLE 1. Infection type (IT) of *Hordeum vulgare* genotypes to four isolates of *Puccinia hordei* and origin of 13 resistant accessions[a]

Accession or line	*Rph* genes[b]	ND89-3	BRS76-12	BRS90-40	VA90-34
Susceptible parents					
Cv. Moore	None	33+	33+	43	4
Cv. Larker	None	33+	3	3	34
Differential lines					
Sudan	*Rph*1	3	33+	3	4
Peruvian	*Rph*2	3	3–	3–	3
Estate	*Rph*3.c	0;1c	3	0;c	0;
Gold	*Rph*4	3	33–	3	34
Magnif	*Rph*5.e	3	3	21c	;1n
Bolivia	*Rph*2+6	3	33–	3	23–c
Cebada Capa	*Rph*7	3	;n	0;1n	33–
Egypt4	*Rph*8	3	3	3c	3
Hor 2596	*Rph*9.i	3–c	;1+n	3	;1+
Triumph	*Rph*9.z	33+	0;cn	3	;1+
Clipper8	*Rph*10	3	3	3–c	;2+c
Clipper67	*Rph*11	3–c	3–c	3–	3
Resistant accessions	…				
Gloria (Mexico)	…	33+	33–	;1–n	0;
Collo "S" (Mexico)	…	3c	34	0;	0;
Giza 119 (Egypt)	…	3–c	33–	0;12cn	;1
CR 270.3.2 (Egypt)	…	33+	33–	;12n	;1
CR 366.13.2 (Egypt)	…	3	33+	0;1	1–
Lenka (Sweden)	…	3c	33+	0;n	0;
Caroline (Sweden)	…	3	33–	10;	0;
Femina (Denmark)	…	33–c	;1=n	0;1	0;
Dorina (Denmark)	…	3c	2+c	0;	0;
Grit (Denmark)	…	3–	0;1	3–	;1
Donan (UK)	…	33+	0;1	33–	;1+
Carre 180 (Algeria)	…	33+	0;12+	0;2+cn	;1
Deir Alla 105 (Jordan)	…	0;1c	33+	;1n	0;1c

[a] IT ratings were based on the scale of Levine and Cherewick (17). ITs of 0 to 2 were considered resistant and ITs of 3 to 4 were considered susceptible. + and – denote more and less sporulation, respectively. Infection type is based on independent tests conducted at Virginia Polytechnic Institute and State University and North Dakota State University. Isolate ND89-3 virulence/avirulence: *Rph*1, 2, and 4 to 11/*Rph*3; Isolate BRS677 virulence/avirulence: *Rph*1, 2, 3, 4, 5, 6, 8, 10, and 11/*Rph*7 and 9; Isolate BRS90-40 virulence/avirulence: *Rph*1, 2, 4, 6, 8, 9, 10, and 11/*Rph*3, 5, and 7; Isolate VA90-34 virulence/avirulence: *Rph*1, 2, 4, 6, 7, 8, and 11/*Rph* 3, 5, 9, and 10.

[b] Gene designations of *Rph*1 to *Rph*11 are based on allele symbols proposed by Franckowiak et al. (10) and *Rph*9.z was based on Borovkova and Steffenson (1).

Evaluation of leaf rust reaction. The parents, BC_1F_1 and F_2 progeny, and a set of host differential lines (*Rph*1 to *Rph*11) were screened for their reaction to isolate VA90-34 in the greenhouse. For each cross, five seedlings of each parent, 168 to 355 F_2 seedlings, and 17 to 53 BC_1F_1 seedlings were evaluated for their reaction to leaf rust infection. A BC_1F_1 population was not available for the accessions Deir Alla 105, Femina, or Lenka. Nevertheless, genetic analysis of each accession in different crosses, including those in both inheritance and allelism studies, provided ample data to support a definite conclusion regarding the number and action of genes present.

Seeds were space planted in plastic pots (75-mm diameter and 65-mm depth) filled with a potting mixture (3:1, peat moss/soil). Pots were placed in wooden flats (35 pots per flat and 5 seeds per pot), and flats were arranged by population on a greenhouse bench. Ten to fourteen days after planting (two-leaf stage), seedlings were inoculated with a mixture of *P. hordei* urediniospores (isolate VA90-34) and talc (≈1 g of spores per 5 g of talc) using a pump. The inoculated plants were placed in a moist chamber maintained near saturation by intermittent misting from a humidifier for 16 h at 20 ± 1°C. Following the mist period, the canvas top of the chamber was opened halfway to allow plants to dry slowly. Plants were placed on a greenhouse bench maintained at 22 ± 3°C.

The 0 to 4 rating scale of Levine and Cherewick (17) was used to score infection types of the parental, BC_1F_1, F_2, and host differential plants 10 to 14 days after inoculation. Infection types of each plant were based on assessment of the first and second leaf of each seedling. Plants with infection types from 3 to 4 were rated susceptible, and plants with infection types from 0 to 2 were rated resistant. Infection types of plants from genetic populations were compared with those of their respective parents and the host differentials to assure proper classification and assignment to resistant and susceptible classes. Observed segregation patterns of resistance and susceptible progeny were used to determine genetic relationships. A chi-square test was used to test the goodness-of-fit for observed segregation patterns to expected genetic ratios. In cases where F_2 progeny were derived from different F_1 plants, a chi-square test for homogeneity was used to determine whether different populations displayed similar genetic behavior. The variance among populations tested was homogeneous, and pooled data are presented in the tables.

RESULTS

Preliminary study of resistance to *P. hordei* in 13 *H. vulgare* accessions. Infection types of the 13 barley accessions, two susceptible parents, and 12 host differential lines to four isolates of *P. hordei* are presented in Table 1. Reaction of the 13 accessions to four isolates with differential virulence patterns allowed for preliminary differentiation among the accessions regarding putative resistance genes. None of the 13 accessions characterized as resistant to isolate VA90-34 were resistant to all of the isolates tested. Isolate ND89-3 with virulence for all genes (*Rph*1 to *Rph*11) except *Rph*3 possesses one of the widest virulence spectra among *P. hordei* pathotypes (15). Isolate BRS76-12 with virulence

for all resistance genes (*Rph*1 to *Rph*11) except *Rph*7 and *Rph*9, and isolate BRS90-40 with virulence for all resistance genes except *Rph*3, 5, and 7 collectively possess virulence for all resistance genes (*Rph*1 to *Rph*11) except for *Rph*7. Deir Alla 105 was the only accession with resistance to isolate ND89-3, and likely possesses gene *Rph*3.c, based on reactions similar to Estate against the four isolates used in this study. Grit and Donan had reaction patterns similar to Hor 2596 and Triumph and may possess alleles at the *Rph*9 locus. Seven accessions were resistant to isolate BRS90-40 and susceptible to isolates ND89-3 and BRS76-12 and, therefore, may possess the same gene. Although Femina, Dorina, and Carre 180 were resistant to all isolates except ND89-3, they exhibited different infection types to one or more of the isolates.

Inheritance studies. Segregation of F_2 and BC_1F_1 progeny for resistance to *P. hordei* from crosses between resistant accessions and susceptible cvs. Moore and Larker is shown in Table 2. The number of resistant and susceptible progeny observed in F_2 populations derived from crosses between cvs. Moore and Larker with resistant accessions Grit, Donan, Gloria, Collo sib. (Collo"S"), Deir Alla 105, Lenka, Giza 119, and CR 270.2.3 was consistent with a 3:1 (resistant/susceptible) ratio. This indicated that resistance in these accessions is governed by a single dominant gene. Observed segregation patterns of their respective BC_1F_1 populations were consistent with the expected 1:1 (resistant/susceptible) ratio for one dominant gene. The number of resistant and susceptible progeny observed in F_2 populations from crosses of cv. Moore with Dorina and Femina was consistent with a 15:1 ratio, indicating that these accessions each possess two independent dominant genes for resistance. The number of resistant and susceptible plants in the BC_1F_1 population for Dorina approximated a 3:1 ratio and supported the two-gene hypothesis. A BC_1F_1 population was not available for Femina.

The number of resistant and susceptible progeny observed in F_2 populations from crosses of cv. Larker with resistant accessions Caroline and CR 366.13.2 was consistent with a 13:3 ratio, indicating the presence of one dominant and one recessive gene in each of these accessions (Table 2). The number of resistant versus susceptible progeny in the respective BC_1F_1 populations of these crosses was consistent with a 1:1 ratio, and supported the two-gene model of one dominant and one recessive gene. Resistance in Carre 180 is governed by a single recessive gene as indicated by the segregation of one resistant to three susceptible progeny in the F_2 population. The lack of resistant progeny in the BC_1F_1 and F_1 (data not presented) generations confirmed the recessive nature of this gene. Because gene number could not be confirmed in the BC_1F_1 generation, the F_3 generation of this cross was evaluated for segregation. Among 40 F_3 families, nine were homozygous resistant, 21 segregated in a 1:3 (resistant/susceptible) manner and 10 were homozygous susceptible. This segregation pattern gave a good fit ($P = 0.93$) to a 1:2:1 genotypic ratio, which was expected for monogenic inheritance of resistance in Carre 180. In summary, leaf rust resistance is governed by a single dominant gene in eight accessions: two dominant genes in two accessions, one dominant and one recessive gene in two accessions, and a single recessive gene in one accession.

Allelic relationships among genes in the 13 resistant parents. Allelism tests were conducted to determine whether resistance genes in the 13 parents were at the same or different loci and to determine if the accessions have unique genes for resistance. Segregation patterns for reaction of F_2 populations to *P. hordei* in crosses among the 13 resistant parents are presented in Table 3. Susceptible F_2 progeny were not observed in crosses among Grit, Donan, Dorina, and Femina, indicating that these parents have at least one gene in common or alleles at the same locus. Likewise, susceptible F_2 progeny were not found in crosses among Gloria, Giza 119, Deir Alla 105, CR 366.13.2, CR 270.2.3, Dorina, Femina, Lenka, Caroline, and Collo "S". This indicated that these resistant parents have at least one gene in common or alleles at the same locus. In crosses where segregation for susceptible progeny occurred, the observed segregation patterns confirmed previous results (Table 2) for gene number and action in most cases. Segregation of resistant and susceptible plants was observed among F_2 progeny for crosses between Carre 180 and all other parents, indicating that the gene in Carre 180 is different from genes in the other resistant parents. The number of resistant and susceptible plants observed in the F_2 populations derived from crosses between Carre 180 with 10 of the 12 resistant parents was consistent with the hypothesis that Carre 180 possesses a single recessive gene. However, in two crosses this gene appeared to be inherited as a dominant factor. The modified segregation patterns (15 resistant to 1 susceptible) observed in crosses between Carre 180 with Collo "S" and Lenka may have resulted from epistasis, suppressor gene action, or both in these genetic backgrounds. Although the infection types of most F_2 plants were easily distinguishable and were classified on the basis of parental and host differential reaction type, it is also possible that some of the progeny were misclassified.

In summary, leaf rust resistance was governed by alleles at a common locus in 10 accessions, and four other accessions possess alleles at a second independent locus. Among these accessions, Dorina and Femina possess alleles at both loci. Segregation patterns in crosses of Caroline and CR 366.13.2 with other resistant parents supported the hypothesis that leaf rust resistance in these accessions is governed by one dominant and one recessive gene. The identity of the recessive gene in Caroline and CR 366.13.2 is unknown. Carre 180 has a recessive gene that is independent of all these loci.

Allelic relationships of genes in the 13 resistant accessions with those in four host differential lines. Crosses between resistant parents and host differential genotypes with effective leaf rust resistance genes to *P. hordei* isolate VA90-34 were evaluated to determine whether resistance in the 13 accessions is conferred by known or unique genes. Development of genetic populations for this study was initiated in the early 1990's. At that time, the only differential lines available were those possessing genes *Rph*1 to *Rph*12 (now designated *Rph*9.z). Among these genes, only *Rph*3, 5, 9, and 10 were effective against isolate VA90-34. The

TABLE 2. Segregation of progeny for reaction to *Puccinia hordei* isolate VA90-34 in crosses of susceptible barley cvs. Moore and Larker with 13 resistant accessions

Cross	Generation	Resistant	Susceptible	Ratio[a]	P[b]
Moore × Grit	F_2	216	65	3:1	0.47
	BC_1F_1	28	21	1:1	0.32
Moore × Donan	F_2	217	67	3:1	0.58
	BC_1F_1	21	20	1:1	0.88
Moore × Gloria	F_2	221	71	3:1	0.79
	BC_1F_1	22	17	1:1	0.42
Moore × Collo "S"	F_2	127	36	3:1	0.39
	BC_1F_1	20	15	1:1	0.40
Moore × Deir Alla 105	F_2	212	83	3:1	0.21
Larker × Deir Alla 105	F_2	196	59	3:1	0.49
Moore × Lenka	F_2	163	58	3:1	0.78
Larker × Giza 119	F_2	276	79	3:1	0.23
	BC_1F_1	9	8	1:1	0.81
Larker × CR 270.2.3	F_2	216	75	3:1	0.76
	BC_1F_1	18	12	1:1	0.27
Moore × Dorina	F_2	197	13	15:1	0.40
	BC_1F_1	26	12	3:1	0.35
Moore × Femina	F_2	249	19	15:1	0.57
Larker × Caroline	F_2	150	31	13:3	0.57
	BC_1F_1	19	16	1:1	0.80
Larker × CR 366.13.2	F_2	223	59	13:3	0.35
	BC_1F_1	8	7	1:1	0.27
Larker × Carre 180	F_2	75	225	1:3	1.00
	BC_1F_1	0	53	…	…

[a] Expected segregation ratio.
[b] Probability of chi-square.

host differential line possessing *Rph*10 was not included in the allelism tests because it is susceptible to *P. hordei* race 8, whereas the 13 parents are resistant to race 8. Segregation patterns of F_2 populations from these crosses are presented in Table 4.

Estate (*Rph*3.c) × resistant parents. Susceptible F_2 progeny were not obtained from crosses of Estate with the nine resistant parents Gloria, Giza 119, CR 270.3.2, Collo "S", Lenka, Deir Alla 105, Dorina, CR 366.13.2, and Caroline. Lack of segregation in these crosses suggests that resistance genes in these parents are either allelic or closely linked to the *Rph*3 locus. The number of resistant and susceptible progeny observed in F_2 populations from crosses of Estate with resistant parents Grit and Donan was consistent with a 15:1 ratio for two independent dominant genes. A 13:3 (resistant/susceptible) F_2 ratio was observed for the cross between Estate and Carre 180, as was expected for one dominant and one recessive gene governing resistance. Although a cross between Estate and Femina was not obtained, no segregation was observed in F_2 populations from crosses between Femina and other accessions possessing *Rph*3 (Table 3). Therefore, Femina likely possesses an allele at the *Rph*3 locus.

Magnif (*Rph*5.e) × resistant parents. Segregation for susceptible F_2 progeny occurred in all crosses between Magnif and the 13 resistant parents; therefore, the genes in these accessions are different from *Rph*5 in Magnif (Table 4). The number of resistant and susceptible progeny observed in F_2 populations from crosses between Magnif and 8 of the 13 resistant parents was consistent with a 15:1 ratio, which corroborated the hypothesis of dominant monogenic inheritance of resistance in these eight accessions. A 61:3 (resistant/susceptible) F_2 ratio was observed for crosses of Magnif with Caroline and CR 366.13.2 as expected for resistance governed by one recessive and two dominant genes. The number of resistant and susceptible progeny observed in F_2 populations from crosses of Magnif with Dorina and Femina was consistent with a 63:1 ratio and supported the hypothesis of three independent dominant genes for resistance. The number of resistant and susceptible progeny observed in the F_2 population from the cross between Magnif and Carre 180 was consistent with a 13:3 ratio as expected for one dominant and one recessive gene.

Hor 2596 (*Rph*9.i) × resistant parents. Lack of segregation for susceptible progeny in crosses of Hor 2596 with Donan, Dorina, Femina, and Grit suggested that these accessions possess an allele at or near the *Rph*9 locus (Table 4). Expected segregation patterns of resistant and susceptible progeny were observed in crosses of Hor 2596 with 8 of the 13 resistant parents and confirmed previous

TABLE 3. Segregation of F_2 progeny from a partial diallel cross of 13 *Hordeum vulgare* accessions for reaction to *Puccinia hordei* isolate VA90-34

Cross	Resistant	Susceptible	Ratio[a]	*P*[b]	Gene no.[c]	Cross	Resistant	Susceptible	Ratio[a]	*P*[b]	Gene no.[c]
Grit					1	× Carre 180	255	48	13:3	0.20	1
× Donan	217	0	NS	…	1	CR 366.13.2					2
× Dorina	312	0	NS	…	2	× Dorina	133	0	NS	…	2
× Femina	240	0	NS	…	2	× Femina	163	0	NS	…	2
× Collo "S"	167	18	15:1	0.41	1	× Collo "S"	272	0	NS	…	1
× CR 270.2.3	173	12	15:1	0.89	1	× CR 270.3.2	327	0	NS	…	1
× Giza 119	198	18	15:1	0.21	1	× Lenka	186	0	NS	…	1
× Gloria	262	12	15:1	0.20	1	× Caroline	171	0	NS	…	2
× Lenka	240	17	15:1	0.81	1	× Donan	159	11	61:3	0.27	1
× Deir Alla 105	148	15	15:1	0.12	1	× Carre 180	243	31	55:9	0.19	1
× Caroline	193	14	61:3	0.16	2	CR 270.3.2					1
× CR 366.13.2	202	13	61:3	0.35	2	× Dorina	188	0	NS	…	2
× Carre 180	202	41	13:3	0.45	1	× Femina	101	0	NS	…	2
Gloria					1	× Collo "S"	288	0	NS	…	1
× Dorina	235	0	NS	…	2	× Lenka	75	0	NS	…	1
× Femina	291	0	NS	…	2	× Caroline	198	0	NS	…	2
× Collo "S"	294	0	NS	…	1	× Donan	243	18	15:1	0.67	1
× CR 270.3.2	291	0	NS	…	1	× Carre 180	178	28	13:3	0.85	1
× Giza 119	290	0	NS	…	1	Donan					1
× Lenka	263	0	NS	…	1	× Dorina	230	0	NS	…	2
× Deir Alla 105	259	0	NS	…	1	× Femina	444	0	NS	…	2
× Caroline	193	0	NS	…	2	× Collo "S"	222	19	15:1	0.29	1
× CR 366.13.2	268	0	NS	…	2	× Lenka	210	16	15:1	0.60	1
× Donan	280	16	15:1	0.54	1	× Caroline	183	6	61:3	0.33	2
× Carre 180	234	52	13:3	0.80	1	× Carre 180	161	36	13:3	0.86	1
Giza 119					1	Carre 180					1
× Dorina	292	0	NS	…	2	× Dorina	222	16	61:3	0.14	2
× Femina	195	0	NS	…	2	× Femina	261	12	61:3	0.82	2
× Collo "S"	295	0	NS	…	1	× Collo "S"	255	21	15:1	0.35	1
× CR 270.3.2	211	0	NS	…	1	× Lenka	190	15	15:1	0.52	1
× Lenka	198	0	NS	…	1	× Caroline	234	28	55:9	0.12	2
× Deir Alla 105	214	0	NS	…	1	Dorina					2
× Caroline	299	0	NS	…	2	× Femina	326	0	NS	…	2
× CR 366.13.2	267	0	NS	…	2	× Collo "S"	330	0	NS	…	1
× Donan	258	18	15:1	0.85	1	× Lenka	248	0	NS	…	1
× Carre 180	261	74	13:3	0.12	1	× Caroline	329	0	NS	…	2
Deir Alla 105				…	1	Femina					2
× Dorina	204	0	NS	…	2	× Collo "S"	329	0	NS	…	1
× Femina	298	0	NS	…	2	× Lenka	279	0	NS	…	1
× Collo "S"	197	0	NS	…	1	× Caroline	309	0	NS	…	2
× CR 270.3.2	244	0	NS	…	1	Lenka					1
× Lenka	122	0	NS	…	1	× Collo "S"	285	0	NS	…	1
× Caroline	199	0	NS	…	2	× Caroline	285	0	NS	…	2
× CR 366.13.2	298	0	NS	…	2	Caroline					2
× Donan	101	8	15:1	0.64	1	× Collo "S"	197	0	NS	…	1

[a] Expected segregation ratio. NS = no segregation.
[b] Probability of chi-square.
[c] Number of putative effective genes expected in each parent.

conclusions regarding gene number and mode of inheritance. This result also indicated that resistance in these accessions was not conferred by alleles at the *Rph*9 locus. Deviation from the expected 61:3 (resistant/susceptible) ratio for F_2 progeny from the cross between Hor 2596 and Caroline likely was the result of small population size.

Triumph (*Rph*9.z) × resistant parents. Segregation for susceptible progeny was not observed in crosses of Triumph with Donan, Dorina, Femina, and Grit, suggesting that these resistant parents possess an allele at or near the *Rph*9 locus (Table 4). Results from this study concerning crosses with Hor 2596 and Triumph corroborate with those of Borovkova et al. (1), who proposed that the resistant gene in Triumph *Rph*9.z (formerly designated *Rph*12) is an allele of the gene found in Hor 2596 (*Rph*9.i). The number of resistant and susceptible progeny observed in F_2 populations from crosses between Triumph and 5 of the 13 resistant parents was consistent with a 15:1 segregation ratio. Therefore, resistance in these accessions was governed by single dominant genes not located at the *Rph*9 locus. A F_2 ratio of 61:3 (resistant/susceptible progeny) was found for crosses of Triumph with CR366.13.2 and Caroline as expected based on the hypothesis that the latter two accessions each have one dominant and one recessive gene for resistance. Resistance in Carre 180 is governed by a single recessive gene independent of the *Rph*9 locus as confirmed by the 13:3 (resistant/susceptible) segregation pattern observed for F_2 progeny from the cross with Triumph.

In summary, 9 of 13 accessions possessed alleles at the *Rph*3 locus and four accessions possessed alleles at the *Rph*9 locus governing resistance to leaf rust. Among these accessions, Dorina and Femina possessed alleles at both loci, whereas Carre 180 possessed a recessive gene independent of these loci. The recessive genes in Caroline and CR 366.13.2 likely are independent of the other genes identified in this study.

DISCUSSION

Leaf rust resistance conferred by the gene *Rph*7 remained effective in the Eastern U.S. for more than 20 years (12). In 1990, virulence to *Rph*7 was identified (26) and, therefore, necessitated a search for new sources of resistance. Unique sources of leaf rust resistance continue to be found among accessions of *H. vulgare* (23,27). In the current study, 13 accessions of spring barley possessing resistance to isolates of *P. hordei* with virulence for genes *Rph*1, 2, 4, 6, 7, 8, and 11 were identified and genetically characterized.

Leaf rust resistance in the accessions Collo "S", CR 270.2.3, Deir Alla 105, Donan, Giza 119, Gloria, Grit, and Lenka is governed by single dominant genes. Dorina and Femina each have two dominant genes conferring resistance, whereas Caroline and CR 366.13.2 each have one dominant and one recessive gene. The mode of inheritance in Carre 180 is different from that of all other accessions in that a single recessive gene confers resistance. Considering that most of the *Rph* genes previously identified in *H. vulgare* are dominant in action, it is interesting that two or three of the genes identified in the current study are recessive in action. Jin and Steffenson (15) identified a recessive gene in addition to *Rph*3 in *H. vulgare* accession PI 531990. They also observed recessive gene action in accessions of *H. spontaneum*. According to a recent study by Chen and Line (3), resistance to *P. striiformis* Westend. f. sp. *hordei* in barley is predominantly governed by recessive genes.

Ten of the thirteen accessions evaluated in this study possess alleles at or tightly linked to the *Rph*3 locus. Jin and Steffenson (15) also observed that *Rph*3 occurred at a high frequency in barley genotypes originating in Egypt and the Mediterranean region. The allele conferring resistance in Deir Alla 105 likely is *Rph*3.c, as found in Estate, based on a similar reaction of these two genotypes to four differential isolates of *P. hordei*. In contrast, the allele at or near the *Rph*3 locus in Caroline, Collo "S", CR 270.2.3, CR 366.13.2, Dorina, Femina, Giza 119, Gloria, and Lenka differ from *Rph*3.c, based on susceptibility of these accessions to isolate ND89-3 which is avirulent to *Rph*3.c. This finding was verified by additional tests of these parents with isolate ND89-3. Alleles producing different reactions to a series of *P. hordei* isolates have been identified previously in barley (4,14,25). Results of the current study support the hypothesis of multiple alleles at loci governing resistance to barley leaf rust (14,20, 24,25). Although virulence to *Rph*3 has been reported in Europe (6), new alleles at this locus could provide control of leaf rust, especially when used in combination with other resistance genes.

TABLE 4. Allelic relationship of genes in 13 *Hordeum vulgare* accessions with genes *Rph*3, 5, and 9 governing resistance to *Puccinia hordei* isolate VA90-34

Cross	Resistant	Susceptible	Ratio[a]	*P*[b]	Genes[c]
Estate					*Rph*3.c
× Gloria	296	0	NS	…	*Rph*3
× Giza 119	269	0	NS	…	*Rph*3
× CR 270.3.2	296	0	NS	…	*Rph*3
× Collo "S"	261	0	NS	…	*Rph*3
× Lenka	220	0	NS	…	*Rph*3
× Deir Alla 105	311	0	NS	…	*Rph*3.c
× Dorina	214	0	NS	…	*Rph*3+9
× CR 366.13.2	264	0	NS	…	*Rph*3+?
× Caroline	235	0	NS	…	*Rph*3+?
× Grit	74	7	15:1	0.37	*Rph*9
× Donan	200	18	15:1	0.22	*Rph*9
× Carre 180	209	57	13:3	0.26	Unknown
Magnif					*Rph*5.e
× Collo "S"	226	19	15:1	0.70	*Rph*3
× CR 270.3.2	300	24	15:1	0.39	*Rph*3
× Deir Alla 105	267	24	15:1	0.16	*Rph*3.c
× Lenka	203	16	15:1	0.52	*Rph*3
× Gloria	262	13	15:1	0.30	*Rph*3
× Giza 119	272	26	15:1	0.74	*Rph*3
× Grit	246	18	15:1	0.70	*Rph*9
× Donan	239	18	15:1	0.62	*Rph*9
× Caroline	295	12	61:3	0.52	*Rph*3+?
× CR 366.13.2	183	8	61:3	0.74	*Rph*3+?
× Dorina	253	6	63:1	0.33	*Rph*3+9
× Femina	287	8	63:1	0.11	*Rph*3+9
× Carre 180	178	31	13:3	0.26	Unknown
Hor 2596					*Rph*9.i
× Grit	319	0	NS	…	*Rph*9
× Donan	239	0	NS	…	*Rph*9
× Dorina	248	0	NS	…	*Rph*3+9
× Femina	254	0	NS	…	*Rph*3+9
× Collo "S"	71	4	15:1	0.74	*Rph*3
× CR 270.3.2	270	13	15:1	0.25	*Rph*3
× Deir Alla 105	254	24	15:1	0.10	*Rph*3.c
× Giza 119	272	20	15:1	0.67	*Rph*3
× Gloria	290	20	15:1	0.88	*Rph*3
× Lenka	88	8	15:1	0.40	*Rph*3
× Caroline	50	6	61:3	0.03	*Rph*3+?
× CR 366.13.2	299	9	61:3	0.14	*Rph*3+?
× Carre 180	266	50	13:3	0.18	Unknown
Triumph					*Rph*9.z
× Grit	315	0	NS	…	*Rph*9
× Donan	99	0	NS	…	*Rph*9
× Dorina	328	0	NS	…	*Rph*3+9
× Femina	316	0	NS	…	*Rph*3+9
× Gloria	198	19	15:1	0.13	*Rph*3
× Collo "S"	251	12	15:1	0.28	*Rph*3
× CR 270.3.2	125	12	15:1	0.23	*Rph*3
× Deir Alla 105	79	5	15:1	0.91	*Rph*3.c
× Giza 119	213	17	15:1	0.47	*Rph*3
× Caroline	191	12	61:3	0.41	*Rph*3+?
× CR 366.13.2	210	13	61:3	0.42	*Rph*3+?
× Carre 180	181	34	13:3	0.27	Unknown

[a] Expected segregation ratio. NS = not segregated.
[b] Probability of chi-square.
[c] Putative *Rph* genes in each cross.

Virulence for *Rph*3 has not been identified in North America or Mexico (9,23); therefore, alleles at this locus can be deployed, preferably in combination with other resistance genes to provide control of barley leaf rust in these areas.

Donan, Dorina, Femina, and Grit possess alleles at or tightly linked to the *Rph*9 locus. Dorina and Femina also have alleles at the *Rph*3 locus. Virulence for *Rph*9.z *(*formerly *Rph*12) has not been identified in North America, nor has this gene been deployed on this continent. A low percentage of *P. hordei* isolates collected in Arizona and California in 1993 had virulence for *Rph*9.i (9). Alleles at the *Rph*9 locus, such as *Rph*9.z, could be combined with other effective resistance genes, such as *Rph*3, to provide resistance to leaf rust in North America and other countries where virulence for these genes has not been identified.

In addition to a dominant allele at the *Rph*3 locus, Caroline and CR 366.13.2 each possess a recessive gene that is independent of the other genes identified in this study. Carre 180 possesses a single recessive gene for resistance to isolate VA90-34 that is independent of all other genes identified in the current study. Further allelism tests and perhaps a molecular mapping project should be initiated to determine if this gene is new.

Resistance to leaf rust was not governed by the *Rph*5 locus in any of the 13 accessions based on segregation for susceptible progeny in all crosses with Magnif. Virulence for *Rph*5 is widely prevalent in Europe (18) and South America (2,9) but has not been identified in North America where this gene has not been used in commercial cultivars. Therefore, *Rph*5 also could be deployed in North America, but probably should be used only in combination with other effective genes.

In summary, resistance to *P. hordei* isolate VA90-34 is governed by single dominant alleles at the *Rph*3 locus in six accessions. Caroline and CR366.13.2 likely have a second unknown recessive gene in addition to *Rph*3. Resistance in two accessions was governed by single dominant alleles at the *Rph*9 locus, whereas two accessions possessed dominant alleles at the *Rph*3 and *Rph*9 loci. Dreiseitl and Steffenson (8) reported a similar occurrence of known *Rph* genes among 93 Czech and Slovak barley accessions. In that study, *Rph*3 was postulated for 17 accessions, *Rph*9.z (formerly *Rph*12) for 26 accessions, and the combination of *Rph*3 and *Rph*9.z for seven accessions. Resistance in Carre 180 is different from that of all other parents and host differentials included in the current study and is inherited as a single recessive gene in most cases.

Further studies are needed to elucidate the identity of unknown genes reported herein and to determine the identity of alleles at the *Rph*3 and *Rph*9 loci for accessions with these putative genes. Results from this study should be useful to barley breeders in assessing current genetic variability for leaf rust resistance in their programs and in providing them with the genetic identity of resistance genes in potentially new sources. Combining unique resistance genes, such as those identified in Carre 180, Caroline, and CR 366.13.2 with other effective resistance genes such as *Rph*3, 5, and 9 and more recently identified genes such as *Rph*16, should provide for more durable resistance than single deployment of these genes. Furthermore, resistance of all 13 accessions to isolate VA90-34 and to other isolates makes them valuable sources of leaf rust resistance in North America.

ACKNOWLEDGMENTS

This research was funded in part by the Virginia Small Grains Board, the Virginia Agricultural Council, and the Virginia Crop Improvement Association.

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