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Evaluation of cultivated and wild barley for resistance to pathotypes of *Puccinia hordei* with wide virulence *

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Abstract

An isolate of *Puccinia hordei* (ND89–3) originally collected in Morocco is virulent on most barley genotypes reported to possess resistance, except cultivar Estate (CI 3410), which possesses the *Rph*3 gene and exhibits a low to intermediate level of resistance (infection type 12). Isolate ND89–3 possesses one of the widest virulence spectrums reported for *P. hordei*. Accessions of *Hordeum vulgare* (1,997 in total) and *H. spontaneum* (885 in total), mostly originating from the Mediterranean region and parts of North Africa, were evaluated with isolate ND89–3 at the seedling stage to identify new sources of leaf rust resistance. Fifty-eight accessions of *H. vulgare*, and 222 accessions of *H. spontaneum* exhibited low infection types to this isolate. Further evaluations of these resistant accessions with isolates of *P. hordei* virulent for *Rph*3, *Rph*7, and *Rph*12 suggested that most of the resistant *H. vulgare* accessions possess the *Rph*3 gene. Data suggested additional sources of effective resistance in *H. vulgare* are rather limited. Five *H. vulgare* accessions and 167 *H. spontaneum* accessions were identified as possible sources of new genes for leaf rust resistance. These accessions likely possess resistance genes that are different from *Rph*1 to *Rph*12, or gene combinations thereof based on their reaction to four leaf rust isolates. Utilization of these accessions in barley breeding will broaden the germplasm resources available for genetic control of *P. hordei.*

Introduction

Leaf rust of barley (*Hordeum vulgare* L.), caused by *Puccinia hordei* G. Otth, is an important disease in many areas of the world. It is likely that this disease will remain significant because new pathotypes of *P. hordei* occur frequently, and none of the known *Rph* genes (*Rph*1–*Rph*12) conferring resistance to leaf rust is effective worldwide. The occurrence of virulence for *Rph*3, *Rph*7, or *Rph*12 in *P. hordei* is of great importance because these resistance genes have been used widely in barley breeding programs. Pathotypes virulent for *Rph*3 and *Rph*12 are common in Europe (Clifford, 1985), and in Australia, virulence for *Rph*12 is known (Cotterill et al., 1992). Virulence for *Rph*7 has been reported in Israel (Golan et al., 1978), Morocco (Parlevliet et al., 1981), and recently, in the United States (Steffenson et al., 1993). In 1989, an isolate of *P. hordei*, ND89–3, was collected from a barley field in Rabat, Morocco by A.P. Roelfs (USDA-ARS, Cereal Rust Laboratory, St. Paul, MN). Our preliminary studies indicated that this isolate was virulent for all known *Rph* genes except *Rph*3, and on the resistance sources identified by Sharp & Reinhold (1982), Vivar et al. (1987), and Yahyaoui et al. (1988). Because this isolate possesses one of the widest virulence spectrums known in *P. hordei*, it should be useful in screening for new sources of leaf rust resistance in barley.

In cultivated barley, available sources of resistance are rather limited, particularly to widely virulent pathotypes in the *P. hordei* population. Among the known sources of resistance, many were derived from landrace cultivars originating from the Mediterranean region where the host and pathogen are indigenous and have coevolved (Anikster & Wahl, 1979). Eval-

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uations of the wild relative of barley, *H. spontaneum* C. Koch, to the leaf rust pathogen revealed that resistance is very common in this species (Manisterski et al., 1986; Moseman et al., 1980, 1990; Wahl et al., 1984). Indeed, the two most recently described leaf rust resistance genes (*Rph*10 and *Rph*11) were from *H. spontaneum* (Feuerstein et al., 1990). The objectives of this study were to compare reactions of *H. vulgare* and *H. spontaneum*, and to identify new sources of resistance to *P. hordei* pathotypes with wide virulence. This was done by evaluating the seedling reactions of cultivated and wild barley accessions, mainly from the Mediterranean region, to isolate ND89–3, and three other isolates with virulence on most of the known sources of resistance in barley.

Materials and methods

Rust isolates

Four isolates of *P. hordei* (ND89–3, BRS76–12, BRS90–40, and ND90–23) were used in this study. Table 1 lists the virulence patterns of these four isolates on leaf rust differential genotypes with known *Rph* genes (Steffenson & Jin, 1992). These isolates share common virulence genes for *Rph*1, 2, 4, 6, 8, 10, and 11; more importantly, however, they possess several critical virulence genes for *Rph*3, *Rph*7, and *Rph*12. Isolate ND89–3 is virulent for all known *Rph* genes except *Rph*3. Isolate ND90–23 (collected from California, USA) is virulent for *Rph*7. Isolates BRS76–12 and BRS90–40 (provided by B.C. Clifford, Welsh Plant Breeding Station, Aberystwyth, UK) possess virulence for *Rph*3 and *Rph*12, respectively.

Plant materials

A total of 1,997 barley accessions from the United States Department of Agriculture (USDA) National Small Grains Collection (NSGC) were evaluated. These accessions were collected from sites ranging from northern Africa to western Asia (mostly from the Mediterranean region), and many were landrace cultivars. A collection of 885 *H. spontaneum* NSGC accessions, mostly from Israel, was also evaluated. This group of wild barley included 124 accessions previously reported to possess leaf rust resistance (Moseman et al., 1990). Plant materials from this region were chosen because the host and pathogen are indigenous, and genetic diversity for resistance is expected in the germplasm from this region.

Rust inoculation and evaluation

Four to five seeds of each accession were sown in a plastic cone (3.8 cm diameter and 21 cm depth) containing a 3:1 peat moss:perlite mixture and grown in a greenhouse at 22 ± 3°C. Urediniospores of *P. hordei* isolates were suspended in a light mineral oil and inoculated onto one week old seedling plants (primary leaf fully expanded) using a rate of 3 mg urediniospores 5 ml oil^{-1} 100 plants^{-1}. Inoculated plants were incubated for 16 hours in a chamber in which the humidity was maintained near saturation by mist from ultrasonic humidifiers. Reactions of each accession were evaluated after an incubation period of 12–14 days in a greenhouse at 22 ± 3°C. Infection types 0, 0;, 1, and 2 were considered indicative of host resistance, and infection types, 3 and 4, of host susceptibility based on the scale of Levine and Cherewick (1952). The above-mentioned barley accessions were first evaluated for their seedling reaction to isolate ND89–3. Accessions or plants within an accession that exhibited resistance to isolate ND89–3 were further evaluated using the three isolates BRS76–12, ND90–23, and BRS90–40 possessing virulence for *Rph*3, *Rph*7, and *Rph*12, respectively.

Results

Resistance in H. vulgare

Fifty-eight accessions of *H. vulgare* exhibited low infection types (0; to 2) to isolate ND89–3 (Table 2). Fifty-three accessions that were resistant to isolate ND89–3 were susceptible to isolate BRS76–12, suggesting that they may possess the resistance gene, *Rph*3. Five accessions, PI 531840, PI 531841, PI 531849, PI 531901, and PI 531941 were resistant to both isolates ND89–3 and BRS76–12. These accessions possess gene(s) for resistance that are likely different from those present in the 12 differential host genotypes since the combinations of virulence genes of isolates ND89–3 and BRS76–12 should overcome the genes *Rph*1 through *Rph*12. Isolates BRS90–40 and ND90–23 may possess additional virulence genes that were not detected using the 12 differential genotypes because three accessions, PI 531840, PI 531841 and PI 531849, were susceptible to these two isolates.

Table 1. Infection types[a] of barley genotypes used as leaf rust differentials to four isolates of *Puccinia hordei*

Differential genotype	Accession number	Recognized *Rph* gene	Isolate of *Puccinia hordei* ND89-3	BRS76-12	BRS90-40	ND90-23
Sudan	CI 6489	*Rph*1	$3^{-}3$	33^{+}	$3^{+}3$	4
Peruvian	CI 935	*Rph*2	$3^{+}3$	$3^{-}3$	33^{-}	34
Estate	CI 3410	*Rph*3	12^{-}	$3^{+}3$	00;	0;1
Gold	CI 1145	*Rph*4	3^{-}	33^{-}	33^{+}	3
Magnif	CI 13860	*Rph*5	43	3	0;	0;
Bolivia	CI 1257	*Rph*6+2	43	3^{-}	3^{-}	4
Cebada Capa	CI 6193	*Rph*7	33^{+}	0;	0;	4
Egypt 4	CI 6481	*Rph*8	33^{+}	33^{+}	33^{-}	4
Hor 2596	CI 1243	*Rph*9	$3^{-}3$	0;	3^{-}	21
Clipper BC8	None	*Rph*10[b]	3^{-}	3^{-}	3^{-}	4
Clipper BC67	None	*Rph*11[b]	3^{-}	3^{-}	3^{-}	4
Triumph	PI 290195	*Rph*12[b]	$3^{-}3$	0;	33^{-}	0;

[a] Infection type ratings were based on the scale of Levine and Cherewick (1952). Infection types 0, 0;, 1, and 2 were considered indicative of host resistance, and infection types 3 and 4, of host susceptibility. Symbols + and - denote more or less sporulation, respectively. The susceptible class also included infection type combinations of 23^{-}.

[b] Gene designations of *Rph*10 and *Rph*11 were based on Feuerstein et al. (1990) and *Rph*12 based on Jin et al. (1993). Leaf rust differentials were based on Steffenson and Jin (1992).

Only two accessions, PI 531901 and PI 531941, were resistant to all four isolates used in this study.

Resistance in H. spontaneum

Resistance was common in *H. spontaneum*, as 222 accessions (ca. 25% of the total number evaluated) exhibited low infection types to isolate ND89–3. Accessions (167 in total) resistant to both isolates ND89–3 and BRS76–12 are listed in Table 3. These accessions likely possess resistance genes that are different from the known *Rph* genes, or gene combinations that include *Rph*3. The majority of these accessions (135 in total) also were resistant to isolates BRS90–40 and ND90–23. There were several distinct clusters of accessions that reacted similar to different leaf rust isolates. For example, one cluster susceptible to isolate BRS90–40 consisted of accessions from PI 391002 to PI 391005, and another cluster susceptible to both isolates BRS90–40 and ND90–23 consisted of several accessions from PI 466453 to PI 466486. This suggests that accessions within a cluster probably share a common genetic background for leaf rust reaction.

Discussion

Most of the accessions of *H. vulgare* that were resistant to isolate ND89–3 and susceptible to BRS76–12 originated from Egypt (Table 2). These accessions shared with Estate (a source of *Rph*3) several morphological traits: reduced plant height, six-rowed spikes with reduced surface waxes, and early maturity. Allelism testing of several randomly selected accessions with Aim, an Egyptian landrace cultivar possessing the *Rph*3 allele, confirmed that these accessions possess the *Rph*3 gene (Y. Jin & B.J. Steffenson, unpublished).

Only five out of 1,997 accessions of *H. vulgare* were resistant to isolates ND89–3 and BRS76–12, and only two accessions were resistant to all four isolates in this study (Table 2). The low frequency of resistance in *H. vulgare* to the virulence combinations of the four isolates of *P. hordei* suggests that additional sources of effective resistance in *H. vulgare* are rather rare.

Data in Table 1 suggest that combinations of *Rph*3 with *Rph*7, *Rph*9, or *Rph*12 genes would confer resistance to the virulence combinations used in this study. Gene combination has not been used widely in the barley:leaf rust pathosystem, but it has proven successful

Table 2. Accessions of *H. vulgare* possessing resistance to isolate ND89–3, and their reactions (infection types[a]) to isolates BRS76–12, BRS90–40 and ND90–23 of *P. hordei*

Accession	BRS76-12	BRS90-40	ND90-23	Accession	BRS76-12	BRS90-40	ND90-23
CI 3550	3	3	3	CI 3559	3⁻2	23⁻	0;
CI 3560	3	0;	0;	CI 3582	23⁻	0;	0;
CI 3737	3⁻2	0;	0;	CI 3741	3⁻2	21	12
CI 3778	23⁻	0;	0;	CI 3781	3	0;1	0;
CI 3792	3⁻2	3	3	CI 3797	3	3⁻2	3⁻2
CI 4122	3	3	3	CI 4251	3⁻2	3⁻2	0;1
CI 4995	3⁻2	3	3	CI 4997	3	3	3
CI 4998	3	3	3	CI 6710	3	0;	0;
CI 6713	3	0;	3⁻2	CI 6714	23⁻	21	12
CI 6717	23⁻	0;1/3[b]	21	CI 7090	3⁻	0;	0;
CI 7229	3⁻2	3	3	CI 9206	3⁻2	0;	0;
CI 10149	3	0;	0;1/3	CI 10162	23⁻	0;	0;
CI 10506	23⁻	0;	0;	CI 11187	3	0;1	0;1
CI 11188	23⁻	0;1	0;1	PI 531840	21	23⁻	3⁻2
PI 531841	0;1	23⁻	3	PI 531849	0;1	3⁻2	3⁻2
PI 531873	3⁻2	0;1/3	0;1	PI 531874	3	0;1/23⁻	3⁻2/0;1
PI 531890	23⁻	0;1	0;1	PI 531892	3⁻2	0;1	23⁻
PI 531897	3	0;1	21	PI 531901	0;1/23⁻	12	0;
PI 531907	3⁻2	0;1	0;1/3	PI 531918	23⁻	0;1	0;1
PI 531929	3⁻2	0;1	0;	PI 531930	3⁻2	0;1	0;1
PI 531935	3⁻2	0;1	0;1	PI 531941	21	12	12/3
PI 531944	3⁻2	0;1	0;1	PI 531945	3⁻	0;1	0;
PI 531946	3⁻2	3	3	PI 531947	3	0;1	3
PI 531948	23⁻	0;1	0;1	PI 531956	3	0;1	0;1
PI 531961	3⁻2	23⁻/0;	0;1/3⁻	PI 531962	3⁻2	21	21
PI 531963	3	0;1	0;1	PI 531969	3	3	0;1/3
PI 531970	3	0;1	0;1	PI 531971	3	3/0;1	3
PI 531972	3⁻2	12	12	PI 531973	23⁻	3/0;1	0;1
PI 531974	3⁻2	0;	0;	PI 531975	3⁻	0;1	0;

[a] Infection type ratings were based on the scale of Levine and Cherewick (1952). Infection types 0, 0;, 1, and 2 were considered indicative of host resistance, and infection types 3 and 4, of host susceptibility. Symbols + and - denote more or less sporulation, respectively. The susceptible class also included infection type combinations of 23⁻.

[b] "/" indicated there was a mixture of plants with high and low infection types within an accession. The most common type was listed first.

in the control of wheat leaf and stem rusts in North America (Roelfs, 1985; Samborski, 1985). Use of the slow rusting type of leaf rust resistance in barley has been successful in Europe (Clifford, 1985). Combinations of effective *Rph* genes with slow rusting might provide durable resistance in barley.

Hordeum spontaneum has been regarded as a valuable genetic resource for barley improvement, especially disease resistance (Moseman et al., 1980). Our study confirmed that many accessions of *H. spontaneum* originating from Israel are resistant to leaf rust as reported by others (Manisterski et al., 1986; Moseman et al., 1990). The high frequency of resistant accessions to leaf rust isolates with broad virulence indicated that resistance genes have been accumulated during the long history of coevolution in the center of the origin for *H. spontaneum* (Wahl et al., 1984). Because the frequency of resistant accessions is high in *H. spontaneum*, many of them should possess two or more genes for resistance to *P. hordei*. Selection

Table 3. PI accessions of *H. spontaneum* possessing resistance to isolates ND89–3 and BRS76–12, and their reactions to isolates BRS90–40 and ND90–23 of *P. hordei*

282583[a]	355444	391058	405166[a]	405235	405344	466324
282597[a]	355445[a]	391059	405167[a]	|	405345	466325
282610	355446	391061	405169[a]	405238	405349	466339[a]
296818[b]	355447	391069	405177	405266	405350	466341
296819[b]	355449	|	405178	405277	405351	466427
296841	391000	391075	405179	405289	405353	466453[b]
296935[a]	391001	391087	405180	405292	405354	466458[b]
296942	391002[a]	391088	405194	405298	405364	466470[b]
354923	391003[a]	391089	405201	405299	405375[b]	466476[b]
354926	391004[a]	391116	|	405300	405390[a]	466480[b]
354927	391005[a]	391121	405206	405301	405397	466481[b]
354928[a]	391006	391122	405208	405304	|	466482[b]
354932	|[c]	405141[a]	405210	405305	405401	466483[b]
354933	391010	405144	405212	405308	420914[b]	466486[b]
354937	391012	405148[a]	|	405311	465980[a]	466524
354940	391024	405151[a]	405223	405327	466244	466538
355434	391044	405154	405227	|	466245	466567
355435	|	405163	405232	405338	466247	531853
355437	391050	405165	405233	405341	466323[a]	

[a] Susceptible to isolate BRS90–40 only.
[b] Susceptible to isolates BRS90–40 and ND90–23.
[c] | = consecutive accession numbers included.

for resistance in this study was based on low infection types at the seedling stage. These accessions could possess other types of resistance, such as slow rusting or adult plant resistance that is not detected by seedling infection types alone. In the light of the occurrence of widely virulent pathotypes in the *P. hordei* populations and limited sources of resistance in *H. vulgare*, *H. spontaneum* should be a valuable genetic resource for the development of leaf rust resistant cultivars.

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