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Partial resistance to leaf rust in a collection of ancient Spanish barleys

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A collection of 569 Spanish barley accessions was screened for resistance to leaf rust (*Puccinia hordei* Otth) in the field at Córdoba during the 2000–2001 season. The level of resistance ranged from very low to very high. In 14 % of the accessions the relative AUDPC (L94 = 100 %) was lower than 10 %. Selected accessions that were most resistant in the field, were tested in the seedling stage under controlled conditions. Macroscopic components of resistance indicated that six lines had a high level of partial resistance close to check cv. Vada and one line a similar level of partial resistance. Histological studies indicated that the resistance was based on a high percentage of early aborted colonies and reduction in colony size without plant cell necrosis. Three of the selected lines showed high percentage of plant cell necrosis associated with established colonies, which indicates a combination of prehaustorial resistance with late acting incomplete posthaustorial resistance. Although the new barley varieties already incorporate some partial resistance, new sources of partial resistance like these are needed to improve durability of the resistance.

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Barley leaf rust, caused by *Puccinia hordei* Otth, is an important disease of barley (*Hordeum vulgare* L.) worldwide. Genetic resistance in the host is the best way to control this disease. Breeders usually rely on hypersensitive resistance that is governed by major genes and is race-specific. This resistance is associated with plant cell necrosis around the infection site (low infection type). This type of resistance has been widely exploited by breeders because of its monogenic nature, but the pathogen is able to overcome this kind of resistance soon after cultivars carrying the resistance are grown at large scale. All genes for hypersensitive resistance in *Hordeum vulgare*, including *Rph7* in the USA, have been defeated by virulent isolates of the pathogen (GRIFFEY et al. 1994).

There is an increasing awareness for the need to search for more durable types of resistance, such as partial resistance. Partial resistance is characterised by a reduced rate of epidemic development despite a susceptible (high) infection type (IT) (PARLEVLIET and VAN OMMEREN 1975). One of the most interesting aspects of partial resistance is its high stability in different environments and its apparent durability (PARLEVLIET 1975).

Landraces may have fair levels of partial resistance. Farmers have made an unconscious selection against extreme susceptibility generation after generation. Natural selection could also take place. The pathogen affects the vigour of their host plant, and hence its rate of reproduction so it is likely that during the long time of coexistence of pathogen and plant population, natural selection has favoured plant genotypes with an adequate level of resistance.

In this paper a collection of 569 ancient Spanish barley lines was screened for resistance to leaf rust in order to determine the level and type of resistance in the collection and to study the mechanisms of the lines with the highest level of partial resistance.

MATERIAL AND METHODS

Field experiment

A collection of 569 lines of spring barley (with a high amount of landraces), kindly provided by the Centro de Recursos Fitogenéticos (CRF), INIA, Spain was studied for levels of partial resistance to leaf rust. Information about lines can be found in the CRF internet website http://www.crf.inia.es/.

The collection was grown in the field at Córdoba in November 2000. Each line was represented by a 1 meter long single row. A susceptible line, L94, was placed adjacent to each line and used as spreader of the disease. A hypersensitive resistant line (L94 + *Rph7*) and several partially resistant checks (Vada, 116-5, C123 and 17.5.16) were repeated placed across the plots. Artificial inoculation was done using a monopustule-derived isolate collected at Córdoba (avirulence/virulence: *Rph3*, *Rph5*, *Rph7*, *RphC*, *RphD*/*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph12*). Inoculation was done in two ways: transferring

sporulating L94 plants inoculated in the greenhouse to the field one month after sowing and dusting over the spreader rows a mixture of urediospores diluted in talcum powder.

Disease severity (%) was assessed three times and AUDPC (area under disease progress curve) was calculated and converted to a value relative to the AUDPC of the susceptible check (L94 = 100 %).

Macroscopic observations

The lines showing AUDPC lower than 20 % of the AUDPC on L94 were selected. Their IT was evaluated at seedling stage in a growth chamber. Five plants per line were grown in pots (7 × 7 × 9 cm) and inoculated with the same monopustule isolate as used in the field trial, mixed with talcum powder (1:10 v/v). Plants were incubated 24 hours in darkness and relative humidity at saturation. The next day, plants were transferred to a compartment at 20°C and 14 hours photoperiod. Infection type was scored 12 days after inoculation according to a 0–9 scale (McNeal et al. 1971).

Forty-five lines with a high IT in seedling stage and low AUDPC in the field were selected in order to measure their macroscopic components of resistance at the seedling stage. Latency period was determined by daily counting the number of uredia visible in a marked area (around 2.5 cm^2). The latency period was taken as the time period from the beginning of incubation to the time at which 50 % of the uredia had appeared (Parlevliet 1975). Infection frequency was determined in the marked area as the final number of uredia per cm^2. Plants were grown in soil in plant boxes (35 × 20 × 8 cm). Ten lines with five plants each were included in each box. Susceptible L94, and partially resistant check Vada, were also included in each box. About eleven days after sowing, the first leaves were fixed in a horizontal position and inoculated in a settling tower. Each box received three mg of spores mixed with talcum powder (1:10 v/v), which resulted in a spore deposition of about 180 spores per cm^2. Lines with the highest level of resistance and with high IT were selected to confirm the results in a similar seedling experiment in three simultaneous replications, and to determine the histological aspects of their resistance.

Microscopic observations

Five days after inoculation three middle segments of 1 to 3 cm^2 per replication and per genotype were collected from the infected leaves of the experiment mentioned above. Three replications were carried out. Leaf segments were prepared for fluorescence microscopy (Rohringer et al. 1977), but instead of Calcofluor we used Uvitex 2B (Ciba-Geigy). The preparations were examined at 100× with a Leica epifluorescence microscope (DM LB, 330 to 380 nm wave length transmission). The colonies were scored and classified according to their stage of development (Niks 1982). Colonies that developed a germ tube and not an appressorium over a stoma were ignored. We defined early aborted colonies as individuals that formed a primary infection hypha and not more than six haustorial mother cells. Colonies that had developed more haustorial mother cells were classified as established. 100 infection units were classified per leaf segment. A filter with 420 to 490 nm transmission was used to observe necrosis of host cells, which display a golden yellow autofluorescence. The length (L), and width (W) of ten arbitrarily chosen established colonies per leaf were measured with an eyepiece micrometer. Colony size (CS) was calculated as the geometric mean of L and W, $CS = SQRT(\frac{1}{4} \times L \times W)$. The statistical analysis of the different percentages was performed on arc sin-transformed data if appropriate.

RESULTS

Resistance levels in the field

Susceptible check L94 reached a disease severity of 60 %. There was a continuous variation in the AUDPC of the Spanish barley lines (Fig. 1). About 24 % of the lines displayed an AUDPC lower than 20 %, relative to the AUDPC on L94 (= 100 %). Twenty-two lines (3.8 %) showed a relative AUDPC of less than 2%, which is about as low as the hypersensitive check *L94 + Rph7* (1.1%) and the partially resistant Vada (1.6 %), 116-5 (3.9 %) and 17.5.16 (0.3 %). High susceptibility was also common, since on 28 % of the lines a relative AUDPC higher than 60 % was observed.

Evaluation for non-hypersensitive resistance

The seventy lines with lowest AUDPC in the field were screened for IT in the seedling stage. Only 20 %

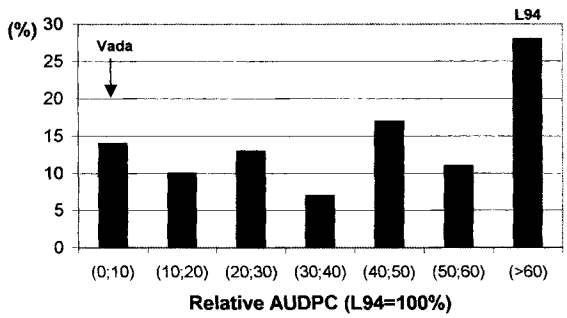

Fig. 1. Distribution of the 569 barley lines according to the relative AUDPC of *Puccinia hordei*.

Table 1. *Macroscopic components of resistance to Puccinia hordei in selected lines of Spanish barleys*

Lines	Seedling			Field
	IT	RLP[a]	RIF[a]	AUDPC
L94	9	100 a[b]	100 a[b]	100
BGE007851	9	115 b	55 cd	8.7
BGE007967	9	117 bc	71 bc	3.9
BGE008927	7-8	114 b	71 ab	0.7
BGE008943	9	118 bc	70 bc	1.8
BGE009139	9	125 c	65 bcd	1.8
BGE009359	9	114 b	78 ab	1.7
BGE011188	9	118 bc	53 d	1.7
Vada	9	115 b	71 bc	1.6

[a] Latency period (RLP), infection frequency (RIF) and area under disease progress curve (AUDPC) are expressed as values relatives to L94 (= 100 %). The actual average values for L94 were 150 h (latency period) and 68 pustules per cm^2 (infection frequency).
[b] Per column, data with the same letter are not statistically different (Duncan, $p < 0.05$).

of these lines showed a low infection type, suggesting that the high levels of resistance identified in the remaining 80 % of the lines is not due to seedling resistance based on hypersensitivity.

Levels of partial resistance

Forty-five lines were selected on the basis of their high IT and low AUDPC, to study the latency period in the seedling stage. Seven lines with the longest latency period and high IT were selected for further studies.

Macroscopic components in the selected lines

Macroscopic components of the resistance are shown in Table 1. All selected lines showed a high IT in seedling stage. All lines showed a significantly longer relative latency period than L94. Relative latency period on line BGE009139 was even longer than on Vada. On all lines, except BGE008927 and BGE009359 the infection frequency was significantly lower than L94.

Microscopic components in the selected lines

Microscopic components are shown in Table 2. The percentage of early aborted colonies not associated with host cell necrosis on all lines were significantly higher than in L94. This percentage was particularly high in lines BGE007851, BGE009139, BGE011188 and Vada (14 % or higher). Many more of the established colonies in lines BGE007851, BGE008927 and BGE009359 were associated with at least some plant cell necrosis than in the rest of the lines (included L94 and Vada) for which the percentage was very low. In general the necrosis only covered a small part of the colony area. Colony size was significantly greater in L94 than in the rest of the lines (including Vada). In the selected lines, colony size was similar as in Vada. Only line BGE009139 supported significantly smaller colonies than BGE007967, BGE008927, BGE009359 and BGE011188.

DISCUSSION

Partial resistance was defined as a resistance that reduces the epidemic build-up despite a high infection type (PARLEVLIET 1975). Field resistance was common in the collection and was not associated with hypersensitivity in the seedling stage in 80 % of the most resistant lines, suggesting the existence of high levels of partial resistance in the collection. It is reported that landraces of different crops usually build up levels of non hypersensitive resistance to rust rather than high frequencies of hypersensitivity resistance. A collection of tetraploid wheat was screened for resistance to wheat leaf rust (*Puccinia triticina*)

Table 2. *Microscopic components of resistance to Puccinia hordei in selected Spanish barley lines in seedling stage*

Lines	% Early aborted colonies without necrosis[a]	% Colonies with plant cell necrosis[a,b]	Mean colony size (mm^2)[a]
L94	1 d	1 b	0.170 a
BGE007851	14 a	36 a	0.079 bc
BGE007967	9 b	1 b	0.106 b
BGE008927	5 c	56 a	0.097 b
BGE008943	8 bc	1 b	0.083 bc
BGE009139	15 a	2 b	0.062 c
BGE009359	8 bc	53 a	0.110 b
BGE011188	14 a	1 b	0.094 b
Vada	18 a	3 b	0.079 bc

[a] Data with the same letter per column are not statistically different (Duncan, $p < 0.05$).
[b] All infection units associated to host cell necrosis were established colonies (had developed at lest six haustorial mother cells).

and in almost all lines a high IT was found, and in most of them moderate levels of partial resistance (ANDENOW et al. 1997). A near-absence of race-specific, major gene resistance and a relatively high frequency of moderate levels of partial resistance to leaf rust was found in a collection of Ethiopian barley landraces (ALEMAYEHU and PARLEVLIET 1996). We did not study the IT in adult plants, so we cannot exclude the possibility of hypersensitive resistance acting only in the adult plant stage, rarely occurring in barley to leaf rust, but suggested by NIKS et al. (2000).

In the commercial varieties of barley the trend is totally different. Breeders tend to employ genes for hypersensitivity resistance. A collection of modern cultivars of barley from Western Europe were tested for leaf rust resistance with the isolate 24 (with similar virulence as the isolate used by us) and 50 % of the lines displayed a low infection type (NIKS et al. 2000), while in the most resistant landraces in this study the percentage was only 20 %. Only 2 out of 25 varieties tested by NIKS et al. (2000) did not have any *Rph* gene. Similarly it was found that only 2 out of 26 varieties of bread wheat did not have any *Lr* gene (SINGH 1993).

The selected lines had as high level of partial resistance as the partially resistant check Vada. Especially high was the level of partial resistance in lines BGE007851, BGE011188 and BGE009139. On the latter, the rust fungus even displayed a significantly longer latency period and smaller colony size than in Vada, and a similar high percentage of early aborted colonies not associated with plant cell necrosis. Still, in Vada, the level of partial resistance components (latency period and percentage of early aborted colonies) was not as high as reported in earlier studies (PARLEVLIET 1975; NIKS 1982). This may be due to the long incubation used in our study. Recently, evidence was found that an incubation in a dark dew chamber for substantially longer time than natural night length, reduces the effectiveness of genes for partial resistance (NIKS 2002).

Presence of host cell necrosis in established colonies, observed in lines BGE007851, BGE008927 and BGE009359, suggested some late acting hypersensitivity genes with an incomplete expression that did not decrease the IT. Only line BGE008927 showed a slight decrease of the infection type. The percentage of early aborted colonies not associated with cell necrosis is a good indicator of the level of partial resistance (prehaustorial resistance) even in genotypes giving a hypersensitive reaction (NIKS and KUIPER 1983). So we can say that the level of partial resistance in line BGE007851 is similar to Vada while in the other two lines the level is lower.

The partial resistance of landraces is usually durable. ZHANG (1995) reported that the resistance of nine Chinese landraces to stripe rust remained effective for more than 50 years.

New sources of partial resistance to barley leaf rust are available for a breeding programme. It is likely that the partial resistance of the selected lines comes from different combinations of minor genes (on different loci). QI et al. (2000) reported abundance of QTLs for partial resistance to leaf rust on the barley genome. Combining these genes through crosses may lead to transgression and increase of the level o partial resistance (PARLEVLIET and KUIPER 1985). In fact, the new varieties of barley already exhibit higher levels of partial resistance to leaf rust than 20 years ago (PARLEVLIET et al. 1980; NIKS et al. 2000). The presently discovered Spanish lines may be useful additional sources of partial resistance to leaf rust.

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