New Sources of Resistance to *Puccinia hordei* in Barley Land Race Cultivars

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ABSTRACT

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New genes for resistance to *Puccinia hordei* appeared to be common in several collections of barley (*Hordeum vulgare*) land race cultivars originating in central and southern Tunisia. Response of four land race cultivars to a number of different isolates of *P. hordei* from the Mediterranean region differentiated them from the known genotypes. A dominant resistance gene that behaved as Pa_3 was found in Tu32. Three of the land race cultivars (Tu17, Tu27, and Tu34) each have a dominant resistance gene that is most likely different from the previously known resistance genes. The dominant resistance genes identified in this study were as effective as Pa_3 and Pa_7 and, hence, should be considered for use as new sources of resistance. Further testing is needed to determine the genetic relationships between these genes.

Additional keyword: leaf rust.

Although leaf rust has not been a significant factor affecting barley production in North Africa and the Middle East, this situation may change because the disease is now commonly observed in the major barley-growing areas.

Several major genes for resistance to *Puccinia hordei* Otth. have been described (1,2,7,8,10). These genes have been designated as Pa, Pa_2, Pa_3, etc., and are assumed to operate on a gene-for-gene basis (3) with corresponding virulence genes in the pathogen. The leaf rust pathogen shows wide virulence on barley cultivars having Pa resistance genes in Europe (1), North America, North Africa, and the Middle East (6,11). The Pa_7 gene is effective throughout Europe, while virulence against Pa_3 occurs rather infrequently (4). Barley lines having the Pa_3 or Pa_7 resistance genes were resistant to various isolates of *P. hordei* from the Mediterranean region (6,9,11). Pa_3, Pa_7, and Pa_9 were also effective against all races of *P. hordei* known to occur in the United States (5). Pa_9 was overcome by many leaf rust isolates from North Africa and the Middle East (11).

The search for new resistance sources among cultivated barleys led to the identification of barley land race cultivars that expressed seedling resistance to isolates of *P. hordei* from the Mediterranean region (11). The objectives of this study were to determine the resistance factor(s) associated with barley land race cultivars and whether or not the resistance genes are the same as the known Pa genes.

MATERIALS AND METHODS

Parent selection. One hundred eighty single heads of barley land race cultivars were from farmers' fields in central and southern Tunisia. They were screened for their reaction to isolates of *P. hordei* from the Mediterranean area. Thirty-four lines had intermediate to resistant reactions to most of the isolates of *P. hordei* tested (11). Four resistant barley lines were selected, and seeds from single heads were multiplied in the field. These lines were given a Tunisian (Tu) number and were further screened against various isolates of *P. hordei* (Table 1).

Selected lines (Tu17 collected from Oasis, southern Tunisia, in 1982 and Tu27, Tu32, and Tu34 collected from El Jem, southeast Tunisia, in 1983) were crossed with four differential genotypes with known Pa genes. Reka1 (Pa2, CI 5051) was completely susceptible to all isolates of *P. hordei* (11) and was used as the susceptible parent. Parents and five to 10 F_1 seeds were planted in Tucson, AZ, where backcrosses and additional single crosses were made. The backcrosses and F_3 lines were later screened in environmentally controlled growth chambers. Screening of the parents and the segregating progenies was conducted in three separate controlled environments with 16-hr daily photoperiod at 15/20 ± 1 C (dark/light).

Inoculum and inoculation techniques. Inocula from monouredial cultures of three leaf rust isolates (TuKe82-5, TuOa82-1, and MoMe84-5) were multiplied on the universally susceptible cultivar Moore (CI 725). Isolates were kept in different growth chambers to minimize the chances of contamination. The three isolates TuKe82-5, MoMe84-5, and TuOa82-1 were collected from Le Kef (Tunisia), Merchouch (Morocco), and the Oasis (Tunisia), respectively. Their virulence patterns on the differential set are shown in Table 2. Isolate TuOa82-1 was considered largely virulent since only two resistance genes were effective against it. TuKe82-5 and MoMe84-5 were largely avirulent because more resistance genes were effective against them. Resistance factors associated with the barley land race cultivars were effective against these isolates.

Inoculation procedures were described previously (6), with the following exception: because of the large number of plants screened, the rust spores were suspended in Soltrol 170 oil in a ratio of 1 mg of spores/1 ml of oil. The spore suspension was then

TABLE 1. Reactions of four Tunisian barley lines and four differential barley genotypes to nine isolates of *Puccinia hordei* from several locations

	Barley genotypes[a]							
Isolates[b]	Rek Pa_{2+}	Hor Pa_9	Est Pa_3	C.C. Pa_7	Tu17	Tu27	Tu32	Tu34
TuMa83-16	S[c]	R	R	R	R	R	R	R
TuKe82-5	S	R	R	R	R	R	R	R
TuOa82-1	S	S	R	R	R	R	R	R
MoMe84-5	S	I	R	R	R	R	R	R
MoRb84-1	S	R	R	R	R	R	R	R
JoAm84-4	S	S	R	R	R	R	R	R
SyAl184-1	S	I	R	R	R	R	R	R
EgGg84-1	S	S	R	R	R	R	R	R
EgSk84-1	S	R	R	R	R	R	R	R

[a] Reka: Reka1, CI 5051 Hor: Hor2596, CI 12434; Est: Estate, CI 34102; C.C: Cebada Capa, CI 61933.
[b] Isolates 1, 2, and 3 are from Tunisia; 4 and 5 from Morocco; 6 from Jordan, 7 from Syria, and 8 and 9 from Egypt.
[c] R = resistant reaction, I = intermedite reaction, S = susceptible reaction.

sprayed on the plants to be screened with a DeVilbiss atomizer attached to a compressed air hose (15–20 psi).

The plants were grown in a 2:1 soil:sand mixture. Two hundred F_2 seedlings from each cross were grown in metal flats (34 × 25 × 8 cm). Seeds were sown in eight rows of 25 seeds per row. Parents, the universal susceptible, backcrosses, and F_3 seedlings were grown in plastic pots (10 cm diameter). Parents and hybrids that were to be transplanted into the field for seed production of the successive generations (i.e., F_2, F_3, and BCF_2) were planted in peat pots (five seeds/pot).

Statistical analysis. Probability values for goodness of fit to expected ratios were calculated using chi-square. In both F_2 and F_3 progenies where more than one F_1 plant was studied, a chi-square test for homogeneity was used to determine whether different F_2 families displayed similar genetic behavior. Combined data are presented in the tables.

RESULTS

Parents. The four land race barley cultivars selected for this study had an effective resistance to most of the isolates of *P. hordei* from the Mediterranean region (Table 1), suggesting that these cultivars may possess new resistance genes to *P. hordei*. Their reactions to different isolates were invariably associated with some chlorosis and, as such, were normally distinguishable from resistance conditioned by Pa_3, Pa_7, and Pa_9, and for that matter, those typical to Pa, Pa_2, Pa_4, Pa_2+, $Pa_2 + Pa_5$, $Pa_2 + Pa_6$. and Pa_8. Table 1 shows the reaction patterns of nine isolates of *P. hordei* that were observed on these selected lines.

When land race cultivars were crossed with barley genotypes having specific resistance genes, the progeny segregated in the F_2 generation, indicating the presence of different resistance genes or gene combinations. The land race cultivars were crossed with the susceptible parent RekaI to determine the number of gene loci for resistance in each cultivar (Table 3). Crosses between the resistant land race cultivars and the three resistant genotypes (Hor 2596, Estate, and Cebada Capa) with known resistance genes were made to determine if the genes for resistance were at a common locus (Tables 4–6).

Segregation in resistant × susceptible crosses. Segregation results of the crosses involving the susceptible parent, RekaI, and four land race barley cultivars are shown in Table 3. In crosses involving RekaI and the four land race cultivars (Tu17, Tu27, Tu32, and Tu34), the F_2 progenies fitted a 3:1 ratio (Table 3) when tested against all isolates.

Segregation in resistant × resistant crosses. With crosses involving Hor2596, resistance gene (Pa_9), different segregation ratios in the F_2 progeny were observed, some of which were not expected (Table 4). Avirulent and F_2 progenies from crosses involving Hor2596 and the land race cultivars Tu17, Tu27, Tu32, and Tu34, fitted 13:3 and 3:1 ratios when tested with avirulent isolate TuKe82-5, and virulent isolate TuOa82-1, respectively (Table 4). The F_2 progeny of (Tu32 × Hor2596) fitted 13:3 rather than a 3:1 ratio when tested with TuOa82-1 (Table 4).

TABLE 2. Virulence patterns of three isolates of *Puccinia hordei* on barley differential cultivars

	Differential host genotypes[a]												
Isolate	1 Est Pa_3	2 C.C. Pa_7	3 Hor Pa_9	4 Ric Pa_{2+}	5 Bolivia $Pa_2 + Pa_6$	6 Quinn $Pa_2 + Pa_5$	7 Mag Pa_5	8 Per Pa_2	9 Sud Pa	10 Eg Pa_8	11 Bat Pa_{2+}	12 Gol Pa_4	13 Rek Pa_{2+}
TuKe82-5	R[b]	R	R	R	R	R	R	S	R	S	R	S	S
MoMe84-5	R	R	R	S	R	R	S	S	R	S	R	S	S
TuOa82-1	R	R	S	S	S	S	S	S	S	S	S	S	S

[a] Est: Estate, CI 3402; C.C.: Cebada Capa, CI 61933; Hor: Hor2596, CI 12434; Ric: Ricardo, CI 63065; Bolivia: Bolivia, CI 12576; Quinn: Quinn, CI 10247; Mag: Magnif, CI 138068; Per: Peruvian, CI 9359; Sud: Sudan, CI 648910; Eg: Egypt, CI 648111; Bat: Batna, CI 339112; Gol: Gold, CI 114513; Rek: RekaI, CI 5051.

[b] R = resistant reaction, I = intermediate reaction, S = susceptible reaction.

TABLE 3. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (Rekal × four land races)

Cross	Parental reaction[a]	Isolate	Observed frequency		Expected ratio	Probability of fit
			Resistant	Susceptible		
Tu17 × RekaI	R/S	TuKe82-5	392	142	3:1	0.42
Tu17 × RekaI	R/S	TuOa82-1	371	126	3:1	0.89
Tu27 × RekaI	R/S	TuKe82-5	155	52	3:1	1.00
Tu27 × RekaI	R/S	TuOa82-1	164	58	3:1	0.76
Tu32 × RekaI	R/S	TuKe82-5	218	68	3:1	0.68
Tu32 × RekaI	R/S	TuOa82-1	175	67	3:1	0.37
Tu34 × RekaI	R/S	TuKe82-5	535	182	3:1	0.84
Tu34 × RekaI	R/S	TuOa82-1	66	25	3:1	0.67

[a] Reaction of first parent/second parent; R = resistant, S = susceptible.

TABLE 4. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (Hor 2596 × four land races)

Cross	Parental reaction[a]	Isolate	Observed frequency		Expected ratio	Probability of fit
			Resistant	Susceptible		
Tu17 × Hor2596	R/R	TuKe82-5	720	39	15:1	0.23
Tu17 × Hor2596	R/R	MoMe84-5	721	51	15:1	0.73
Tu17 × Hor2596	R/S	TuOa82-1	762	243	3:1	0.57
Tu27 × Hor2596	R/R	TuKe82-5	333	27	15:1	0.38
Tu27 × Hor2596	R/S	TuOa82-1	72	17	3:1	0.24
Tu32 × Hor2596	R/R	TuKe82-5	168	15	15:1	0.35
Tu32 × Hor2596	R/S	TuOa82-1	209	43	13:3	0.54
Tu34 × Hor2596	R/R	TuKe82-5	176	16	15:1	0.30
Tu34 × Hor2596	R/S	TuOa82-1	140	50	3:1	0.73

[a] Reaction of first parent/second parent; R = resistant, S = susceptible.

The cultivar Estate has the dominant resistance gene Pa_3 (8), which was effective against all isolates of *P. hordei* from North Africa and the Middle East (11).

The F_2 progeny from Tu17, Tu27, and Tu34 crossed with Estate fitted a 15:1 ratio (Table 5). In the F_2 progeny of Tu32 × Estate, there were no susceptible plants detected when tested with the virulent isolate TuOa82-1. However, a 15:1 ratio was observed when the F_2 progeny was tested with the avirulent isolate, TuKe 82-5 (Table 5). The latter ratio may be due to the low number of plants in the test or may have resulted from misclassification or mixed seed.

Crosses (Table 6) were also made with the resistant cultivar Cebada Capa, which is resistant to almost all isolates of *P. hordei* worldwide (4,6,11). No susceptible plants were observed in the F_2 progeny of Tu17 × Cebada Capa when tested with the avirulent isolates, indicating a gene in common between within the parents. A 15:1 ratio was observed when these F_2 progeny were tested with TuOa 82-1 isolate (Table 5).

Segregation in F_1, F_3, and backcross generations. To verify F_2 ratios, F_3 and backcross hybrids in crosses with RekaI were tested (Table 7). The F_3 progeny of Tu17 × RekaI fit a 1:2:1 ratio, and no segregation was observed in BC_2. A segregation ratio of 1:2:1 was also observed for the F_3 progeny of Tu27 × RekaI, Tu32 × RekaI, and Tu34 × RekaI.

DISCUSSION

Many barley land races grown in central and southern Tunisia have an adequate level of resistance to the leaf rust pathogen. Barley cultivars grown in these regions are often mixtures of heterogenous genotypes. They may have originated from cultivars discarded from commercial production, seed collections that have been exchanged for goods among nomad tribes along the southern parts of North Africa, or seeds that were handed down from generation to generation within the farming communities.

Mixed barley cultivars or land races, regardless of their origin, are useful sources of resistance. Incorporating this valuable material into a breeding program is relatively easy since there are no sterility problems or other abnormalities that occur with interspecific hybridization. Furthermore, undesirable agronomic traits that are usually derived from wild relatives do not have to be bred out when using land races.

Crosses were made between barley genotypes with four known resistance genes and four land races to determine if the resistance factor(s) to *P. hordei* in these lines were dominant or recessive, and whether they were controlled by one, few, or many genes. The three monouredial isolates of *P. hordei* used to test the segregating populations differed in their virulence. The avirulent isolate allowed detection of the largest number of resistance gene(s). The

TABLE 5. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (Estate × four land races)

Cross	Parental reaction[a]	Isolate	Observed frequency		Expected ratio	Probability of fit
			Resistant	Susceptible		
Tu17 × Estate	R/R	TuKe82-5	710	46	15:1	0.91
Tu17 × Estate	R/R	MoMe84-5	642	46	15:1	0.69
Tu17 × Estate	R/R	TuOa82-1	377	22	15:1	0.61
Tu27 × Estate	R/R	TuKe82-5	53	6	15:1	0.32
Tu27 × Estate	R/R	TuOa82-1	158	15	15:1	0.25
Tu32 × Estate	R/R	TuKe82-5	77	2	(63:1)[b]	1.00
Tu32 × Estate	R/R	TuOa82-1	162	0	no seg.	
Tu34 × Estate	R/R	TuKe82-5	317	14	15:1	0.16

[a] Reaction of first parent/second parent; R = resistant, S = susceptible.
[b] Ratios in parentheses were not expected, but gave the best fit.

TABLE 6. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (Cebada Capa × Tu17)

Cross	Parental reaction[a]	Isolate	Observed frequency		Expected ratio	Probability of fit
			Resistant	Susceptible		
Tu17 × C. Capa	R/R	TuKe82-5	640	0	no seg	...
Tu17 × C. Capa	R/R	MoMe84-5	552	no seg	...	...
Tu17 × C. Capa	R/R	TuOa82-1	565	34	15:1	0.62

[a] Reaction of first parent/second parent; R = resistant, S = susceptible.

TABLE 7. The reaction of barley seedling progeny to two isolates of *Puccinia hordei* (RekaI × four land races)

Cross	Parental generation	Parental reaction[a]	Isolate	Observed frequency			Expect ratio	Probability of fit
				Resistant	Segregation	Susceptible		
Tu17 × RekaI	F_1	R/S	TuKe82-5	13	...	...		
Tu17 × RekaI	F_1	R/S	TuOa82-1	19	...	...		
Tu17 × RekaI/Tu17	BC_2	R/S/R	TuKe82-5	15	...	...	no seg.	
Tu17 × RekaI/Tu17	BC_2	R/S/R	TuOa82-1	18	...	...	no seg.	
Tu17 × RekaI	F_3	R/S	TuKe82-5	8	12	2	1:2:1	0.17
Tu17 × RekaI	F_3	R/S	TuOa82-1	16	26	13	1:2:1	0.78
Tu27 × RekaI	F_1	R/S	TuKe82-5	10	...	...		
Tu27 × RekaI	F_1	R/S	TuOa82-1	9	...	...		
Tu27 × RekaI	F_3	F/S	TuKe82-5	11	19	13	1:2:1	0.68
Tu27 × RekaI	F_3	R/S	TuOa82-1	10	13	4	1:2:1	0.25
Tu32 × RekaI	F_1	R/S	TuOa82-1	10	...	...		
Tu32 × RekaI	F_3	R/S	TuOa82-1	16	28	15	1:2:1	0.91
Tu34 × RekaI	F_1	R/S	TuKe82-5	9	...	...		
Tu34 × RekaI	F_1	R/S	TuOa82-1	10	...	...		
Tu34 × RekaI	F_3	R/S	TuKe82-5	12	22	14	1:2:1	0.78
Tu34 × RekaI	F_3	R/S	TuOa82-1	3	24	5	1:2:1	0.46

[a] Reaction of first parent/second parent; R = resistant, S = susceptible.

differentially virulent isolate allowed further classification of segregating progenies.

The result of cultivar inoculation with a specific pathogen isolate is an interaction of corresponding resistance and virulence genes giving an infection type. This, in turn, gives rise to some degree of resistance. If the critical corresponding genes in the pathogen are virulent, the host is susceptible, whether or not it has resistance genes. In cases of quantitative inheritance, several interacting genes in both the host and pathogen may be required to give effective resistance. This premise is used throughout in interpreting the results.

In crosses with the susceptible cultivar RekaI (Table 3), a monogenic mode of inheritance was suggested with the avirulent isolates F_3, and backcross results (Table 7) support this hypothesis. When the four land race cultivars were crossed to RekaI, the observed F_2, F_3, and backcross segregation ratios implied monogenic inheritance. These cultivars have a dominant resistance gene.

F_2 progeny tests of Tu17, Tu27, and Tu34 crossed to either Hor2596 or Estate suggested the presence of a dominant gene in each of these cultivars. These genes were different from Pa_3 and Pa_9. Likewise, Tu32 possessed a dominant resistance gene as suggested by the F_2 progeny Tu32 × Hor2596. However, no susceptible plants were observed when Tu32 was crossed to Estate. The absence of recombinant types implies that the parents have a resistance gene in common, as was the case with Cebada Capa, La Estanzuela, Gondar, and Dabat barley cultivars (4). Two closely linked loci could be involved rather than a single locus. To distinguish between the resistance factor(s) involved in Tu32 × Estate cross, screening the F_3 progeny with an isolate virulent on either parent would be necessary.

The merit of using isolates of different virulence levels can be seen in the cross of Tu17 × Cebada Capa. The absence of susceptible plants in the F_2 progeny, when tested with the avirulent isolates, suggests that Tu17 and Cebada Capa have a gene in common, probably Pa_7, or that two closely linked loci could be involved. The F_2 progeny, when inoculated with the virulent isolate, had a 15:1 ratio implying the presence of two dominant genes. The most likely explanation is that Tu17 and Cebada Capa may have the gene(s) conferring resistance to TuKe82-5 and MoMe84-1 in common. Then each cultivar has different independently inherited genes for resistance to TuOa82-1.

The rate at which the *P. hordei* pathogen adapts to a resistant host can be reduced by using diversified sources of resistance. For this diversification to be efficient, it should be controlled relative to the virulence composition of the pathogen. This requires a thorough monitoring of the pathogen population. Resistance diversification can be accomplished by various strategies and thus increase the durability of resistance.

Genetic studies indicate that the barley land race cultivars have effective sources of resistance to *P. hordei*. Genetic analysis of crosses between land race and known resistance sources of barley cultivars suggests that, with the exception of Tu32, the resistance genes in these barley cultivars were different from those genes previously identified. Tu17, Tu27, and Tu34 each have a dominant resistance gene. Additional genetic studies should be conducted to determine the relationships between the resistance genes in the land race cultivars.

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