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Identification of a new pathotype of *Puccinia hordei* with virulence for the resistance gene *Rph7*

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Abstract

Barley leaf rust resistance gene *Rph7*, derived from barley accession Cebada Capa, is the most effective R-gene for resistance to *Puccinia hordei*. Virulence for this gene was known in USA, Israel and Morocco but not yet in Europe. We found an unexpected leaf rust infection in the field at Córdoba, Spain in 2004 on *Rph7* carrying lines. This virulence for *Rph7* was confirmed in growth chamber experiments, being the first report of *Rph7* virulence in European populations of *Puccinia hordei*. A collection of 680 barley accessions was screened for resistance against this new isolate. Twelve accessions showed segregation with individual plants showing resistance based on hypersensitivity (low infection type). These individual resistant plants were selected and grown in the greenhouse to obtain seeds.

Keywords Barley, *Hordeum vulgare*, leaf rust, *Puccinia hordei*, *Rph7*.

Introduction

Barley leaf rust, caused by the *Puccinia hordei* Otth, is one of the most important foliar diseases of barley throughout the world. The use of resistant barley varieties has proven an effective method to prevent yield losses which may reach 32% in susceptible cultivars (Griffey et al., 1994). Because of environmental and health risks, and the need to reduce production costs there is the tendency to reduce the use of fungicides, in benefit of genetic resistance. At present, 19 major race-specific genes for resistance to leaf rust named *Rph1* to

Rph19 have been described in barley (Weerasena et al., 2004). However, few of these major genes have been deployed in commercial cultivars (Cotterill et al., 1994, Dreiseitl and Steffenson, 2000). *Rph7* was believed to be fully effective in Europe (Niks et al., 2000), although virulence to it has been reported in Israel (Golan et al., 1978), Morocco (Parlevliet et al., 1981), and the United States (Steffenson et al., 1993).

During the growing season 2003-2004 selected barley plants showing resistance to our standard leaf rust isolate CO-01 (virulence/avirulence *Rph1,2,4,6,8,9,12/3,5,7*) were planted in the field at CIFA experimental station, Córdoba for seed multiplication and genetic studies. During the growing season, we noticed that some of these selected plants were unexpectedly infected by leaf rust (compatible infection type).

The objectives of the present study were: 1) to determine the virulence spectrum of the new *P. hordei* isolate, and 2) to identify new sources of race-specific resistance in barley germplasm from Spain and the Fertile Crescent.

Material and methods

Virulence spectrum identification

Uredinia were collected from barley plants at the field of Córdoba, Spain from which a monosporic isolate was derived and used across the experiment. The isolate was multiplied on the susceptible barley line L94.

The virulence spectrum of this new isolate was determined on a set of differential genotypes possessing the leaf rust resistance genes *Rpg1-Rph9* (Table 1) (Steffenson et al., 1993). An additional differential genotype possessing the leaf rust resistance gene *Rph7* (L94-Pa7) was included to the differential set. To confirm the reaction of Cebada Capa (*Rph7*) and L94-Pa7, three additional monosporic isolates CO-01, Al-02 and 1.2.1 known to be avirulent to *Rph7* were used (Table 1). The susceptible cultivar L94 was used throughout the experiments as control.

Table1. Infection types of four *Puccinia hordei* isolates on barley seedlings

Genotype	Recognized *Rph* gene(s)[1]	Isolates[2]			
		CO-04	CO-01	Al-02	1.2.1
Sudan	*Rph1*	9	9	9	9
Peruvian	*Rph2*	9	8	9	9
Estate	*Rph3*	8	6	7	6
Gold	*Rph4*	9	9	9	9
Magnif 104	*Rph5*	9	2	8	9
Bolivia	*Rph2+6*	9	9	9	9
Cebada Capa	*Rph7*	9	1	4	2
Egypt 4	*Rph8*	9	9	9	9
Ab 14 Koln	*Rph9*	9	9	9	8
L94-*Pa7*	*Rph7*	9	2	2	3
L94	-	9	9	9	9

[1] Seeds of the differential barley genotypes provided by Dr. R.E. Niks, the Netherlands.
[2] Isolates CO-01 and CO-04 were collected in Córdoba, Spain, on 2001 and 2004 respectively; isolate AL-02 was collected in Alhama de Granada, Spain, on 2002; isolate 1.2.1 was kindly provided by Dr. R.E. Niks, the Netherlands.

Screening for resistance in a germplasm collection

Seed samples of 680 accessions of barley germplasm from Spain and the Fertile Crescent were kindly provided by the Centro de Recursos Fitogenéticos (CRF), INIA, Spain, the International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria, and United States Department of Agriculture (USDA), USA (Table 2).

Table 2. Origin and source of the barley landraces screened against the new *Rph7* virulent isolate.

Origin	Number of accessions	Source
Israel	4	USDA
Jordan	29	ICARDA + USDA
Lebanon	15	ICARDA
Palestinian Territory	23	ICARDA + USDA
Spain	569	CRF
Syria	40	ICARDA

About 10-15 seedlings per accession were grown in 7x7x11 cm pots. Plants were grown in a growth chamber at 20 ºC and white fluorescent light (12 h light / 12 h dark). The inoculation was carried out by dusting freshly collected urediniospores of the new isolate CO-04 diluted 10 times with talcum powder, over the seedlings when the second leaf of the seedlings had emerged. The inoculated plants were kept in an inoculation chamber for 20 hours at 20 ºC

with a relative humidity of about 100% and darkness. Plants were then transferred to a growth chamber at the same growing conditions as mentioned above.

IT was recorded 12-14 days after inoculation following the 0-9 scale of McNeal et al. (1971) where: 0 = No uredinia or other macroscopic sign of infectiton; 1 = Few faint hypersensitive flecks; 2 = No uredinia, but clear hypersensitive necrotic flecks present; 3 = Flecks with small uredinia surrounded by necrosis; 4 = Small to medium uredinia often surrounded by necrosis and chlorosis, low sporulation; 5 = Medium uredinia often surrounded by necrosis and chlorosis, reasonable (or: fair) sporulation; 6 = Medium-sized to large uredinia surrounded by necrosis and chlorosis, reasonably sporulation; 7 = Medium-sized to large uredinia surrounded by chlorosis but not necrosis, good sporulation; 8 = Medium-sized to large uredinia surrounded by a little chlorosis but not necrosis, good sporulation; and 9 = Large uredinia without chlorosis or necrosis, very good sporulation. Infection types 0-6 are considered indicative of resistance, and 7-9 of susceptibility.

Results and Discussion

Isolates CO-01, Al-02 and 1.2.1 showed the expected reactions on the differential lines, with incompatible reaction on Cebada Capa and L94-*Pa7* confirming the effectiveness of *Rph7* to the avirulent isolates. The new isolate (CO-04) showed compatible infection type on all the differential genotypes used in the present study including Cebada Capa and L94-Pa7 confirming its virulence on *Rph7* (Table 1).

Virulence changes in rust populations can result from sexual recombination, introduction or mutations (McIntosh, 1988). Reinhold and Sharp (1982) stated that in Mediterranean areas, where summer months are dry, the fungus may be dependant on sexual recombination to complete its life cycle, since in Europe/USA it also does not complete its life cycle, still it does not need Ornithogalum to become fully endemic throughout the year, thus resulting in higher frequency of new physiologic races. In Israel, Golan et al. (1978) reported new virulence types of *Puccinia hordei* emerging from sexual recombination on the alternate hosts, *Ornithogalum narbonense, O. montanum*, and *O. brachystachys* showing virulence to all known race-specific genes including *Rph7*. However, Steffenson et al. (1993) reported that the infection of *O. umbellatum* with *P. hordei* was never observed in Virginia State, USA where *P. hordei* pathotypes with virulence to *Rph7* were detected and they concluded that mutation was the most plausible explanation for the origin of *Rph7* virulence in North America. We did not study the occurrence of sexual stage of *P. hordei* but are aware that

Ornithogalum species described as alternate hosts of *P. hordei* are present at Córdoba province where we collected isolate CO-04.

A second possibility is that this isolate (CO-04) was introduced to Southern Spain from North Africa, mainly from Morocco, where virulence to *Rph7* is known (Parlevliet et al., 1981). The Moroccan *Rph7* virulent isolate (Niks et al., 1989) was found to have an abnormal morphology of the substomatal vesicles (SSV). However, germlings of our CO-04 isolate showed the typical SSV morphology of *P. hordei* (Niks, 1986), similar to that of isolates CO-01, Al-02 and 1.2.1 (data not shown).

The origin of isolate CO-04 virulent to *Rph7* is not known. The identification of *P. hordei* pathotype with virulence to *Rph7* is significant because this is its first report in Europe. The appearance of this new pathotype emphasizes the need of regular virulence surveys and searching for new sources of resistance.

Table 3. Infection type (IT) of selected individual plants in barley accessions against the new *Rph7* virulent isolate of *P. hordei* (CO-04)

Accession	Origin	Infection type
PI572573	Israel	2
PI420922	Jordan	6
PI420923	Jordan	3
IG29091	Jordan	5
IG31459	Jordan	5
IG32763	Jordan	3
PI420919	Jordan	5
IG36019	Jordan	6
IG36046	Jordan	5
PI186425	Palestinian Territory	5
IG32574	Syria	4
IG35374	Syria	5
L94		9

The level of resistance in the germplasm collections screened against this new CO-04 isolate is not high. Most of the accessions (98.2% of the collection) showed compatible infection type (IT $\geq$7). However, in the remaining 1.8% of the collection (12 accessions), segregation was observed with individual plants showing low IT (IT $\leq$ 6) with clear hypersensitivity (Table 3). These individual plants were selected for seed multiplication and

for further studies. Jin et al. (1995) also found that resistance to *Rph7* virulent isolates was rare in *H. vulgare* but fairly common in *H. spontaneum*.

We may conclude that selected plants carry new resistance genes different from the tested leaf rust resistance genes. This because our test isolate has virulence corresponding to the widely used *Rph* genes in the international leaf rust resistance breeding programs including the highly effective resistance gene *Rph7* (Niks et al., 2000).

Further studies are needed to elucidate the identity of gene(s) reported here and to determine if these genes are alleles or linked to known genes for leaf rust resistance. Selected plants are being crossed to the susceptible cultivar L94 to study the inheritance of the detected gene(s) in F_2. Also they will be crossed to genotypes known to carry different major race-specific leaf rust resistance 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 new sources of leaf rust resistance.

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