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Variation in the pathogenicity of 20 Algerian isolates of *Pyrenophora graminea* Ito & Kur. on nine barley *(Hordeum vulgare* L.) varieties

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Summary. Pathogenic variability in isolates of *Pyrenophora graminea*, the casual organism of leaf stripe of barley (*Hordeum vulgare L.*), collected from different barley-growing areas of Algeria was investigated by artificially inoculating 19 isolates from Algeria and 1 from Syria on nine barley varieties. The isolates were found to exhibit variation and physiological specialization and variation to particular barley varieties. All isolates had different virulence spectra and could be divided into 12 homogeneous groups. The most virulent group was composed of six isolates each, virulent on six of the nine barley varieties. The isolate from Syria was the least virulent. The results demonstrated that the population of *P. graminea* was highly variable at country level and within regions. The barley varieties used differed in the genetic factors making them resistant to the pathogen and in this way it was possible to discriminate among the isolates. Varieties NK 1272 and M 23 were resistant to all isolates. This set of genotypes can be used as differentials in the future and as sources of resistance for any geographic and sequential gene deployment strategy.

Key words: pathogenic variability, *Pyrenophora graminea,* Algerian isolates, barley, *Hordeum vulgare.*

Introduction

Leaf stripe of barley, caused by *Pyrenophora graminea* Ito & Kuribayashi (conidial stage: *Drechslera graminea* Rabh. ex Schlt.), synonym of *Helminthosporium gramineum* Rabh., is a destructive disease in most barley-growing regions in the world (Richardson *et al.*, 1976; Johnston *et al.*, 1982; Porta-Puglia *et al.*, 1985).

Leaf stripe is a monocyclic systemic disease. The pathogen is carried from year to year as resting mycelium in the pericarp and seed coat of infected kernels (Drechsler, 1923). Upon germination of the seed, the fungus resumes its activity and may infect the growing seedling. The first symptoms of the disease may be seen on seedlings, but characteristic symptoms become prominent only after tillering. Later in the cycle, most of the leaves of infected plants develop brown stripes. The spikes fail to extrude from their sheets, heading is difficult or cannot take place and the seed produced is shriveled.

Field trials suggest the existence of a wide variation in the reaction of barley cultivars to the pathogen, ranging from complete resistance to high susceptibility (Kline, 1971; Tekauz, 1983; Knudsen, 1986; Boulif and Wilcoxson, 1988; Delogu *et al.*, 1989). These findings are due to the genetic

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variability of barley genotypes or of *P. graminea* isolates or both. Considerable variation in the pathogenicity of different isolates of *P. graminea* has been reported by researchers (Christensen and Graham, 1934; Arny, 1945; Kline, 1972; Mohammad and Mahmood, 1976; Tekauz, 1983; Hammouda, 1988; Gatti *et al.*, 1992). The last authors also found large biochemical variation.

Physiological specialization to particular barley varieties was first reported in the United States in 1925 (Johnson, 1925). Four to five physiological races of *P. graminea* have been identified (Knudsen, 1986).

At present, identification of the causal agent is based on the morphology of the conidiophores and conidia. However, it is difficult to distinguish among isolates using morphological characters alone. Recently, molecular markers have been introduced as an aid to a better characterization of fungal species and to distinguish genetically between isolates of the same species (Michelmore and Hulbert, 1987).

Few researchers have assessed the limits of resistance or susceptibility of barley genotypes to *P. graminea*. Shands and Arny (1944) defined plants with 15% or less infection after inoculation by the pathogen as resistant, and plants with 60% infection or more as susceptible. Scott and Knott (1974) developed a statistical test based on the mean infection rate of barley genotypes, in which isolates were classified as avirulent (causing 0-6% infected plants), intermediate (13-16%), and virulent (>16%).

Several methods are used to test the reaction of barley cultivars to *P. graminea:* the sandwich method (Houston and Oswald, 1948; Mohammad and Mahmood, 1974), the floral inoculation and grain inoculation at maturity (Kline, 1971); exposure of plants to natural inoculum (Knudsen, 1980); and, more recently, inoculation by toxin filtrate (Haegi et al, 1994). Nilsson (1976) found that the method with low incubating temperatures gave the best results.

A knowledge of the pathogen population structure is essential for an efficient approach to breeding for resistance. The purpose of this study was to assess isolate variability and pathogen virulence on different barley varieties.

Table 1. List of barley varieties inoculated with *Pyrenophora graminea*.

Variety	Origin of seeds	Reaction to *P. graminea*
Arig 8	INRA-Morocco	Susceptible
Alpha	ICARDA	Resistant
Tichedrett	Algeria	Susceptible
Rihane 03	ICARDA	Intermediate
Harmal	ICARDA	Resistant
M23	USA	Resistant
Saida	Algeria	Susceptible
Jaidor	France	Intermediate
NK 1272	USA	Resistant

Materials and methods

Nine barley genotypes of diverse origin and with different reactions to leaf stripe (Table 1) were artificially inoculated with 19 Algerian and one Syrian isolate of *P. graminea* (Table 2). The inoculum

Table 2. Sites where isolates of *Pyrenophora graminea* were collected in Algeria and Syria.

Isolate No.	Location	Isolate No.	Location	Country
1	Rahmoune/Constantine	11	Yagou/Batna	Algeria
2	Grarem/Mila	12	Khenchela	"
3	Caid Laid/Mila	13	Kais/Khenchela	"
4	Guelma	14	Setif	"
5	Tamlouka/Guelma	15	Arrerridj	"
6	Hamla/Bouaghi	16	Boiimaiza/Skiid-a	"
7	Sedrata/Ahras	17	Ain Berda/Annaba	"
8	Ain Seynour/S. Ahras	18	Rahouia/Tiaret	"
9	Ksar Sbahi	19	Sougeur/Tiaret	"
10	Batna	20	Breda	Syria

from Algeria was collected from different regions in the 1994-1995 season. Isolation consisted in removing small pieces from infected barley leaves, dipping them in 5% sodium hypochlorite for 3 min, washing them two or three times in distilled and sterilized water and drying them on filter paper. The disinfected pieces were then plated in Petri dishes (3-4 pieces per dish) containing 20 ml potato dextrose agar (PDA). After 7-8 days of incubation, monoconidia were transferred to another PDA plate and incubated for 10 days. Seeds of the varieties used in this study were treated in the same way as the leaves.

Artificial inoculation was by the sandwich method. Approximately 50 kernels per variety were germinated by the sandwich method at 4 ℃ for 13 days in the dark in Petri dishes (1 dish per variety) containing a mycelial mat. Germinated seeds were picked out from a plate and planted in the field in a 1-m row at the INRA Research Center at Meknès, Morocco. A split-plot design with 4 replicates was used. Varieties were the main plots and isolates were the sub-plots. At heading, the total number of plants produced and the number of diseased plants were recorded. The data were transformed to arcsin $x^{1/2}$ (where x was the percentage of diseased plants).

Analysis of variance was performed using the SAS statistical package, while hierarchical cluster analysis was performed using the NTSYS program (single linkage with squared Euclidean distances). Because of the wide variation between replications, evaluation and analysis were based on maximum incidence. In view of the polygenic nature of the inheritance of resistance to *P. graminea* as stated by many authors (Suneson, 1950; Knudsen, 1980), and variability in the reaction of the germplasm, barley varieties showing less than 15% of maximum incidence were classified as resistant.

Results and discussion

Leaf stripe in the field developed very well on susceptible barley varieties, showing the effectiveness of the inoculation method used. Analysis of variance showed significant differences both among isolates of *P. graminea* and among barley varieties. The variety x isolate interaction was also highly significant, suggesting that the varieties reacted differently to different isolates (Table 3) and consequently showed pathogenic specialization in physiological races and the existence of different genetic resistance factors in the host varieties. Our results confirmed the physiological specialization hypothesis in *P. graminea* reported by many authors. One hundred percent incidence levels were observed on some replications of susceptible varieties. The large variations observed between replications can be explained by the fact that some kernels were strongly invaded by large amounts of inoculum and some lost their seed coat during the soaking process, confirming the observation made by Mohammad and Mahmood (1974).

Based on the mean incidence level (Table 4) and the classification of isolates as virulent or avirulent using the 15% incidence limit (Table 5), all isolates tested had different virulence spectra. Isolate 20 from Syria had a low virulence on all varieties used. Isolate 7 was virulent on Arig 8 only. Isolates 3, 4, 14, 15 and 18 were virulent on two of

Table 3. Analysis of variance of the barley stripe disease incidence on nine barley varieties artificially inoculated with 20 isolates of *Pyrenophora graminea*.

Source of variation	Degree of freedom	Mean squares	F-value	Coefficient of variation (%)
Variance total S-bloc	35	2989.50	00.00	
Varieties	8	10807.08	18.26	
Replications	3	1323.84	2.24	
Error l	24	591.84		130.0
Total variance	719	447.23		
Isolates	19	1258.94	5.83	
Varieties x Isolates	152	541.09	2.51	
Variance total S-Bloc	35	2989.50	13.85	
Error 2	513	215.91		87.5

Table 4. Mean disease incidence[a] on barley varieties inoculated with 20 *P. graminea* isolates.

Isolate	Varieties								
	Arig 8	Alpha	Tichedrett	Rihane	Harmal	M23	Saida	Jaidor	NK 1272
1	30.5	47.2	18.1	11.8	18.7	3.9	25.8	44.4	0.0
2	24.9	21.4	20.7	24.7	11.6	4.2	24.4	6.3	0.0
3	12.4	33.4	5.0	10.5	31.1	2.8	13.5	13.0	0.0
4	35.1	4.2	38.2	10.0	2.8	0.0	8.6	2.6	0.0
5	20.8	5.1	25.7	9.9	6.2	0.0	25.9	9.5	0.0
6	18.4	36.2	25.2	16.3	4.2	3.6	10.1	2.6	0.0
7	21.1	0.0	1.4	11.9	0.0	0.0	1.0	6.2	0.0
8	32.2	22.2	61.3	9.2	28.0	0.0	15.2	0.0	0.0
9	24.9	3.6	54.4	26.9	3.6	6.1	43.6	2.3	0.0
10	61.1	18.7	24.5	29.5	18.4	0.0	32.9	14.3	0.0
11	71.0	50.0	5.5	31.3	25.0	0.0	45.0	74.7	0.0
12	69.7	75.5	57.8	18.4	6.2	0.0	47.6	29.3	0.0
13	19.0	31.5	25.8	7.7	15.7	0.0	14.5	18.6	0.0
14	44.3	3.6	14.4	9.6	21.9	2.1	15.0	3.7	0.0
15	58.5	0.0	77.1	15.4	14.6	2.3	8.0	8.1	0.0
16	57.0	16.7	66.1	29.3	67.0	0.0	47.0	15.2	0.0
17	25.0	0.0	38.2	21.8	15.9	1.1	18.8	34.4	0.0
18	22.4	13.1	24.1	7.1	7.6	0.0	10.9	0.6	0.0
19	27.8	36.3	63.4	13.0	27.7	9.4	72.7	0.0	0.0
20	15.3	13.6	15.0	1.1	15.4	0.5	4.5	2.3	0.0

[a] Least significance differences at P=0.05: isolates, 6.7; varieties, 7.9; isolate x varieties, 20.6.

Table 5. Virulence (H = high; L = low) of 20 isolates of *Pyrenophora graminea* on 9 barley varieties.

Isolate	Variety									High/Low
	Arig 8	Alpha	Tichedrett	Rihane	Harmal	M23	Saida	Jaidor	NK 1272	
1	H	H	H	L	H	L	H	H	L	6/3
2	H	H	H	H	L	L	H	L	L	5/4
3	L	H	L	L	H	L	L	L	L	2/7
4	H	L	H	L	L	L	L	L	L	2/7
5	H	L	H	L	L	L	H	L	L	3/6
6	H	H	H	H	L	L	L	L	L	4/5
7	H	L	L	L	L	L	L	L	L	1/8
8	H	H	H	L	H	L	L	L	L	4/5
9	H	L	H	H	L	L	H	L	L	4/5
10	H	H	H	H	H	L	H	L	L	6/3
11	H	H	L	H	H	L	H	H	L	6/3
12	H	H	H	H	L	L	H	H	L	6/3
13	H	H	H	L	L	L	L	H	L	4/5
14	H	L	L	L	H	L	L	L	L	2/7
15	H	L	H	L	L	L	L	L	L	2/7
16	H	H	H	H	H	L	H	L	L	6/3
17	H	L	H	H	H	L	H	H	L	6/3
18	H	L	H	L	L	L	L	L	L	2/7
19	H	H	H	L	H	L	H	L	L	5/4
20	L	L	L	L	L	L	L	L	L	0/9

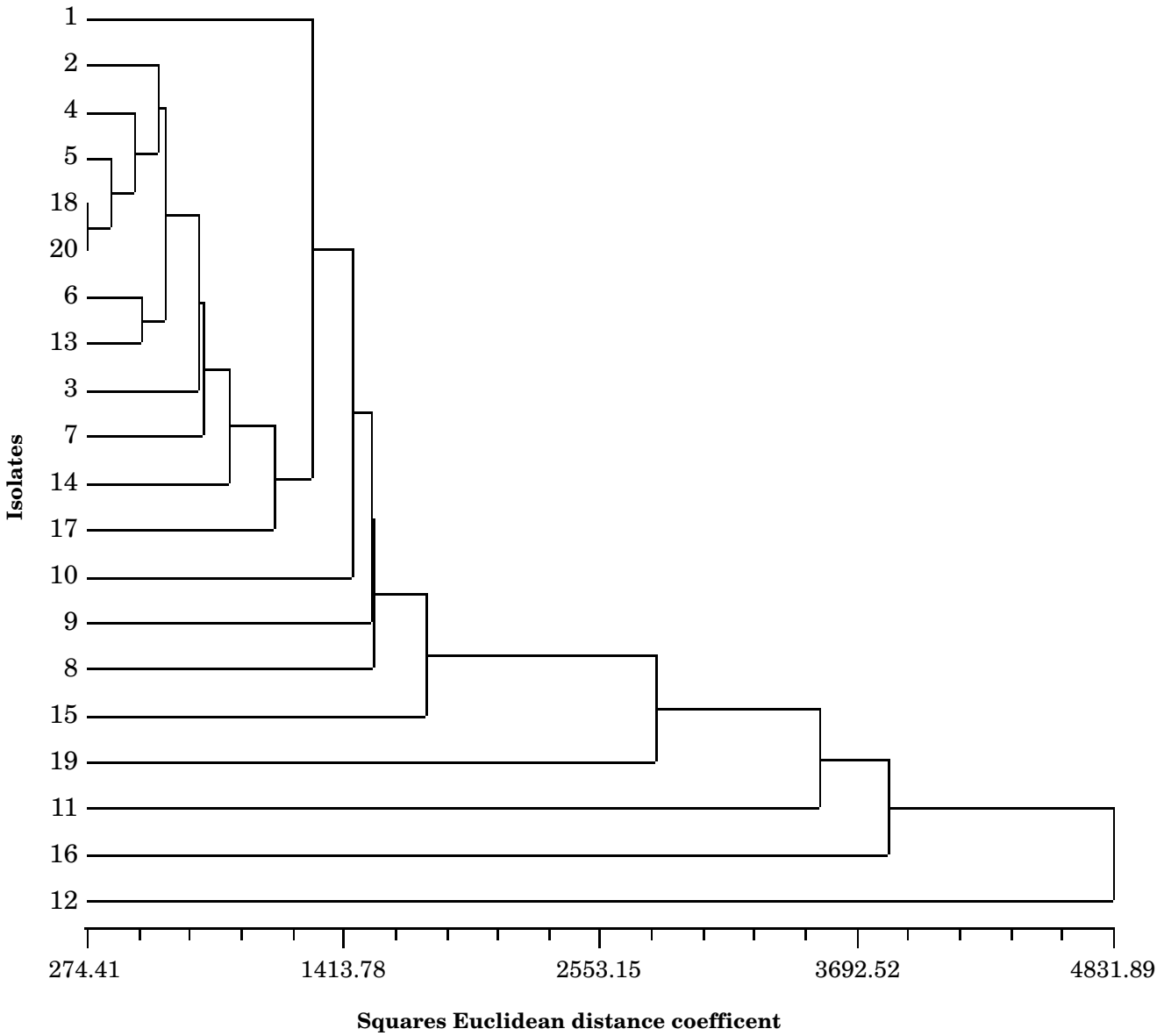

Fig. 1. Dendrogram of 20 isolates of *Pyrenophora graminea*.

the nine barley varieties and were differentiated by their reaction to different pairs of the barley varieties Alpha, Tichedrett and Saida. Isolate 5 was virulent on the varieties Arig 8, Tichedrett and Saida. Four isolates (6, 8, 9, 13) were each virulent on four varieties and could be discriminated using the varieties Rihane, Alpha, Saida, Jaidor and Harmal. Two isolates (2, 19) were virulent on five barley varieties. Isolates 1,10, 11,12,16 and 17 and 19 were the most virulent since they developed a susceptible disease reaction on six of the nine barley varieties. All the tested isolates could be discriminated by their reaction on seven varieties, since genotypes NK 1272 and M 23 were highly resistant to all isolates.

Using cluster analysis, the isolates were classified in 14 groups (Figure 1). Isolates 2, 4, 5, 18 and 20 formed the first group and included isolates col-

lected from different regions of Algeria and even the isolate from Syria. Most of these isolates were collected from Eastern high plateau. Isolates 6 and 13 were classified in the second group while all the remaining isolates had increased values of the distance coefficients and could not be grouped. These results showed clearly that the population of *P. graminea* was highly variable at country level and from region to region. However, isolates of *P. graminea* were generally more specific to regions than are other foliar diseases such as rusts or powdery mildew. This can be explained by the fact that *P. graminea* is exclusively seed-borne and that barley seed in Algeria is not transported very much from region to region and also because the pathogen of barley stripe is a monocyclic disease having a low level of epidemic potential (Richardson, 1976; Knudsen, 1986).

Regarding the reaction of barley varieties to the pathogen, Arig 8 was susceptible to all Algerian isolates but was resistant to the isolate from Syria. Tichedrett was susceptible to 15 isolates and Alpha and Saida were susceptible to 11 isolates. Rihane and Jaidor, two cultivars newly released in Algeria, were resistant to 12 and 15 isolates, respectively. The varieties NK 1272 and M23 showed resistance to all isolates. The varieties Harmal and Alpha, reported to be resistant to this pathogen by Boulif and Wilcoxson (1988), were susceptible to at least 9 isolates, suggesting that the isolates in Algeria were different from those in Morocco. These barley varieties appear to have different genes conferring resistance to *P. graminea*.

Any attempt to breed for resistance to this pathogen should be based on geographical and sequential use of available sources of resistance. The inheritance of resistance in some varieties is currently being investigated. The results obtained further showed that the nine varieties used in this study differentiated among all the isolates and therefore constituted a set of differentials that can be used in Algeria and in North African countries.

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