Virulence Spectrum to Barley in Some Isolates of *Pyrenophora teres* from the Mediterranean Region

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ABSTRACT

Harrabi, M., and Kamel, A. 1990. Virulence spectrum to barley in some isolates of *Pyrenophora teres* from the Mediterranean region. Plant Dis. 74: 230-232.

Thirty-three isolates of *Pyrenophora teres*, the causal agent of net blotch of barley (*Hordeum vulgare*), were collected from Tunisia, Algeria, Morocco, Egypt, and Cyprus. Their virulence spectra were determined using 10 differential barley cultivars. A cluster analysis indicated that the isolates belonged to four groups. The North African isolates had a higher mean virulence and a low variance across all cultivars; these isolates can be considered as complex races. All isolates were virulent on cultivar Martin, which is widely grown in Tunisia. None of the cultivars tested were highly resistant to all isolates investigated.

Barley net blotch, caused by *Pyrenophora teres* Drechs. (anamorph *Drechslera teres* (Sacc.) Shoem., syn. *Helminthosporium teres* Sacc.), is a disease that can cause serious yield losses of barley. Abdelhak et al (1) reported yield losses of up to 100% in Egypt. In Tunisia, the disease reached epidemic proportions in 1985 and 1987 (Harrabi, *unpublished*), and increased prevalence of the disease has been reported in several North African and Middle Eastern countries (3,5).

Accepted for publication 18 August 1989 (submitted for electronic processing).

The pathogen is a highly variable organism and there are several virulence types. Bjarko (2) tested 26 isolates of *P. teres*—15 from the Middle East and 11 from Montana—and separated the Middle Eastern isolates into seven virulence types and the Montana isolates into five virulence types. In Australia, three virulence types have been reported (6). McDonald and Buchannon (7) reported two virulence types among isolates from Canada, Mexico, North Dakota, and California. Tekauz and Mills (10) found new isolates in Canada that were virulent to two previously resistant cultivars. Durable resistance to *P. teres* may, therefore, be difficult to achieve.

The genetics of resistance to *P. teres* in barley has not been fully investigated. Only five genes for resistance have been reported. Khan and Boyd (6) found a single gene (Pta), in CI 2330, and concluded that the previously reported genes Pt1 and Pt2 were allelic. Schaller (9) found a single incompletely dominant gene (Pt) in Tifang. Mode and Schaller (8) identified three genes (Pt1, Pt2, and Pt3) in six resistant cultivars. The high variability of *P. teres* has important implications for breeding programs.

Our study investigated virulence types in isolates of *P. teres* collected from countries in the Mediterranean region where the disease is economically important.

MATERIALS AND METHODS

Description of isolates. Thirty-three isolates of *P. teres* originating in five countries and three geographical regions were evaluated on specific differential barley cultivars. The distribution of the isolates was as follows: 23 from Tunisia, 2 from Algeria, 3 from Morocco, 3 from Cyprus, and 2 from Egypt (Table 1).

Isolation and maintenance of isolates. Leaf tissue with typical net blotch symptoms was surface-sterilized in a 1% sodium hypochlorite solution for 2 min, placed onto plates containing 20% V-8 agar, and incubated for 2 days at 20 C.

During incubation, 10-hr periods of fluorescent light (170 $\mu E \cdot m^{-2} \cdot s^{-1}$) were alternated with 14-hr periods of darkness. Conidia produced from the leaf tissue were transferred singly onto petri dishes containing V-8 agar and incubated for 10–15 days under the conditions described above.

Tests for virulence. Ten barley cultivars (Table 2) from different barley breeding programs were used to evaluate isolates of *P. teres*. The cultivars were chosen on the basis of their differential reactions and diverse origins from among 300 cultivars and lines tested prior to this study. A universal susceptible control (cv. Martin, from Tunisia) was included in the experiment.

Twenty seeds per cultivar were surface-disinfested with 1% sodium hypochlorite for 8 min and 95% ethanol for 15 sec. The seeds were then planted in metal flats (60 × 40 × 4 cm). Each flat contained two rows of each cultivar. The flats were arranged in a completely randomized design with four replications. The experiment was repeated two times.

A suspension made with conidia from 10-day-old *P. teres* cultures was adjusted to about 4×10^4 conidia per milliliter and then atomized onto 10-day-old seedlings. Inoculated plants were placed in a darkened dew chamber maintained at about 20 C for 24 hr, then returned to the growth chamber. After 7 days, disease ratings were made on a 0–4 scale, where 0 = no observable infection; 1 = pinpoint lesions, no chlorosis; 2 = slightly elongated lesions, slight chlorosis; 3 = elongated lesions crisscrossed with netlike venation, moderate chlorosis; and 4 = well-developed lesions, extensive chlorosis and necrosis.

Results were analyzed by means of a cluster-analysis program from the BMDP statistical software (4), using mean disease rating for each cultivar across the four replications. The purpose of cluster analysis was to place isolates into groups suggested by the data, not defined a priori. As a result, isolates in a given cluster would tend to be similar to each other in terms of disease rating and across all cultivars.

The similarity was measured by the Euclidean distance between the responses according to the following formula: $D_{KL} = (\bar{X}_K - \bar{X}_L)/(1/n_K + 1/n_L)$, where D_{KL} is any distance between clusters C_K and C_L, $\bar{X}_K$ is the mean vector for cluster C_K, $\bar{X}_L$ is the mean vector for cluster C_L, and n_K and n_L are the number of observations in clusters C_K and C_L, respectively.

RESULTS AND DISCUSSION

The isolates were clustered into four groups (Table 3). Cluster 1 was composed of isolates from northern Tunisia, Morocco, and Algeria. This cluster had the highest virulence ratings, with a mean disease rating of 3.3. Most of the isolates in cluster 1 were collected from subhumid areas. Cluster 2 was composed of both isolates from Egypt, two from Cyprus, and isolates collected from the semiarid areas of Tunisia. This cluster was made up of isolates of intermediate virulence, with a mean disease rating of 2.6. The frequency of isolates within this cluster was 33%, the highest of all the clusters. All isolates in cluster 3 were collected from intermediate rainfall areas in Tunisia, except isolate NB 26/1984, which came from a local land race barley population grown in an oasis in southern Tunisia. Microclimatic conditions in the oasis are characterized by high humidity

Table 1. Isolates of *Pyrenophora teres* collected from different geographic regions in the Mediterranean area

Isolate/year collected	Where collected		Cultivar on which collected
	Country	Locality	
NB 9/1987	Tunisia	Kelibia	Local
NBI 13/1987	Algeria	Guelma	Saida
NB 29/1987	Tunisia	Bir Mecharga	Martin
NBI 21/1987	Morocco	Merchouch	KLDN/ICARDA
NBI 8/1987	Cyprus	Athalassa	Kantara
NBI 27/1987	Morocco	Meknes	Local
NBI 19/1987	Morocco	Settat	Brasserie Maroc
NBI 14/1987	Egypt	Unknown	Unknown
NB 250/1987	Tunisia	Siliana	Local
NBI 23/1987	Algeria	Oued Smar	Saida
NBI 5/1987	Egypt	Sakha	Giza 117
NB 212/1987	Tunisia	Kef	Local
NBI 11/1987	Cyprus	Laxia	Six-row local
NB 262/1987	Tunisia	Zaghouan	Six-row local
NB 225/1987	Tunisia	Kef	Local
NB 34/1987	Tunisia	Zaghouan	Martin
NB 264/1987	Tunisia	Zaghouan	Martin
NB 31/1987	Tunisia	Fahs	Local
NB 28/1987	Tunisia	Bourbia	Martin
NBI 10/1987	Cyprus	Laxia	Kantara
NB 132/1987	Tunisia	Beja	Martin
NB 22/1985	Tunisia	Fahs	Local
NB 23/1985	Tunisia	Sidi Rabah	Local
NB 24/1985	Tunisia	Lorbus	Local
NB 25/1985	Tunisia	Sidi Amor	Local
NB 26/1984	Tunisia	Telmime (oasis)	Local
NB 27/1985	Tunisia	Tebika	Local
NB 28/1985	Tunisia	Soliman	Local
NB 29/1985	Tunisia	Tebourba	Martin
NB 30/1985	Tunisia	Beja	Local
NB 31/1985	Tunisia	Goubellat	Martin
NB 32/1985	Tunisia	Mater	Martin
NB 33/1985	Tunisia	Tunis	Unknown

Table 2. Mean disease rating of 33 isolates of *Pyrenophora teres* on 10 barley cultivars used as differentials

Cultivar	Row type	Origin	Mean disease rating[x]
Martin	6	Tunisia	3.8 c[y]
Turk	6	United States	3.5 c
Strain 205	6	Ethiopia	2.5 b
WI2291/EH70-f_3 ICB78-670-7AP-0A9	2	ICARDA[z]	2.4 ab
Deir Alla 106/Strain 205 ICB77-99-1AP-0AP	6	ICARDA	2.4 ab
Line 251/14	6	Denmark	2.4 ab
Martin × Universe TC74-52-2Bj-0Bj	6	Tunisia	2.3 ab
Herawi	6	Egypt	2.2 ab
WI2197/Cam ICB77-19-1AP-5AP-1AP-0AP	2	ICARDA	2.1 ab
Emir//Apam/Hc1905 ICB78-817-3AP-0AP	2	ICARDA	1.7 a

[x] Values are the means of 33 isolates based on a 0–4 scale, where 0 = no observable infection; 1 = pinpoint lesions, no chlorosis; 2 = slightly elongated lesions, slight chlorosis; 3 = elongated lesions, crisscrossed with netlike venation, moderate chlorosis; and 4 = well-developed lesions, extensive chlorosis and necrosis.

[y] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.

Table 3. Cluster analysis of mean disease rating of 33 isolates of *Pyrenophora teres* on 10 barley cultivars

Cluster	Isolates in cluster	Frequency of isolates	Mean disease rating[y]
1	NB 9/1987, NBI 13/1987, NB 29/1985, NB 29/1987, NBI 21/1987, NBI 23/1987, NB 212/1987, NBI 27/1987, NB 25/1985, NB 32/1985	0.31	3.3 a[z]
2	NB 250/1987, NB 30/1985, NBI 8/1987, NBI 11/1987, NBI 19/1987, NB 225/1987, NB 34/1987, NB 24/1985, NBI 14/1987, NB 262/1987, NBI 5/1987	0.33	2.6 ab
3	NB 264/1987, NB 22/1985, NB 28/1985, NB 33/1985, NB 26/1984, NB 28/1987	0.18	2.2 bc
4	NBI 10/1987, NBI 132/1987, NB 31/1985, NB 31/1987, NB 23/1985, NB 27/1985	0.18	1.6 c

[y] Values are the means of four replications of all isolates based on a 0–4 scale, where 0 = no observable infection; 1 = pinpoint lesions, no chlorosis; 2 = slightly elongated lesions, slight chlorosis; 3 = elongated lesions, crisscrossed with netlike venation, moderate chlorosis; and 4 = well-developed lesions, extensive chlorosis and necrosis.

[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

because of irrigation and a greenhouse effect under tall, densely grown palm trees. Isolates in cluster 3 had a frequency of 18% and a mean disease rating of 2.2. Isolates in cluster 4 originated in different areas with varying climatic conditions. They had a frequency of 18% and a mean disease rating of 1.6.

Martin, a local cultivar from Tunisia, was susceptible to all isolates and thus was considered universally susceptible. None of the cultivars tested were highly resistant to all isolates. Emir//Apam/Hc1905 from the International Center for Agricultural Research in the Dry Areas (ICARDA) had the lowest mean disease rating—significantly lower than Martin, Turk, and Strain 205 (Table 2). The remaining cultivars had intermediate reactions across all isolates.

Table 4 shows the mean disease rating and variance of each isolate when tested on the 10 barley differentials. A mean disease rating of 3.0 or more and low variance indicated a high level of virulence across all cultivars. Isolates NB 9/1987, NB 29/1987, NB 212/1987, and NB 250/1987 from Tunisia; NBI 13/1987 and NBI 23/1987 from Algeria; NBI 21/1987 and NBI 27/1987 from Morocco; and NBI 5/1987 from Egypt have mean disease ratings of 3.1–3.8 and variances of 0.16–0.81. These isolates have high virulence to the 10 cultivars. NB24/1985, from a semiarid area in Tunisia, had the highest variance but an intermediate mean disease rating. Isolate NB132/1987 had the lowest mean disease rating and a low variance, indicating that it is not highly virulent to the 10 cultivars.

P. teres is a major constraint on increased barley production. A knowledge of its virulence spectrum may aid in designing proper breeding strategies. Given the high variability between isolates of *P. teres* found in our study, barley breeding programs should also concentrate on nonspecific types of resistance. National barley breeding programs and those of international centers such as ICARDA and CIMMYT (Centro Internacional de Mejoramento de Maíz y Trigo) could develop this resistance in barley cultivars by using the multilocation testing approach in different countries. Resistant or intermediate cultivars selected from the multilocation disease nurseries distributed by ICARDA could be crossed together to pyramid resistance genes into adapted cultivars. To achieve nonspecific resistance, progeny resulting from these crosses could be tested across many countries with varying virulence types. This type of resistance cannot be achieved without the proper knowledge of the virulence spectrum of the pathogen and the sources of host resistance.

Table 4. Mean disease rating and variance of 33 isolates of *Pyrenophora teres* tested on 10 barley cultivars

Isolate	Mean disease rating[y]	Variance[z]
NB 9/1987	3.8	0.16
NBI 13/1987	3.6	0.49
NB 29/1987	3.6	0.49
NBI 21/1987	3.6	0.49
NBI 23/1987	3.3	0.16
NB 212/1987	3.3	0.16
NBI 27/1987	3.1	0.81
NB 250/1987	3.1	0.46
NB 29/1985	3.1	1.21
NBI 5/1987	3.1	0.75
NBI 8/1987	3.0	1.00
NBI 19/1987	2.9	1.44
NBI 14/1987	2.9	1.00
NB 25/1985	2.9	1.21
NB 32/1985	2.8	1.95
NBI 11/1987	2.6	0.71
NB 225/1987	2.6	0.92
NB 26/1984	2.6	0.93
NB 264/1987	2.5	0.71
NB 262/1987	2.4	1.37
NB 30/1985	2.3	1.12
NB 31/1987	2.3	1.10
NB 22/1985	2.2	0.83
NB 34/1987	2.2	1.28
NB 27/1985	2.1	0.94
NB 28/1985	2.0	1.11
NB 33/1985	2.0	1.55
NB 24/1985	2.0	2.66
NB 28/1987	1.9	0.98
NB 23/1985	1.6	1.26
NB 31/1985	1.4	1.60
NBI 10/1987	1.3	1.32
NB 132/1987	1.2	0.83
LSD ($P = 0.05$)	0.8	

[y] Values are the average of the disease rating of 10 cultivars replicated four times based on a 0–4 scale, where 0 = no observable infection; 1 = pinpoint lesions, no chlorosis; 2 = slightly elongated lesions, slight chlorosis; 3 = elongated lesions, crisscrossed with netlike venation, moderate chlorosis; and 4 = well-developed lesions, extensive chlorosis and necrosis.

[z] Infection type of each cultivar replicated four times ($n = 40$).

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