Inheritance of Resistance to Net Blotch in Barley in Morocco

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ABSTRACT

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Barley cultivars currently grown in Morocco do not have satisfactory resistance to net blotch, caused by Pyrenophora teres. This study determined the inheritance of resistance in spring barley to net blotch in 11 F_{2:5} populations in the field in Morocco. Three populations were evaluated in both the field and glasshouse to compare inheritance of resistance of seedlings to single isolates in the glasshouse with natural inoculum occurring in the field. In the field, resistance in cvs. Heartland and Minn 7 was inherited in a monogenic manner in crosses involving moderately susceptible, susceptible, and highly susceptible cultivars. The degree of susceptibility of the susceptible parent appeared to have little effect on the resistance level in the progeny. AC-SAD 176 and Anoidium had single resistance genes and Manchurian had two independent genes that conditioned resistance. Crosses among resistant cvs. Heartland, Minn 7, and ACSAD 176 segregated 7: 8: 1 (homozygous resistant: segregating: homozygous susceptible) indicating different independent genes that condition resistance. Heartland appeared to possess a recessive gene that conditioned resistance to isolate I-12 in a cross involving Rabat 071 and a dominant gene to isolate I-1 in crosses involving Arig 8 and ACSAD 176. Since resistance to P. teres in five cultivars was simply inherited, incorporation of these genes into barley adapted to Morocco should be feasible.

Barley, Hordeum vulgare L., the predominant cereal of Morocco, occupies about 2 million hectares. Drought and diseases, especially net blotch, are important restraints on yield (3). Net blotch, caused by Pyrenophora teres Drechs., conidial state Drechslera teres (Sacc.) Shoemaker, is the most important disease of barley in Morocco, and local cultivars lack satisfactory resistance. It is an important foliar disease of barley in other Mediterranean countries and elsewhere (4,5,10).

The first genetic study on net blotch resistance in barley was by Geschele (8), who suggested that resistance was inherited in a simple Mendelian fashion. Schaller (16) identified resistance gene Pt, an incompletely dominant gene that conferred resistance in the cv. Tifang (CI 4407-1) in a cross with Atlas (CI 4118).

In later studies, Mode and Schaller (14) reported additional genes for resistance, including *Pt2* and *Pt3*, that were incompletely dominant against California isolates of *P. teres*; cv. Tifang (CI 4407-1) had *Pt1*;

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Ming (CI 4797), Manchurian (CI 739), and Harbin (CI 4929) had *Pt2*; and Canadian Lake Shore (CI 2750) and CI 4922 had *Pt3* and *Pt3*. Gray (9), using isolates that differed from those of Mode and Schaller (14), found a single dominant resistance gene in Canadian Lake Shore and in CI 9825

Resistance was conferred by a single dominant gene in Rojo, Hoyo Epuyen, and an accession of Hordeum spontaneum in Argentina (7); and in CI 5791 in Canada (13). Khan and Boyd (11) found a fourth gene for resistance, designated Pta, that was carried by five resistant lines: Tifang, Ming, Manchuria (CI 2330), CI 5791, and CI 9819. Resistance in CI 5791 and CI 9819 (both Ethiopian lines) was governed by duplicate genes, one of which was Pta. They concluded that Pta was different from genes Pt1, Pt2, and Pt3 identified in California. Selim et al. (18) found two dominant duplicate genes in CI 11458, CI 11460, and Estate (CI 3410), and a single gene in Giza 118, Sahrawi, Trivannes (CI 1109), CI 4292, CI 5822, and CI 5791. Bockelman and Sharp (1) found the single resistance genes Rptla in Tifang and Rpt3d in CI 7584, and two additional resistance genes, Rpt1b and Rpt2c, in CI 9819. Recently, Wilcoxson et al. (22), using breeding lines of the Minnesota program, found that inheritance of resistance to P. teres was monogenic in each line and at least two different loci were involved.

Sources of resistance to net blotch have been identified in field evaluations in Morocco. Among 143 U.S. barley cultivars and lines, 19 were resistant (3) and in another test with lines from the USDA world barley collection, 1,664 lines were resistant to moderately resistant (2). Apparently, no studies have been made on the inheritance of resistance to barley net blotch in Morocco.

The purpose of this research was to obtain information on inheritance of resistance to net blotch in eight barley cultivars. A secondary objective was to obtain information on glasshouse versus field inheritance studies and to ascertain the influence of degree of susceptibility of the susceptible parent used in crosses on the resistance level observed in progeny.

MATERIALS AND METHODS

In Morocco, both the teres and maculata forms of *P. teres* occur (19). Since the teres form is probably more common it was used in this study.

Based on literature and on preliminary evaluations in glasshouse and field plantings in Morocco, eight cultivars with different levels of resistance and susceptibility to net blotch were chosen as parents (Table 1). Parents reported to carry high levels of resistance in the field were Heartland (21,22), Minn 7 (3), and Manchurian (CI 1251) (17). The parents reported to be moderately resistant in Morocco were Anoidium (2) and ACSAD 176. The susceptible parents were older Moroccan cvs. Rabat 071 and Arig 8. Rabat 071, Arig 8, and ACSAD 176 were also selected for use in this investigation because they are well adapted to Morocco and have good agronomic characters. In addition, ACSAD 176 is high yielding. The other susceptible parent was Morex, a malting cultivar previously widely grown in Minnesota (15) that has been moderately susceptible in the field in Morocco.

Inheritance of resistance to net blotch was studied in 11 populations in the field at the Sidi Laydi Experimental Station, Morocco, and in three populations in the glasshouse in Morocco. Crosses were made in fall 1990 and F₁ plants were grown during winter 1991 in a glasshouse at St. Paul, MN. Seed from several F₁ plants was bulked to obtain the F₂ grown in summer 1991 at St. Paul, MN. At maturity, individual F₂ plants were harvested to obtain seed for planting F₃ families in Morocco

Heartland and Minn 7, the most resistant cultivars, were crossed to Rabat 071, Arig

8, and Morex, the susceptible cultivars. Manchurian and Anoidium were crossed to Rabat 071, the most susceptible cultivar. Heartland, Minn 7, and ACSAD 176 were crossed to each other to determine the similarity of their resistance genes.

Field experiment. In 1991 to 1992, F_{2:3} families and parents of the eleven populations (Table 2) were hand-planted in two 2.5-m rows spaced 30 cm apart at the Sidi Laydi Experimental Station, Morocco. Net blotch did not develop due to drought. Irrigation was applied to assure seed production and 30 to 40 spikes of each family were harvested in bulk without selection. In 1992 to 1993, the F_{2:4} populations and parents were planted. Unfortunately, a second dry year and unusually high temperatures precluded development of net blotch even with irrigation. Seed for planting F_{2:5} lines for each cross was harvested from 30 to 40 spikes from each F_{2:4} family.

In fall 1993, F_{2:5} lines and parents were hand-planted in four 2.5-m rows spaced 30 cm apart at the Sidi Laydi Experimental Station. In addition, the susceptible parents Rabat 071 and Arig 8 were planted in two 2.5-m rows with four replications per F_{2:5} population. Spreader rows of Moore barley (CI 7251) were planted between and around each population. The spreader rows were inoculated at the mid-tillering growth stage by scattering straw of Rabat 071 that was severely infected with *P. teres* among the Moore spreader rows.

The net blotch reactions of $F_{2:5}$ families and parents were assessed at about heading on the flag and penultimate leaves when disease was fully developed on Rabat 071, the most susceptible cultivar according to the seedling assessment scale of Tekauz (20): infection reactions (IR) were evaluated on plants in the families with a 1 to 10 scale in which values of 1 to 3 indicate a resistant reaction, 4 to 5 moderately resistant, 6 to 7 moderately susceptible, and 8 to 10 susceptible. Resistant plants had small lesions (>3 mm in size) with no chlorosis, moderately resistant plants had larger lesions with some chlorosis, moderately susceptible plants had large lesions with chlorosis and some netting, and susceptible plants had large lesions with netting, severe chlorosis, and necrosis that often caused death of the entire leaf. The $F_{2:5}$ families were classified as resistant (plants were scored 1 to 5), segregating (plants displayed <6 and >6 IR values), or susceptible (plants were scored 6 to 10); the reaction of the parental lines was used as a reference. Because the lines were in the F_5 generation, the genetic composition of the segregating lines would consist of more homozygous and fewer heterozygous plants than lines in the F_3 generation.

Glasshouse experiment. Progeny of the crosses Heartland × Rabat 071, Heartland × Arig 8, and Heartland × ACSAD 176 (Table 3) were evaluated in the glasshouse in 1993 to 1994 in Morocco. About 30 seeds of each F_{2:3} family were planted in three 10-cm-diameter plastic pots (10 seeds per pot) containing a 2:1 ratio of sterilized soil to vermiculite. There were four replications of the parents. The glasshouse was maintained at 20 to 25°C with a natural photoperiod (11 h of daylight).

Reactions to two isolates of P. teres were studied because they differentiated resistant Heartland from the susceptible cultivars. The isolates, I-1 and I-12, were obtained from near Settat and Nador City, Morocco, respectively, in 1993. They were isolated onto V8 juice agar, and then monoconidial isolates were maintained on susceptible barley cv. Moore in a glasshouse until experiments were made. Then monoconidial cultures of each isolate were grown at 20°C with a 12-h photoperiod on 17.7% V8 juice agar. Conidia were scraped from 17-day-old cultures into sterile water containing Tween 20 (1 drop per 500 ml of water) and standardized at 40,000 conidia per ml with a hemacytometer. Inoculum was sprayed with Olympic Polysprayers (Olympic Co., Mainland, PA) onto the second and third leaves of 17-day-old barley plants until they were dripping wet. Inoculated plants were kept wet at 95 to 100% relative humidity in plastic tents for 48 h.

Net blotch infection ratings were assessed 10 days after inoculation on the second and third inoculated leaves according to the Tekauz (20) 1 to 10 qualitative scale described above. F₃ families

Table 1. Origin, CI or PI number, and infection reaction (IR) of resistant and susceptible barley cultivars infected with *Pyrenophora teres* in this study

Parent	IR^a	CI or PI number	Origin	
Resistant				
Heartland	1 to 3	PI 145351	Canada	
Minn 7	1 to 3	• • •	U.S.	
Manchurian	4 to 5	CI 1251	Manchuria	
Anoidium	4 to 5	CI 7269		
ACSAD 176	4 to 5	•••	Syria	
Susceptible				
Rabat 071	8 to 10	CI 4979	Morocco	
Arig 8	8 to 10	• • •	Morocco	
Morex	6 to 7	CI 15773	U.S.	

^a Based on reactions in the field and glasshouse: 1 to 3 = resistant; 3 to 4 = moderately resistant; 6 to 7 = moderately susceptible; 8 to 10 = susceptible.

were classified as homozygous resistant (IR 1 to 5), segregating, or homozygous susceptible (IR 6 to 10) with the reaction of parental lines used as a reference. Individual plants within segregating lines were classified as resistant (IR 1 to 5) or susceptible (IR 6 to 10). Observed ratios were tested against theoretical ratios for different numbers of segregating genes; the chisquare test was used for goodness of fit.

RESULTS AND DISCUSSION

Field experiment. Net blotch reactions of the parents were similar to what had been reported or previously observed. Rabat 071 and Arig 8 displayed typical susceptible reactions (IR 8 to 10) at the tillering stage of growth, and Morex was moderately susceptible (IR 6 to 7). Manchurian, Anoidium, and ASCAD 176 were moderately resistant (IR 4 to 5). Heartland and Minn 7 were resistant (IR 1 to 3).

Six $F_{2:5}$ populations—Heartland × Rabat 071, Minn 7 × Rabat 071, Anoidium × Rabat 071, Minn 7 × Arig 8, Heartland × Morex, and Minn 7 × Morex—segregated 1 homozygous resistant: 2 segregating: 1 homozygous susceptible family, indicating that one major gene controlled resistance in each population (Table 2). The Manchurian × Rabat 071 population segregated 7 homozygous resistant: 8 segregating: 1 homozygous susceptible family, indicating that either of two genes controlled resistance in Manchurian. No expected ratio fit the segregation in the Heartland × Arig 8 population (Table 2).

The population of Minn 7 × Heartland segregated 7 resistant: 8 segregating: 1 susceptible, indicating that each parent carried a single resistance gene and that the two genes segregated independently (Table 2). When the two resistant cvs. Heartland and Minn 7 were crossed with moderately resistant ACSAD 176, the populations again segregated 7 resistant: 8 segregating: 1 susceptible (Table 2), indicating single nonallelic resistant genes in each cultivar.

We hypothesize that Heartland and Minn 7 possess different major genes that were effective against P. teres in crosses to cultivars with three different levels of susceptibility (Rabat 071, Arig 8, and Morex). Further, Anoidium appeared to have a single gene and Manchurian two independent genes that conditioned resistance in crosses to Rabat 071, as previously reported (11, 14). Heartland had earlier exhibited resistance in the field in the northern Great Plains (21,22), and Minn 7 was resistant in the field in Morocco (3). Single-gene and a two-gene inheritance of resistance to P. teres in lines in the field had been reported in Canada (13) and Egypt (18).

The degree of susceptibility of Rabat 071, Arig 8, and Morex had some effect on the resistance expression of progeny lines. In crosses involving Rabat 071, the severity of net blotch in the most susceptible progenies was less than in Rabat 071. In

contrast, Arig 8 and Morex, which were less susceptible than Rabat 071, each produced a few progenies in crosses with the resistant parents that were more severely diseased than either susceptible parent.

Glasshouse experiment. Net blotch was observed on the second and third leaves of seedlings of cvs. Rabat 071, Arig 8, and ACSAD 176 infected with isolates I-1 and I-12 within 48 h of inoculation; severe leaf necrosis and chlorosis were present after approximately 10 days. Rabat 071 was moderately susceptible to isolate I-1 (IR 6 to 7) and susceptible to isolate I-12 (IR 8 to 10). Arig 8 was susceptible to isolate I-1 (IR 8 to 10) and moderately susceptible to I-12 (IR 6 to 7). ASCAD 176 was moderately susceptible to isolate I-1 (IR 6 to 7) and moderately resistant to isolate I-12 (IR 4 to 5). Heartland was resistant to both isolates (IR 1 to 3). All plants of Rabat 071 were diseased, indicating the effectiveness of the inoculation technique.

The families in the Heartland × Rabat 071 population infected with isolate I-12 segregated 1 resistant: 2 segregating: 1 susceptible (Table 4). When individual plants in the segregating $F_{2:3}$ families were grouped into resistant and susceptible

classes and combined across all families. the observed ratio was 1 resistant: 3 susceptible, indicating a single recessive gene for resistance (Table 3). This finding differs from other reports wherein genes conferring resistance to P. teres were either dominant or incompletely dominant (1,11, 13,18).

Families in the Heartland × Arig 8 population infected with isolate I-1 segregated 1 resistant: 2 segregating: 1 susceptible, indicating that a single gene conferred resistance in Heartland (Table 4). However, when plants within segregating families were classified as resistant and susceptible and combined across families, the expected fit to the 3:1 ratio was poor because of an excess of susceptible plants in segregating families (Table 3).

Families of the Heartland x ACSAD 176 population infected with isolate I-1 segregated 1 resistant: 2 segregating: 1 susceptible (Table 4). Plants within segregating F_{2:3} families segregated 3 resistant: 1 susceptible (Table 3). Thus, both family and plant data support the hypothesis that resistance in Heartland in this cross to isolate I-1 was controlled by a single dominant gene.

The evidence for major gene control of resistance in the experiments on seedlings in the three populations was relatively unambiguous, and consistent with earlier investigations in which single dominant or incompletely dominant genes were reported to condition resistance (1,12,14,22). In one case, our results were different. Resistance in the population Heartland × Rabat 071 infected with isolate I-12 was conditioned by a single recessive gene. We suggest that this conclusion remain tentative until F_1 plants of Heartland \times Rabat 071 can be evaluated with additional isolates.

The lack of fit to the expected ratios in the Heartland × Arig 8 populations cannot be explained. The number of susceptible lines in the field exceeded the expectation for a monogenic segregation ratio. In the glasshouse, the number of segregating families exceeded the expected number.

This study demonstrated that simply inherited resistance is available for use in Morocco. The genes in Heartland, Minn 7. and ACSAD 176 were different, independent, and at single loci. Also, a single gene appeared to be responsible for resistance in Anoidium, whereas two independent genes

Table 2. Net blotch reactions of F_{2.5} families from resistant/susceptible and resistant/resistant barley populations infected with Pyrenophora teres in the

	F _{2:5} family reaction						
Population	Resistant	Segregating	Susceptible	Total families	Theoretical ratio	Chi-square	P
Resistant × Susceptible							
Heartland × Rabat 071	18	31	18	67	1:2:1	0.373	0.90 to 0.80
Minn 7 × Rabat 071	27	51	31	109	1:2:1	0.744	0.70 to 0.60
Anoidium × Rabat 071	29	56	29	114	1:2:1	0.143	0.95 to 0.90
Minn 7 × Arig 8	15	26	15	56	1:2:1	0.280	0.90 to 0.80
Heartland × Morex	26	47	34	107	1:2:1	2.770	0.30 to 0.20
Minn 7 × Morex	16	32	16	64	1:2:1	0.000	< 0.99
Manchurian × Rabat 071	53	53	7	113	7:8:1	0.472	0.80 to 0.70
Heartland × Arig 8	12	32	62	106	?		0.00 to 0.70
Resistant × Resistant							
Minn 7 × Heartland	46	47	6	99	7:8:1	0.297	0.90 to 0.80
Heartland × ACSAD 176	40	44	7	91	7:8:1	0.350	0.90 to 0.80
Minn 7 × ACSAD 176	37	37	7	81	7:8:1	1.115	0.60 to 0.50

Table 3. Frequency of resistant and susceptible F₃ plants within segregating F_{2:3} families of barley populations infected with Pyrenophora teres in the glasshouse

	Plants					
Population	Resistant	Susceptible	Total	— Theoretical ratio	Chi-quare	P
Heartland × Rabat 071 (I-12) ^a	268	786	1,054	1:3	0.10	0.80 to 0.70
Heartland × Arig 8 (I-1)	727	297	1,024	3:1	8.57	<0.005
Heartland × ACSAD 176 (I-1)	1,046	363	1,429	3:1	0.43	0.60 to 0.50

^a Isolate used in inoculation.

Table 4. Reaction of F_{2:3} families from resistant × susceptible barley populations to Pyrenophora teres isolates at seedling stage in the glasshouse

$\mathbf{F}_{2:3}$ families reaction							
Population	Resistant	Segregating	Susceptible	Total families	Hypothesis tested	Chi-square	P
Heartland \times Rabat 071 (I-12) ^a Heartland \times Arig 8 (I-1)	16 40	34 51	18 25	68 116	1:2:1 1:2:1	0.10 5.59	0.95 to 0.90 0.10 to 0.05
Heartland × ACSAD 176 (I-1)	31	41	24	96	1:2:1	3.06	0.30 to 0.20

^a Isolates used in inoculation to classify F_{2:3} lines.

accounted for resistance in Manchuria. Because these resistances are simply inherited, their incorporation into barley adapted to Morocco should not be difficult. Heartland and Minn 7 may be the preferred parents because they are improved lines and both possess a high level of resistance to a range of isolates of P. teres. This is in contrast to Manchurian, which has poor agronomic characters, and to Anoidium, which is agronomically poor and very late in maturing.

In another study, the pathogenic variability of P. teres was indicated to be very high in Morocco (6). In that study, isolates were found that were virulent on each of the resistant cultivars included in this investigation, which suggests that the pathogen could adapt to new cultivars with resistance based on these sources. Accordingly, it may be advantageous to incorporate more than one gene for resistance into cultivars to provide broader resistance. With lines obtained from the populations studied here and selected isolates from Morocco, it should be possible to combine genes from Heartland, Minn 7, and AC-SAD 176 rather quickly. The use in different regions in Morocco of barley cultivars that carry different single genes for resistance is probably not a good strategy, since few Moroccan farmers use certified seed and the transfer of barley seeds from one region to another is common and uncontrolled.

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