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Search for partial resistance against *Puccinia hordei* in barley landraces from the Fertile Crescent

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With 2 figures and 4 tables

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Abstract

A collection of 111 barley landraces from the Fertile Crescent was screened for resistance to barley leaf rust in the field and under controlled conditions. Large variation was observed for disease severity under field conditions. Accessions with high resistance because of hypersensitivity were identified. Also segregation was observed in some accessions, with individual plants showing hypersensitive reactions (IT ≤ 6). Partial resistance due to a reduction of infection in spite of a compatible infection was commonly found (19%). Resistance of 12 accessions selected for their low disease severity and high IT, was shown to be due to a prolonged latency period and increased percentage of early aborted colonies not associated with host cell necrosis. A high correlation was observed between the microscopic and macroscopic components of partial resistance.

Key words: *Hordeum vulgare* — *Puccinia hordei* — barley — landraces — leaf rust — histology

Barley leaf rust caused by the fungus *Puccinia hordei* Otth, is an important foliar disease in temperate regions throughout the world. The use of resistant barley cultivars has been an effective method to control the disease and to reduce yield losses, which may reach 40% in the susceptible cultivars (Griffey et al. 1994).

Evaluations of the *Hordeum* gene pool (*H. vulgare*, and its wild progenitor *H. vulgare* ssp. *spontaneum*) have resulted in the identification of 19 major race-specific resistance genes named *Rph1-Rph19* (Weerasena et al. 2004). However, only a few of these genes have been deployed in commercial cultivars. Genes *Rph2, Rph3, Rph4, Rph7* and *Rph12* have been used in Europe (Dreiseitl and Steffenson 2000), whereas, *Rph2*, *Rph6*, and *Rph7* have been deployed in the United States (Steffenson et al. 1993). The resistance controlled by these genes is not durable and is assumed to operate on a gene-for-gene basis with avirulence factors in the pathogen population. Virulence to *Rph3* and *Rph9* has been identified in Europe, South America and the Middle East (Brooks et al. 2000). *Rph7* was believed to be effective in Europe (Niks et al. 2000), but virulence to it has recently been identified in Spain (Shtaya, unpublished) and has been reported in Morocco (Parlevliet 1976), Israel (Golan et al. 1978, Brodny and Rivadeneira 1996), and the United States (Steffenson et al. 1993).

This short life of resistance has caused breeders to look for other types of resistance such as partial resistance which appears to be more durable and race-non-specific (Parlevliet and Van Ommeren 1975).

The objectives of this study were to screen a collection of barley landraces from its centre of origin (the Fertile Crescent) looking for new sources of durable resistance to barley leaf rust and to study their components of resistance.

Materials and Methods

Plant materials: Seed samples of 111 *Hordeum vulgare* landraces from the Fertile Crescent were kindly provided by the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, and United States Department of Agriculture (USDA), USA (Table 1).

Inoculum: A monosporic isolate (CO-01) with virulence/avirulence factors *Rph1,2,4,6,8,9,12/3,5,7* was derived from a *Puccinia hordei* population collected at Córdoba, Spain on barley fields. The isolate was maintained in liquid nitrogen, and multiplied on susceptible barley line L94 before being used across all the experiments.

Field experiment: Field testing was performed at the CIFA experimental farm at Córdoba, Spain during the growing season 2003–2004. Accessions were sown in November 2003 in three complete randomized blocks. Each accession was represented by 25–30 seeds in a single row, 1 m long per replicate. A spreader row, of the very susceptible line L94, was sown in the alleyways, perpendicular to the tested accessions. Leaf rust epidemic was initiated by artificial inoculation of the spreader rows at growth stage DC 43 (Zadoks et al. 1974) by dusting a mixture of urediospores and talcum powder. Disease severity (DS) was at the end of the growing season as the percentage of leaf area covered by the rust uredinia.

Seedling studies: In a first experiment, DS and infection type (IT) of all 111 accessions were studied in the seedling stage under controlled conditions. About 12–15 seedlings per accession were grown in $7 \times 7 \times 11$ cm pots in three replicates. The inoculation was carried out by dusting fresh urediospores of isolate CO-01, diluted 10 times with talcum powder, over the seedlings when the second leaf of the seedlings had emerged. After inoculation, seedlings were incubated overnight in complete darkness and at a relative humidity of 100%. Seedlings were then transferred to a growth chamber at 18–22°C and white fluorescent light (12 h light/12 h dark).

Twelve days after inoculation, DS was estimated as the percentage of the first leaf area covered by the rust uredinia. Infection type (IT) on a 0–9 scale (McNeal et al. 1971) was also recorded on the first leaf.

In a second experiment, components of resistance of 12 selected accessions were determined. Selected accessions were those that showed a disease severity $<15\%$ in the field and compatible interaction (IT ≥ 7) in the seedling stage (first experiment). Seeds of the selected accessions were sown in soil in plastic trays ($35 \times 20 \times 8$ cm) with three replicates of three plants each. In each tray, eight accessions plus 'Vada' (high level of partial resistance) and

Table 1: Origin and source of the barley landraces used in this study

Origin	No. accessions	Source
Israel	4	USDA
Jordan	29	ICARDA + USDA
Lebanon	15	ICARDA
Palestinian Territory	23	ICARDA + USDA
Syria	40	ICARDA

L94 (very susceptible) checks were included. Eleven days after sowing, the first leaf of each plant was placed in a horizontal position with the help of metal staples and inoculated with isolate CO-01 of *P. hordei*. The inoculation was carried out 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 200 spores/cm^2 deposition (Niks and Rubiales 1994). The inoculated plants were kept in an inoculation chamber for 12 h at 20°C with a relative humidity of about 100% and in darkness. Plants were then transferred to a growth chamber at 18–22°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 (McNeal et al. 1971). LP was determined by daily 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 the number of uredia/cm^2. The actual LP and IF were converted into relative latency period (RLP) and relative infection frequency (RIF), taking the LP and IF of line L94 as 100% in each box.

Histological studies: Five days after inoculation, a central leaf segment of nearly 2 cm^2 per plant was collected. Leaves were fixed and cleared by boiling for 1.5 min in lactophenol/ethnol (1 : 2, v/v) and stored overnight in this mixture at room temperature. Segments were then washed once with 50% ethanol for 30 min, once 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. They, then were stained with a 0.1% solution of Uvitex 2B in the same buffer. This was followed by rinsing four times with water. Segments were then immersed in a solution of 25% glycerol for a minimum of 30 min (a few drops of lactophenol were added to the solution to prevent deterioration by fungi) and stored until observed. Leaf segments were examined at 100 × with Leica epifluorescence equipment (DM LB, 330–380 nm wave length transmission). At least 100 infection units were evaluated per leaf segment, and classified according to their stage of development (Martínez et al. 2001). Early aborted colonies were 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 calculating the length (L) and the width (W) of 20 colonies. These colonies were randomly chosen. CS was calculated using the formula:

$$CS = \frac{\pi LW}{4}.$$

Data analysis: Analysis of variance (ANOVA) was calculated by using PROC GLM in an SAS program (SAS Institute 1988). Comparisons between lines were made by the Duncan-test.

Results

Under field conditions, the susceptible check L94 showed 47% DS and the partially resistant cv. 'Vada' only 4% DS (Fig. 1). High susceptibility was common in the collection with seven accessions being even more susceptible than L94. DS was < 15% in 21 accessions. Nine of these accessions with low DS in the field, showed incompatible infection (low IT). However, 12 accessions showed compatible infection (high IT) and low severity in the seedling test and in the field (Table 2).

In seedling tests, most of the accessions (92.8%) displayed compatible interaction (IT ≥ 7). Segregation was observed in eight accessions, with individual plants showing low IT. The susceptible check L94 showed 33% DS and the partially resistant cv. 'Vada' 15% (Fig. 2).

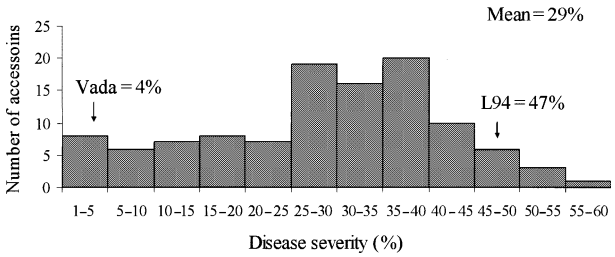

Fig. 1: Frequency distribution of the accessions according to the disease severity of *Puccinia hordei* in the field

Table 2: Macroscopic components of resistance to leaf rust (*Puccinia hordei*) isolate CO-01 in selected accessions of barley landraces from the Fertile Crescent in the seedling stage and in the field

Accessions	Seedlings in growth chamber				Adult plants in the field (DS)
	IT[1]	RLP[2]	RIF[2]	DS[2]	
IG31396	9	117ab[3]	46bc	15bc	14b
IG32710	9	122ab	70abc	20b	11b
IG32733	9	118ab	77ab	16bc	10b
IG32747	9	122ab	69abc	16bc	13b
IG110861	9	124ab	52bc	20b	13b
IG110870	8	131a	28c	17bc	12b
IG110887	9	126ab	58abc	9c	5b
IG110906	8	127ab	28c	19b	4b
IG115774	9	114b	67abc	19b	9b
IG125768	9	117ab	53bc	20b	14b
IG125775	9	118ab	68abc	22b	10b
PI186425	9	118ab	61abc	21b	8b
'Vada'	9	123ab	51bc	15bc	4b
L94	9	100c	100a	33a	47a

[1]IT on a scale of 0–9 (McNeal et al. 1971).
[2]Relative latency period (RLP) and relative infection frequency (RIF) referred to L94 = 100%. The actual values for L94 were LP = 141h and IF = 70 pustules/cm^2. DS = disease severity.
[3]Data with the same letter per column are not statistically different (Duncan-test, P = 0.05).

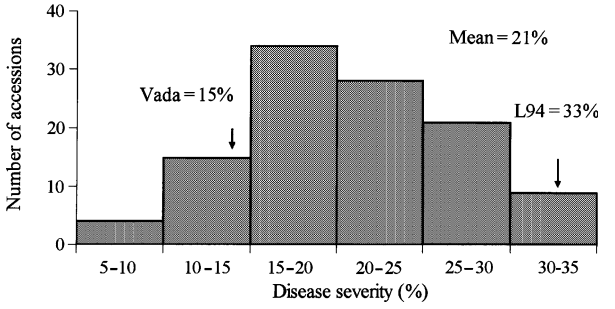

Fig. 2: Frequency distribution of the accessions according to the disease severity of *Puccinia hordei* in climatic room (seedlings)

Table 3: Microscopic components of resistance to *Puccinia hordei* in selected accessions of barley landraces in the seedling stage

Accessions	% of early aborted colonies		% of established colonies		Colony size (CS)[1]
	EA + [1]	EA− [1]	EST + [1]	EST− [1]	
IG31396	2a[2]	44ab	0.3b	53.7bc	0.076bcd
IG32710	0.8abc	22b	0.8 b	76b	0.080bc
IG32733	0c	29ab	0b	71bc	0.072bcd
IG32747	0.5bc	27ab	0b	72.5bc	0.081bc
IG110861	0c	48a	0b	52c	0.041e
IG110870	0c	28ab	9a	63bc	0.068bcde
IG110887	0.7abc	26ab	1b	72.3bc	0.059cde
IG110906	0c	37ab	0b	63bc	0.061bcde
IG115774	0c	23b	0b	77b	0.091b
IG125768	0c	29ab	0b	71bc	0.065bcde
IG125775	1.7ab	28ab	0b	70.3bc	0.078bc
PI186425	0c	39ab	0b	61bc	0.060bcde
Vada	0c	42ab	0b	58bc	0.048de
L94	0c	2c	0b	98a	0.132a

[1]Expressed are percentage of early aborted colonies associated with host cell necrosis (EA +), percentage of early aborted colonies without host cell necrosis (%EA−), percentage of established colonies with host cell necrosis (EST +) and percentage of established colonies without host cell necrosis (% EST−) and mean colony size in mm^2 (CS).
[2]Data with the same letter per column are not statistically different (Duncan-test, P = 0.05).

Table 4: Correlation coefficients (r) among traits in the selected accessions of barley landraces

Traits	DS (seedlings)	DS (field)	RLP (seedlings)	RIF (seedlings)
DS (seedlings)				
DS (field)	0.74*			
RLP (seedlings)	−0.67*	−0.75*		
RIF (Seedlings)	0.95*	0.54**	−0.69*	
EA− (seedlings)	−0.35***	−0.63*	0.56**	−0.74*

DS, disease severity; RLP, relative latency period; RIF, relative infection frequency.
*P = 0.001; **P = 0.01; ***P = 0.1

Macroscopic results of the selected accessions with low DS in the field and high IT in seedlings are shown in Table 2. All the selected accessions showed longer latency periods (higher RLP) than the susceptible check L94, and were similar to the partially resistant 'Vada'. RIF of these accessions was lower than that of L94, although not always significantly so, and always similar to that of Vada. A strong negative correlation was observed between RLP and RIF, and between RLP at the seedling and DS in both seedlings and adult plants in the field (Table 4).

Percentages of early aborted or established colonies associated with host cell necrosis was very low in all selected accessions and checks. All selected accessions had a percentage of early aborted colonies not associated with host cell necrosis (%EA-), higher than the susceptible check L94 and similar to the partially resistant 'Vada' (Table 3). Also, all selected accessions showed smaller colony sizes (CS) than the susceptible check. High correlation was observed between the EA- at seedling stage and RLP at the seedling stage (Table 4).

Discussion

In most European countries, landraces of major crops exist only in gene banks (Ceccarelli et al. 2000), but in some areas of West Asia and North Africa farmers still rely on landraces for major crops such as barley. Barley is a typical self-fertilizing annual crop and landraces of such crops are expected to consist of more or less homozygous plants. For barley, the relatively high number of seeds needed per ha, makes the conscious selection of specific plants by the farmer barely possible. It also means that seeds from a very large number of plants are used for the next crop. Under these conditions a very low frequency of cross-pollination and incidental survival of volunteer plants from another landrace of a previous sowing could easily lead to the low level of within-landrace variation observed in infection type (IT) seen here. Fekadu and Parlevliet (1997) found large variation within and between Ethiopian barley landraces for many quantitative traits, including partial resistance.

Level of partial resistance in the collection is high since most of the accessions had a degree of rust reduction, whereas hypersensitive resistance was observed only in 8% of the accessions. Levels of partial resistance in some of the selected accessions were similar to those of 'Vada'. However, Niks et al. (2000) found that modern European barley cultivars have a higher level of partial resistance than old cultivars. This accumulation of partial resistance was due to breeding programmes and selection methods for partial resistance.

The high level of partial resistance of the selected accessions, observed in the seedling stage, was due to a reduced infection frequency and prolonged latency period. Microscopically, this was found to be associated with a high level of early aborted colonies not associated with cell necrosis (EA-) and reduced colony size, in agreement with Parlevliet and Van Ommeren (1975), Niks (1982) and Statler and Parlevliet (1987).

From our results we can conclude that barley landraces from the Fertile Crescent represent a substantial reservoir for partial resistance, which can be used in breeding programmes for partial resistance accumulation. Additional genetic studies should be conducted to determine the relationship between the resistance gene(s) in the selected landraces, with low infection type together with their relationships with other known major resistance genes.

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