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Screening for Resistance to Leaf Rust (*Puccinia hordei*) in a Collection of Spanish Barleys

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A collection of 418 Spanish barley accessions was screened for resistance to leaf rust in the field at Córdoba, Spain during the 2002–2003 season. Six accessions displaying the lowest disease severity with no macroscopically visible necrosis were selected for further studies on components of resistance. Five of them showed a significantly higher relative latency period than the susceptible line L94 and were similar to the partially resistant Vada. All of them showed lower relative infection frequency and smaller colony size than the susceptible L94 under controlled conditions. Histological studies indicated that the resistance in four of these accessions was based on a higher percentage of early aborted colonies not associated with host cell necrosis. In the remaining two accessions, resistance was based on hypersensitivity.

Key Words: *Puccinia hordei*, barley, histology, partial resistance.

Introduction

Leaf rust, caused by *Puccinia hordei* Otth, is one of the most important foliar diseases of barley (*Hordeum vulgare* L.). Severe losses as high as 30–40% have been reported in several countries (Griffey *et al*. 1994). Leaf rust can be controlled by the use of fungicide application or by the use of resistant varieties. Because of environmental and health risks, and the need to reduce production costs, there is a tendency to reduce the use of fungicides in favour of genetic resistance. Nineteen major genes (*Rph*) for resistance against *Puccinia hordei* have been identified and mapped in barley landraces and wild barley (*H. spontaneum*) (Weerasena *et al*. 2004). The resistance caused by these genes is typically race-specific, expressed as a hypersensitive reaction, and of limited durability. Only *Rph7* is still fully effective in Europe (Niks *et al*. 2000), but virulence for this gene has been reported in Morocco (Parlevliet *et al*. 1981), USA (Steffenson *et al*. 1993) and Israel (Brodny and Rivadeneira 1996).

The non-durability of this resistance has caused breeders to look for more durable types of resistance such as partial resistance (PR). PR has been identified in barley and is expressed as a reduced rate of epidemic development despite a compatible interaction (high infection type) (Parlevliet 1975). In the barley — *P. hordei* pathosystem, this partial resistance is inherited polygenically (Qi *et al.* 1998), and is presumed to be predominantly race-nonspecific and durable (Parlevliet and Van Ommeren 1975, Parlevliet 1983). Partially resistant barley infected with *P. hordei* exhibit a longer latency period (LP), smaller and fewer uredinia, and less spore production than susceptible cultivars (Parlevliet 1975).

The objectives of this work were to identify new sources of resistance against *P. hordei* in Spanish barley germplasm and to study the mechanisms of resistance.

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Material and Methods

Field studies

A collection of 418 spring barley accessions, kindly provided by the Centro de Recursos Fitogenéticos (CRF), INIA, Spain was screened for rust resistance under field conditions. The seeds were sown in November 2002 in a randomized complete block design with three replicates at the CIFA-IFAPA experimental station at Córdoba, Spain. Each line was represented by 25–30 seeds in a one meter row per replicate. The very susceptible line L94 was grown in a single, long row perpendicular to the rows of the tested accessions to act as a rust spreader. Vada, which has a high partial resistance, was sown every twenty rows as a check.

An isolate of leaf rust (*Puccinia hordei*) was collected from barley at Córdoba, Spain, from which a monospore culture (CO-01) was derived (virulence/avirulence factors *Rph1,2,4,6,8,9,12*/*3,5,7*), and used across the experiments. Leaf rust occurs too infrequently in the area of study to rely on natural infection, so infection in the field was ensured by 1) incubating inoculated seedlings of L94 in a greenhouse and then transferring them to the field near the spreader plants and 2) inoculating spreader rows at growth stage DC 43 (Zadoks *et al*. 1974) by dusting a mixture of urediospores and talcum powder.

Disease severity (DS) was estimated twice during the growing season as the percentage of leaf area covered by the rust uredinia.

Seedling studies

Six of the most resistant accessions (DS < 30%) were selected for further studies on components of resistance (Niks and Rubiales 1994). Seeds of the selected accessions were sown in soil in plastic trays (35 × 20 × 8 cm) with three replicates of three plants each. In each box six accessions plus Vada and L94 were included. Eleven days after sowing, the first leaf of each plant was stapled in place in a horizontal position and inoculated with isolate CO-01 of *P. hordei* in a settling tower by dusting a mixture of freshly collected spores and talcum powder (1 : 10, v/v). Each tray was inoculated with 3 mg of spores that resulted in about 180 spores/cm^2 deposition. The inoculated plants were kept in a dark inoculation chamber for 12 hours at 20°C with a relative humidity of about 100%. Plants were then transferred to a growth chamber at 20°C and white fluorescent light (12-h light/12-h dark).

The components of resistance measured in this experiment were: Infection type (IT), latent period (LP) and infection frequency (IF). IT was recorded 12 days after inoculation following the 0–9 scale of McNeal *et al.* (1971). LP was determined daily by counting the number of uredia visible in a marked area (2–3 cm^2), using a 6× pocket lens. The LP_{50} was calculated as the time from the inoculation to the time at which 50% of the uredia had appeared (Parlevliet 1975). The final number of uredia was used to determine the IF calculated as number of uredia/cm^2. LP and IF were expressed as relative latency period (RLP) and relative infection frequency (RIF) by converting actual values to a percentage of the L94 values.

Histological studies

Five days after inoculation, a central leaf segment (2 cm^2 per plant) was collected. Leaves were fixed and cleared by boiling for 1.5 min in lactophenol/ethanol (1 : 2 v/v) and stored overnight in this mixture at room temperature. Segments were then washed with 50% ethanol for 30 min, with 0.05 M NaOH for 30 min, rinsed three times in water (10 min each), and soaked in 0.1 M Tris/HCl buffer (pH 8.5) for 30 min. Leaf segments were then stained with 0.1% solution of Uvitex 2B in the 0.1 M Tris/HCl buffer. Segments were rinsed four times with water, immersed in a solution of 25% glycerol a minimum of 30 min., and examined at 100× magnification with Leica epifluorescence equipment (DM LB, 330 to 380 nm wave length transmission).

At least 100 infection units were observed per leaf segment and classified according to stage of development. Quantitative analyses of phases of the infection process of *P. hordei* in barley beyond appressorium formation showed that the reduced infectibility of partially resistant barley seedlings is a result of significant early abortion of colonies. Early abortion occurs at about the time of first haustoria formation, when the young colonies have formed up to five or six haustorial mother cells (Niks 1982). Since the fluorescence staining method does not permit observation of the haustoria, early aborted colonies were then defined as individuals that formed a primary infection hypha and not more than six haustorial mother cells. Those colonies that formed more than six haustorial mother cells were classified as established colonies. Colony size (CS) was estimated by randomly selecting 20 established colonies and measuring the length (L) and the width (W). CS was calculated using the formula for elliptic surfaces $CS = \pi LW/4$.

Gene postulation

Accessions with low IT were inoculated in the seedling stage with five isolates of *P. hordei* representing a wide virulence range. The virulence/avirulence factors and the origin of the isolates used in the experiment are shown in Table 1. The inoculation was carried out as described above. Only IT was recorded, considering IT 0–6 indicative of resistance and IT 7–9 of susceptibility. Inoculation with each isolate was carried out on different days, to reduce the risk of cross-contamination of the isolates.

Data analysis

Analysis of variance was calculated by using the PROC GLM in SAS (SAS Institute 1988). Comparisons between lines were made by the Duncan test.

Results

Reaction in the field

The susceptible check L94 showed 87% DS, and the partially resistant cv. Vada only 12% DS (Fig. 1). DS in

Table 1. Reaction of seedlings of selected barley accessions to five isolates of *Puccinia hordei*

Accession	Isolates				
	CO-01[1)]	CO-04[2)]	AL-02[3)]	1.2.1[4)]	RI-04[5)]
BGE008930	7[6)]	9	7	7	7
BGE011198	7	9	7	8	7
Estate (*Rph3*)	6	9	7	6	9
Magnif (*Rph5*)	2	9	8	9	6
Cebada Capa (*Rph7*)	1	8	4	2	2
Trumpf (*Rph12*)	9	8	9	6	9
L94	9	9	9	9	9

1) Isolate CO-01 was collected in Spain, virulent/avirulent on *Rph1,2,4,6,8,9,12/3,5,7*.

2) Isolate CO-04 was collected in Spain, virulent/avirulent on *Rph1,2,3,4,5,6,7,8,9,12*.

3) Isolate AL-02 was collected in Spain, virulent/avirulent on *Rph1,2,3,4,5,6,8,9,12/7*.

4) Isolate 1.2.1 was collected in Holland, virulent/avirulent on *Rph1,2,4,5,6,8,9/3,7,12*.

5) Isolate RI-04 was collected in Denmark, virulent/avirulent on *Rph1,2,3,4,6,8,9,12/5,7*.

6) Infection Type according to the 0–9 scale of McNeal *et al.* (1971) where 0–6 indicates resistant and 7–9 susceptible.

barley accessions ranged from very high to very low, and the frequency distribution was markedly skewed towards high DS. Only 27 accessions displayed DS < 40%. Six of them, with DS ≤ 30% (1.4% of the collection), were selected to study their reaction to leaf rust at the seedling stage (Table 2).

Macroscopic observations

Five of the six tested accessions showed a significantly longer relative latency period (RLP) than L94 and were similar to Vada (Table 2). The relative infection frequency (RIF) of the selected accessions was significantly lower than on L94 and was similar to Vada. The increase in the LP and reduction in IF was particularly marked in accessions BGE008930 and BGE011198 which showed a compatible interaction (IT ≥ 7), but with infection type slightly lower than in the remaining accessions, indicating that in these two accessions uredia were associated with some chlorosis.

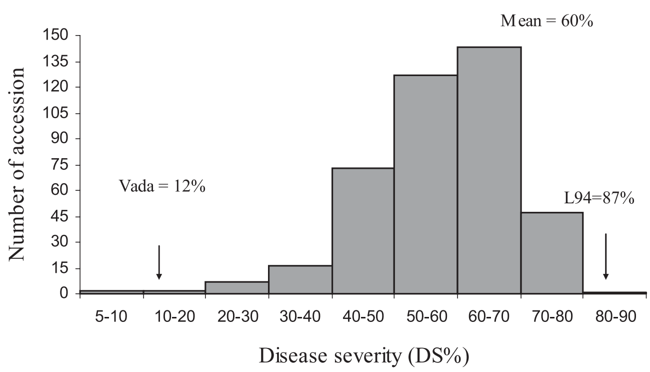

Fig. 1. Frequency distribution of the 418 barley accessions according to the leaf rust disease severity in the field.

Table 2. Macroscopic components of resistance to leaf rust (*Puccinia hordei*) isolate CO-01 in selected accessions of Spanish barley

Accessions	Seedlings in growth chamber			Adult plants in the field (DS%)[3)]
	IT[1)]	RLP[2)]	RIF[2)]	
BGE007942	9	112cd[4)]	60b	15cd
BGE008930	7	204a	6d	5d
BGE009089	9	115c	58b	20bc
BGE009139	9	121c	75b	27b
BGE010359	9	121c	69b	27b
BGE011198	8	160b	39c	8cd
Vada	9	121c	65b	12cd
L94	9	100d	100a	87a

1) Infection type (IT) according to the 0–9 scale of McNeal *et al.* (1971).

2) Relative latency period (RLP) and relative infection frequency (RIF) referred to L94 = 100%. The actual values for L94 were 137 h (latency period) and 72 pustules per cm^2 (infection frequency).

3) Estimated as the percentage of leaf area covered by uredia.

4) Data with the same letter per column are not statistically different (Duncan, $P < 0.05$).

Microscopic observations

The percentage of early abortion associated with plant cell necrosis (EA+) was markedly high in accessions BGE008930 and BGE011198 (Table 3). Also some of the established colonies in these two accessions were associated with cell necrosis. The percentage of early aborted colonies not associated with plant cell necrosis (EA−) was higher in all tested accessions than in L94, although in none was as high as Vada. The average colony size in all the selected accessions was smaller than in L94, but only BGE0089030 and BGE11198 were significantly smaller than Vada.

Gene postulation

Table 1 shows the infection types of the selected barley accessions and L94 to five isolates of *P. hordei*. Reaction of the accessions to the five isolates did not allow precise identification of the *Rph* gene(s) present but allowed us to discern that the resistance gene(s) responsible for the hypersentivity is something other than *Rph3*, *Rph5*, *Rph7* or *Rph12*.

Discussion

Breeders have often used monogenic, race-specific, hypersensitive resistance in their breeding programmes. In most cases, new races with increased virulence to the new genes develop rapidly after the introduction of resistant cultivars. Therefore, it is important to work with other types of resistance, such as the partial resistance, which is more likely to be durable.

Under field conditions, the reduction of the epidemic build-up of the disease was not common in the collection, which agrees with the results previously obtained by Martínez *et al.* (2001). The results also indicate that none of

Table 3. Classification of colony development and measurement of final colony size based on microscopic evaluation of *Puccinia hordei* growth in seedlings of selected Spanish barley accessions

Accessions	Development stage[1)]				Colony size (mm^2)
	EA+	EA−	EST+	EST−	
BGE007942	0c[2)]	14.3bc[2)]	1.4c[2)]	84.3b[2)]	0.188b[2)]
BGE008930	68.5a	11.8bc	19.5a	0.2e	0.038f
BGE009089	0.1c	11.1cd	0c	88.8b	0.156c
BGE009139	0c	15.7bc	0c	84.3b	0.163c
BGE010359	0c	17.9b	0c	82.1bc	0.122d
BGE011198	41.1b	7.8d	15.9b	35.2d	0.096e
Vada	0c	26.3a	0c	73.7c	0.132d
L94	0c	0e	0c	100a	0.235a

1) Expressed are percentage of early aborted colonies associated with plant cell necrosis (EA+), percentage of early aborted colonies without plant cell necrosis (EA−), percentage of established colonies associated with plant cell necrosis (EST+), percentage of established colonies without plant cell necrosis (EST−) and mean colony size in mm^2.

2) Data with the same letter per column are not statistically different (Duncan, $P < 0.05$).

the selected accessions have a level of partial resistance higher than that of Vada. Parlevliet and van Ommeren (1984) found that partial resistance may show large variation between experiments and locations. In a collection of modern European barleys, Niks *et al.* (2000) found that many of the accessions have a level of partial resistance that is equal to or higher than Vada. They also found a high correlation in percentage of early abortion without plant cell necrosis at the seedling and adult plant stage. Through association mapping, Niks *et al.* (2001) reported that the high level of partial resistance detected in European barley cultivars was probably due to three QTLs previously reported by Qi *et al.* (1998). The infection frequency was lower for the partially resistant accessions than for the susceptible L94 and was similar to the partially resistant Vada. Parlevliet and Kuiper (1977) found a high correlation between infection frequency in the seedling stage and partial resistance in the field.

The high level of early aborted colonies associated with plant cell necrosis (EA+) in the accessions BGE008930 and BGE011198 (Table 3), together with their moderate level of established colonies associated with plant cell necrosis (EST+), suggest the presence of hypersensitive resistance genes. This level of hypersensitivity is, however, not detectable macroscopically as a great reduction in IT, but results in a high IT (IT 7–8) slightly lower than that of L94 and Vada (IT 9). It was not possible to postulate the *Rph* resistance genes present in these accessions with the isolates used. A more complete set of isolates covering the whole virulence pattern would be needed. We conclude that the hypersensitive reaction of these selected accessions was not due to *Rph3*, *Rph5*, *Rph7* and *Rph12* resistance genes.

Histological studies can help both to discern the various resistance mechanisms and to combine them in a genotype in the hope to increase durability. Histology allows us also to unravel levels of prehaustorial resistance in the presence of hypersensitive resistance that might not be macroscopically detected. In genotypes giving a hypersensitive reaction, the presence of early aborted colonies not associated with host cell necrosis is a good indicator of the level of partial resistance (Niks and Kuiper 1983). In addition to its slight level of hypersensitivity, accession BGE008930 carries levels of PR similar to other accessions as indicated by the level of early aborted colonies without necrosis (11.8%) similar to those of BGE007942, BGE009089, BGE009139 and BGE010359.

From this study we conclude that the components that best discriminate between susceptible and partially resistant cultivars in the seedling stage were differences in LP, the amount of early aborted colonies without host cell necrosis, and the infection frequency. These results agree with those reported by Niks (1982). From our results we can conclude that the level of partial resistance in the Spanish germplasm is not high. Accessions BGE009089, BGE009139 and BGE010359 may be used as additional sources of partial resistance to leaf rust. Combining the partial resistance, through intermating or crossing with other partially resistant varieties, may lead to increased levels of partial resistance. Additional isolates of *P. hordei* with different virulence patterns need to be examined to postulate the resistance genes in accessions BGE008930 and BGE11198.

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