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Search for partial resistance to leaf rust in a collection of ancient Spanish wheats

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A collection of 917 accessions of Spanish durum and bread wheat was screened for resistance to leaf rust (*Puccinia triticina*) under field conditions at three locations. Resistance levels ranged from very low to very high, high susceptibility being most frequent. Relative disease severity (referred to the most susceptible accession = 100 %) was lower than 20 % in about 6 % of the accessions in each location. In the collection most of the lines (84 %) displayed a susceptible infection type. A final selection of seven accessions (one of them durum) displaying low severity level in the field and high infection type in a growth chamber was chosen for further studies. High levels of partial resistant with longer latency period and high percentage of early aborted colonies without necrosis were found. They might be used in breeding programmes.

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Wheat breeding for leaf rust resistance in modern agriculture has traditionally been based on *Lr* genes for hypersensitive resistance. This approach yielded quick and effective results to control the disease, but pathogen populations commonly were able to overcome the resistance genes by new virulent races. There is an increasing concern about the lack of durability of disease resistance (JOHNSON 1992). Several strategies can be adopted to prolong the durability of the ephemeral *Lr* resistance genes, such as gene pyramiding, diversification and application of cultivar mixtures (MCDONALD and LINDE 2002).

Another approach is the search for different types of resistance that are not based on hypersensitivity. Partial resistance has been defined as a resistance resulting in a reduced epidemic development despite a susceptible infection type (PARLEVLIET and VAN OMMEREN 1975) and it is considered durable. An incomplete hypersensitivity resistance may also result in a reduced epidemic development, being rather quantitative, but is still recognizable by the association with some necrosis of the infected tissue. Partial resistance has been described in many pathosystems, including wheat-wheat leaf rust. It inherits generally polygenically, but not necessarily so (JACOBS 1990; JACOBS and BUURLAGE 1990), because some genes seem to have a rather strong effect, and hence behave as major genes, the presence of which can be concluded from the phenotype of individual plants. An example of such a gene is the *Lr34* (formerly known as *LrT2*) gene (DYCK and SAMBORSKI 1982).

A collection of old cultivars might be a good reservoir for genes for quantitative resistance, as breeding was mainly based on selection for field resistance (ZHANG 1995).

The purpose of the present study was to identify sources of quantitative non-hypersensitive resistance in a collection of ancient Spanish wheat cultivars.

MATERIAL AND METHODS

917 local varieties and landraces of bread and durum wheat from different parts of Spain, kindly provided by the Centro de Recursos Fitogenéticos, INIA, Spain, were grown at three different locations in Southern Spain during the season 96/97. These were Córdoba, Jerez and Granada. Information about these lines can be found in the CRF internet website http://www.crf.inia.es/. 573 lines were bread wheat and 344 durum wheat. Each line was represented by a 1 m long single row. No artificial inoculation was performed as leaf rust infections occur commonly in the area (MONTES et al. 1988). Disease severity (%) was assessed five times from April till June. The final disease severity was referred to that of the most susceptible accession.

In addition, infection type of all the accessions was studied in the seedling state in a growth chamber. Five plants per accession were grown in 7 × 7 × 9 cm pots and inoculated in the second-leaf stage with a local isolate of *Puccinia triticina* (virulent on *Lr2b*, *Lr2c*, *Lr10*, *Lr11*, *Lr12*, *Lr14a*, *Lr14b*, *Lr18*, *Lr20*, *Lr21*, *Lr22*, *Lr23*, *Lr33*, *Lr35*, *Lr37*, *Lr44* and *LrB*).

Plants were incubated 24 h in darkness with saturated RH and moved to a compartment at 20°C and 14 h photoperiod. Infection type (IT) (McNeal et al. 1971) was scored 14 days after inoculation.

Thirty-three accessions out of the 917, showing high IT in the seedling test and high resistance in the field in the three locations, were selected for further studies. Their reaction to leaf rust was studied at the same three locations in the field in 1997–98, and their components of resistance measured in seedlings. The field experiment was organised and evaluated in the same way as the 96/97 experiment. For the seedling test, the plants were grown in soil in plant boxes (35 × 35 × 10 cm). Three consecutive replications were performed. We tested four leaves per line including in each box a susceptible (Little Club) and partially resistant check (Akabozu). Eleven days after sowing, first leaves of each seedling were fixed in a horizontal position with the adaxial side upward. Per plant box, 4 mg of urediospores of the local isolate was mixed with talcum powder (1:9, vol/vol) and applied using a settling tower. The inoculum density was about 130 spores/cm^2.

After inoculation the plant boxes were incubated 24 hours in darkness at 100 % relative humidity and 20°C, and afterwards transferred to a compartment at 20°C and 14 hours of photoperiod.

Infection type, latency period, and infection frequency were determined. Infection type was recorded 12 days after inoculation. Latency period was determined by counting daily the number of uredia visible in a marked area on the leaves till the number of uredia no longer increased. 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. Infection frequency was determined on the marked areas of the leaves. The final number of uredia was used to calculate the number of uredia per cm^2.

From these experiments, the seven lines in which fungus had developed the longest latency period and the lowest infection frequency were selected for further studies at the macroscopic level with different isolates and for microscopic observations. These tests were performed in seedlings and adult plants. Wheat cultivars Little Club (susceptible) and Akabozu (partially resistant) were included as references. In seedlings we incubated the material for 12 hours while in adult plant the incubation was for 24 hours.

For the adult plant experiments, plants were grown individually in 12 × 12 cm pots in a greenhouse. All plants were sown at several dates in order to obtain plants at the same development stage. Adult plant experiments were done in two stages: when the sixth leaf just expanded (DC30, Zadoks et al. 1974) and in flag leaf (DC 48-59, ears just emerged, young but fully expanded flag leaves) at the time of inoculation. Three series were performed, of four pots each. Inoculation was performed by dusting urediospores mixed with talcum powder over the plants. One milligram of urediospores was used per pot.

Components of resistance were again measured as described above, both in seedlings and adult plants after inoculation with isolate *Puccinia triticina* B9414-1CA3 (virulent on *Lr1*, *2c*, *3*, *3bg*, *11*, *12*, *13*, *14a*, *14b*, *16*, *18*, *21*, *22*, *26*, *33*, *34*, *37*, *44(I)* and *LrB(I)*).

Five days after inoculation, central segments of 1 to 3 cm^2 were collected from young first, sixth and flag leaves respectively. Three leaves were sampled of each accession of each series. Segments were prepared as whole mounts for fluorescence microscopy (Rohringer et al. 1977), but instead of Calcofluor we used Uvitex 2B (Ciba-Geigy). The preparations were examined at 200× with a Leica epifluorescence equipment (DM LB, 330 to 380 nm wave length transmission). At least 100 sporelings per leaf segment were scored and classified according to their stage of development (Niks 1982). Sporelings that developed a germ tube but not an appressorium over a stoma were ignored. We defined early aborted sporelings as individuals that formed a primary infection hypha and not more than six haustorial mother cells (Niks 1982). Sporelings that had developed more haustorial mother cells were classified as established. Filter with 420–490 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 for percentage of sporelings aborted or associated with necrosis was performed on arc sin-transformed data.

RESULTS

The susceptible check showed 80 % of disease severity at Córdoba and 70 % at Jerez and Granada. The correlation coefficient of the RDS on the 917 accessions over the three locations was about 0.5. In about 6 % of the lines relative disease severity (RDS) in the field was lower than 20 % the severity of the susceptible check (Fig. 1). High susceptibility was very frequent. 67 % of the lines displayed a RDS higher than 40 %. Durum and bread wheat showed a similar average RDS (about 50 %). In seedling tests most of the lines (82 %) displayed a susceptible IT. 41 % of the lines displaying field resistance (RDS lower than 20 %) showed a high IT in the seedling test. Thirty lines were selected with low RDS in the field and high

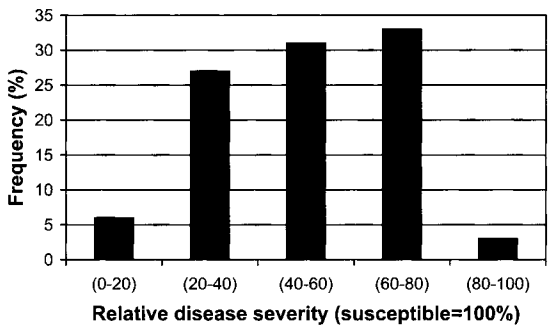

Fig. 1. Distribution of the lines according to field severity during the season 96/97 in Córdoba.

IT in the growth chamber. The RDS was particularly low in lines BGE012036, BGE012609 and BGE-013781 (data not shown).

Out of those thirty lines, seven were selected on which the pathogen displayed a long latency period. These seven lines were studied for the macroscopic and microscopic components of the resistance.

Macroscopic components of the resistance in the selected lines are shown in Table 1. Latency period was longer in adult plant than in seedling. In seedlings the latency period on lines BGE012036 and BGE012609 was significantly longer than on susceptible check Little Club, but shorter than on the partially resistant reference line Akabozu. On the sixth leaf and on the flag leaf, all lines except BGE012609 had a relative latency period significantly longer than Little Club and similar to Akabozu. On line BGE012036 the latency period was even significantly longer than on Akabozu.

In seedling stage, only on line BGE012036 the relative infection frequency was significantly lower than on Little Club, and similar to that on Akabozu. This line also had a reduced infection frequency relative to Little Club in the adult plant stage.

Infection type was high in the seedling stage of all lines (Table 1). The infection type of line BGE011932 was intermediate (IT6) in the sixth leaf stage (good sporulation but associated with some necrosis). In flag leaves the IT of this line was reduced to 2 (only necrotic flecks). The IT of the line BGE012609 was also lower in flag leaf (IT6).

The results of the microscopic observations are shown in the Table 2. In lines BGE011932 and BGE012609, infections tended to be much more frequently to be associated with necrosis than in the other lines, especially in the adult plant stages. This is in agreement with the lower IT observed macroscopically (Table 1). Lines BGE012036, BGE013781 and Akabozu had a higher percentage of early aborted colonies than the susceptible check in seedling stage, but overall level of early abortion was low (1 to 7 %). In the sixth leaf stage, only line BGE012036 had a higher percentage of infection units that had aborted early without host cell necrosis than the Little Club. In the flag leaf stage, lines BGE004788, BGE011932, BGE012036, BGE013781 and Akabozu had a higher percentage of early aborted colonies than Little Club. The high percentage of early aborted infection units without plant cell necrosis is remarkable in the lines BGE013781 (50 %) and BGE012036 (64 %).

Colonies were smaller in adult plant than in seedling stage (Table 2). In seedlings of lines BGE012036, BGE013804 and especially Akabozu

Table 1. *Macroscopic components of resistance (latency period, infection frequency and infection type) in the selected accessions of wheat inoculated with the wheat leaf rust isolate Puccinia triticina B9414-1CA3*

	Seedling stage			Sixth leaf			Flag leaf		
Genotype	RLP[1]	RIF[2]	IT[3]	RLP[1]	RIF[2]	IT[3]	RLP[1]	RIF[2]	IT[3]
Little Club	100 d[4](143)	100 (52)a	9	100 c(166)	100 a(30)		100 d(198)	100 a(38)	9
BGE012609	113 b	99 a	8	105 bc	70 ab	8	111 c	61 ab	6
BGE018644	99 d	86 ab	9	108 b	69 ab	8	116 bc	62 ab	9
BGE013804	105 cd	85 ab	9	112 b	37 b	8	113 c	60 ab	9
BGE004788	102 d	99 a	9	110 b	65 ab	9	112 c	87 a	9
BGE011932	101 d	105 a	9	109 b	39 b	6	-	-	2
BGE013781	101 d	84 ab	9	109 b	70 ab	8	128 b	64 ab	9
BGE012036	109 bc	62 bc	9	131 a	42 ab	8	141 a	42 b	9
Akabozu	128 a	56 c	9	113 b	49 ab	8	120 bc	74 a	9

[1] RLP: Latency period (relative to Little Club). The actual value of latency period (in hours) is presented in brackets.
[2] RIF: Infection frequency (relative to Little Club). The actual value of infection frequency (pustules per cm^2) is presented in brackets
[3] IT: Infection type, on a scale of 0 to 9.
[4] Duncan analysis, level of significance 0.05. Within a column, figures that are followed by a letter in common are not significantly different.

Table 2. *Microscopic components of the resistance of selected wheat lines inoculated with wheat leaf rust isolate Puccinia triticina B9414-1CA3 at five days after inoculation*

	Seedling stage[a]			Sixth leaf[b]			Flag leaf[c]		
Genotype	%EA-[1]	%necrosis[2]	C.size[3]	%EA-[1]	%necros.[2]	C.size[3]	%EA-[1]	%necros.[2]	C.size[3]
Little Club	1 cd[4]	2 d	100 a (0.334)	4 bc	2 ef	100 ab (0.113)	6 e	6 b	100 a (0.031)
BGE012609	2 bcd	5 bc	76 abc	2 c	3 de	111 a	11 de	59 a	41 ab
BGE018644	2 bcd	2 bcd	94 ab	5 bc	2 ef	97 abc	13 cde	3 b	60 ab
BGE013804	4 abc	3 bcd	64 c	13 ab	7 cd	51 d	16 cde	4 b	41 abc
BGE004788	2 bcd	2 cd	83 abc	10 abc	11 c	58 cd	26 cd	10 b	56 bcd
BGE011932	1 d	34 a	85 abc	12 ab	73 a	33 d	31 bc	61 a	28 bcd
BGE013781	5 ab	2 bcd	88 abc	8 abc	16 c	37 d	50 ab	8 b	16 cd
BGE012036	7 a	5 b	70 bc	20 a	26 b	21 d	64 a	4 b	13 d
Akabozu	5 ab	1 d	33 d	13 ab	0 f	62 bcd	25 cd	4 b	21 bcd

[a] Average of three series, three primary leaves per series.
[b] Average of three series, three sixth leaves per series.
[c] Average of three series, three flag leaves per series.
[1] Percentage of early aborted infection units without necrosis.
[2] Calculated as the percentage of infection units with necrosis.
[3] Mean colony size.
[4] Duncan analysis. Level of significance 0.05. Within a column, figures that are followed by a letter in common are not significantly different.

colonies were smaller than in the susceptible check. In sixth leaves of lines BGE004788 and BGE011932 colony size was significantly smaller than in Little Club. In flag leaves, all lines but BGE004788 displayed a reduced colony size.

DISCUSSION

The study of the levels of resistance in the collection of wheat lines demonstrated the fact that in wheat, hypersensitive and non hypersensitive types of resistance against leaf rust occur side by side. A large number of accessions that were resistant in the field, showed low infection types in the seedling test. Other lines showed a susceptible infection type both as seedlings and in the adult plant stage, but relatively low levels of infection in the field (partial resistance). Partial resistance was associated with a prolonged latency period, especially in adult plant stage in agreement with BROERS (1989) and high levels of early abortion of infection structures not associated with host cell necrosis and with a reduced colony size (JACOBS 1990; JACOBS and BUURLAGE 1990). The most extreme representative of this type was line BGE012036. A third category was formed by some lines that showed a susceptible type of reaction in the seedling stage, but lower, more hypersensitive reactions in the adult plants stages. Lines BGE011932 and BGE012609 are examples of this latter category. In the literature, there are many examples of hypersensitive adult plant resistance to *Puccinia triticina* in wheat (PARK and MCINTOSH 1994). Line BGE011932, in which the reaction type drops to IT2 in the flag leaf stage (Table 1), might carry a gene like *Lr13* or *Lr37*, that acts at an early stage of the infection process, and hence results in a complete resistance. Line BGE012609, in which the reaction type drops to IT6 (Table 1), might carry a resistance gene like *Lr12*, *Lr22a* or *Lr35*, that acts at a more advanced stage of infection, and give a moderate infection type. It is difficult to determine the level of partial resistance in lines with such adult plant hypersensitive resistance, but we could get an indication by the level of partial resistance already identified in seedlings where the adult plant genes are inactive. Partial resistance is usually more strongly expressed in adult plant stage, but seedling tests can provide good estimations (BROERS 1989; RUBIALES and NIKS 1995). In addition to that, histological studies can contribute to asses the levels of partial resistance hidden by hypersensitive resistance genes by determining the levels of prehaustorial resistance (estimated by percentage of early aborted infection units not associated with host cell necrosis) that acts before hypersensitive resistance (NIKS and KUIPER 1983). A long latency period was observed on line BGE012609 in seedling stage that does not correlate with the percentage of early abortion in adult plant. This line may have partial resistance only expressed in seedling stage. Similarly, a QTL for partial resistance that is just expressed in the seedling stage, has recently been reported in the pathosystem barley-*Puccinia hordei* (QI et al. 1998). In contrast, line BGE011932 seems to display partial resistance in adult plants but not in seedlings.

The correlation in disease severity of the lines over between locations was only moderate (about 50 %). This indicates the presence of different pathotypes of the leaf rust at those locations. We found, for example, that avirulence against *Lr20* and *Lr23* occurs in Jerez but not in Córdoba nor in Granada (data not presented). There may also be differences in environmental conditions between the locations. Many resistance genes are affected by the temperature. This is true for both partial resistance (DENISSEN 1991; RUBIALES and NIKS 1995) and some genes for hypersensitive resistance (KLOPPERS and PRETORIUS 1994; RAMAGE and SUTHERLAND 1995).

High levels of partial resistance were indicated by long latency period and high percentage of early aborted colonies that were not associated with plant cell necrosis. BROERS and DE HAAN (1994) reported a positive relationship between the level of partial resistance in landraces and the severity of the wheat leaf rust in several European countries. MONTES et al. (1988) pointed out that wheat leaf rust is a common disease in Andalusia. The landrace and cultivars, having a long growing season, would allow more spore generation of the pathogen, and hence, higher disease severities to be reached. Therefore, in those landraces there may have been a greater selection pressure for quantitative types of resistance.

The levels of partial resistance against the wheat leaf rust were particularly high in the studied lines. In bread wheat accessions BGE004788, BGE013804, BGE013781 and durum line BGE018644, the level of partial resistance was similar that of Akabozu. The partial resistance of bread wheat accession BGE012036 was even considerably higher than in Akabozu. The very high levels of early abortion without plant cell necrosis (up to 64 %) in accession BGE12036 are unprecedented. These lines might be a useful sources of partial and hopefully durable resistance to leaf rust.

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