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 racespecific, 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.

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
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/cm² 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 cm²), using a 6× pocket lens. The LP₅₀ 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/cm². 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 cm² 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>
<thead>
<tr>
<th>Accession</th>
<th>Isolates</th>
<th>1.2.4 (IT)</th>
<th>RI-04 (IT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGE008930</td>
<td>7[4]</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>BGE011998</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Estate (Rph3)</td>
<td>6</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Magnif (Rph5)</td>
<td>2</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Cebada Capa (Rph7)</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Trumpf (Rph12)</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>L94</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

1) Isolate CO-01 was collected in Spain, virulent/avirulent on *Rph1,2,4,6,8,9,12/3,5,7,9,12*.  
2) Isolate CO-04 was collected in Spain, virulent/avirulent on *Rph1,2,3,4,5,6,8,9,12*.  
3) Isolate AL-02 was collected in Spain, virulent/avirulent on *Rph1,2,3,4,5,6,8,9,12*.  
4) Isolate AL-02 was collected in Spain, virulent/avirulent on *Rph1,2,3,4,5,6,8,9,12*.  
5) Isolate RI-04 was collected in Denmark, virulent/avirulent on *Rph1,2,3,4,5,6,8,9,12*.  
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 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 reduction in IF was partic-

ularly marked in accessions L94 and was similar to Vada (Table 2). The increase in the relative latency period (RLP) than L94 and was similar to Vada (Table 2). 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.

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

<table>
<thead>
<tr>
<th>Accessions</th>
<th>Seedlings in growth chamber</th>
<th>Adult plants in the field (DS%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGE007942</td>
<td>112c</td>
<td>60b</td>
</tr>
<tr>
<td>BGE008930</td>
<td>204a</td>
<td>6d</td>
</tr>
<tr>
<td>BGE009089</td>
<td>115c</td>
<td>58b</td>
</tr>
<tr>
<td>BGE009139</td>
<td>121c</td>
<td>75b</td>
</tr>
<tr>
<td>BGE010359</td>
<td>121c</td>
<td>69b</td>
</tr>
<tr>
<td>BGE011198</td>
<td>160b</td>
<td>39c</td>
</tr>
<tr>
<td>Vada</td>
<td>121c</td>
<td>65b</td>
</tr>
<tr>
<td>L94</td>
<td>100d</td>
<td>100a</td>
</tr>
</tbody>
</table>

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 cm² (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).

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

<table>
<thead>
<tr>
<th>Accessions</th>
<th>Development stage</th>
<th>Colony size (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGE007942</td>
<td>0.2c</td>
<td>0.122d</td>
</tr>
<tr>
<td>BGE008930</td>
<td>68.5a</td>
<td>84.3b(2)</td>
</tr>
<tr>
<td>BGE009089</td>
<td>0.1c</td>
<td>88.8b</td>
</tr>
<tr>
<td>BGE009139</td>
<td>0c</td>
<td>84.3b</td>
</tr>
<tr>
<td>BGE010359</td>
<td>0c</td>
<td>82.1bc</td>
</tr>
<tr>
<td>BGE011198</td>
<td>41.1b</td>
<td>0.096c</td>
</tr>
<tr>
<td>Vada</td>
<td>0c</td>
<td>73.7c</td>
</tr>
<tr>
<td>L94</td>
<td>0c</td>
<td>100a</td>
</tr>
</tbody>
</table>

1) Expressed as 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 mm².
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 BGE0111198 (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 interjecting 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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Literature cited


Resistance to leaf rust in Spanish barleys


