Physiological Specialization of *Septoria tritici* in North-Africa

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Keywords : *Septoria tritici* - Durum wheat - Physiological specialization.

Summary

Five isolates of Septoria tritici, *collected from durum wheat originating from four locations of Tunisia: Tunis, Fretissa, Bizerte, Pont Bizerte and from Algeria, were inoculated on a set of eight durum wheat cultivars. The isolates were investigated in terms of their morphological characteristics as well as their variability. Physiologic specialization of the pathogen was clearly demonstrated as a result of the differential interaction between the durum wheat cultivars and the isolates. The five isolates could be grouped into three distinct groups according to four evaluation methods: the linear inlection index (LII), pycnidia density (PD), percentage of attack severity (PAS) and percentage of necrosis as evaluated by loss of chlorophyll rate (CR). The results showed that the LII and PAS are more useful to discriminate between isolates than the PD and CR.*

Résumé

Cinq isolats de Septoria tritici *sur blé dur collectés de quatre régions de Tunisie: Tunis, Frétissa, Bizerte, Pont de Bizerte et un isolat d'Algérie ont été utilisés pour étudier leur réaction sur huit variétés de blé dur. Les isolats ont été identifiés en terme de leur caractéristiques morphologiques et pathologiques. La spécialisation physiologique du pathogène a été clairement démontrée comme résultat de l'interaction entre les variétés différentielles et les isolats. Les cinq isolats pourraient être classés en trois groupes distincts en se basant sur quatre méthodes d'évaluation: l'indice d'infection linéaire (LII), la densité des pycnides (PD), le pourcentage de sévérité de l'attaque (PAS) et la teneur en chlorophylle (CR). Les résultats montrent que le LII et PAS sont plus utilisés pour mieux discriminer les isolats que la PD et le CR.*

Introduction

Mycosphaerella graminicola (Fuckel) (anamorph: *Septoria tritici* Rob. ex. Desm.) causes *Septoria tritici* blotch on wheat which is a serious threat to wheat production in the Mediterranean countries. Yield losses from slight to 60%, have been attributed to naturel infection (9, 26). Losses occasionally may be as high as 70% (15). Yield losses are often accompanied by corresponding decreases in grain weight (5, 9, 14, 26). The severity of the disease is closely related to rainfall frequency and cool weather conditions (25). Consequently, severe epidemics have been sporadic in most geographic areas.

Breeding for resistance is generally considered the best method of control. Genetic resistance in durum wheat *(Triticum durum* L.) has been reported by several authors (4, 12, 13, 21, 24, 27). Different models of inheritance were suggested including monogenic control (5, 14, 20, 22), control through two additive genes (14) and control by several genes (7).

Physiologic specialization has not been studied extensively. Although many authors have showed the absence of physiologic races of *Septoria tritici* (1, 2, 3, 8, 14, 17, 18, 19); others have demonstrated the contrary (6, 11, 16, 23, 26).

Morales (13) tested the host-pathogen interaction using four isolates of *S. tritici* from 16 durum wheat cultivars. No physiologic specialization of the pathogen was reported. Then, Perello et al. (17) tested 10^3 isolates of *Septoria tritici* originating from different areas in Argentine. They concluded that there were no true physiologic races. Shipton et al (27) found at least three races. In Argentine, preliminary searches revealed a certain degree of physiologic specialization (6, 16).

Saadaoui (23) tested 19 isolates collected from different wheatgrowing areas of Morocco and inoculated them to a set of seven wheat cultivars used as differentials. He concluded that the virulence groups identified could be considered as physiologic races of *Septoria tritici* in the convential sense.

In view of` the importance of physiologic specialization for a sound breeding program, the present study was undertaken to determine whether physiological races of *S. tritici* occur in the North-African region.

Material and methods

Isolation and inoculum production

Five isolates of *Septoria tritici* were cultured from infected wheat leaves collected from Tunis, Fretissa, Bizerte, Pont Bizerte and Algeria. These isolates were obtained from leaves with typical lesions. Conidia were released from pycnidia by immersing a leaf in approximately 5 ml of sterile distilled water and the resulting conidial suspension was streaked onto the surface of PDA plates (20 g of Potato-Dextrose-Agar,

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Received on 10.04.96 and accepted for publication on 02.08.96.

5 g of glucose in 1 liter distilled water). Cultures were grown at 22°C for 10 h under UV and white fluorescent lighting (22 microEm^{-2}). After 3-5 days, several creamy pink-colored colonies from each plate were transfered to other PDA plates for multiplication.

Inoculum for each isolate was prepared from culture plates (10 days-old). Suspensions were adjusted to approximately 1x10^{7} conidia per milliliter with a hemacytometer. Their morphological variability was studied on the basis of color of spores, size of pycnidiospores and production of spores.

Hosts

Septoria isolates were inoculated to a set of eight cultivars used as differentials. The durum wheat cultivars are: Razzak, Maghrebi, Karim 80, Ben Bechir 79, Badri, Amal, CHEN'S'-CD 26406-3B-5Y-OM-16Y-OB and CHEN'S'-CD 26406-1B-1Y-2Y-OM-1Y-OM originating from the Intemational Maize and Wheat Improvement Center (CIMMYT) and selected in Tunisia.

The experiment was carried out in the growth chamber under controlled conditions of 20°C for 12 h under UV and white fluorescent light of 22 microm^{-2}s^{-2}. The seeding was done, on a sterilized sand and in aluminium trays. A randomized design with three replicates was used. The eight varieties were sown in the same tray. The susceptible control was the wheat cultivar Morocco.

The seedlings were inoculated at the two leaves stage by spraying with a spore suspension of 10^{7} spores per milliliter as determined by hemacytometer counting. The trays were placed in a dew-chamber (relative humidity 100%) for 48 h before transferring to the growth chamber.

Disease assessment

Three criteria were used to characterize the isolates morphologically:

1. Sporulation was determined by counting the number of spores per milliliter using a hemacytometer.

2. Size of spores by measuring the length and width of 10 spores taken at random.

3. Cultures color: the culture which varied between rose-coloured and black were described visually on PDA medium.

The disease was scored after full symptom development 21 days after inoculation. Virulence assessment was estimated by four evaluation criteria: linear infection index (LII), calculated as a ratio between total lesions length and total leaf length. Pycnidia density (PD), calculated as the number of pycnidia per unit of leaf area. The percentage of attack severity (PAS) based on a 10-step scale ranging from 0=0% of infection to 9=90% of leaf area destroyed. The last criterium is chlorophyll rate (CR) determined using the following fomula:

$$CR = \frac{E652 \times V \times 10}{X \times 36}$$

E652: value of absorbance on spectrophotometer at 652 nm.

CR: chlorophyll rate (mg chl/g dried weight).

V: necessary volume of aceton for extraction (ml).

X: weight of dried extract (g).

To determine chlorophyll rate, we take wheat leaves samples from every treatment (check, leaves inoculated separately with different isolates). Each sample was grinded mixed with 5 ml of cooled aceton (100%) and put in a bottle in the freezer. The next step is the mixing in order to get a very fine residue which will allow us to extrate soluble chlorophyllian pigment. After, the centrifugation of 3000 trs/mn was done at temperature of 5°C and for 10 mn. The floater was collected in erlenmeyer and stored in the freezer. The residue was again mixed in cooled aceton of 80%. After the second centrifugation, all the previous steps were remade to obtain residue without chlorophyll. The volume of all floater was determined and the residue will be dried at 60°C to get dry weight of the utilized sample. To determine chlorophyll rate, 1 ml of the floater was mixed with 9 ml of aceton (80%), 1 ml of this mixture was taken to be introduced in spectrophotometer. The ajustment at zero is done with aceton solution of 80%. For statistical analysis, an analysis of variance (ANOVA) was performed with the main emphasis on isolate x cultivar interaction.

Results and discussion

All Septoria isolates demonstrated significant differences for the three morphological characters considered.

The color of culture is a differential criterium which explain the wave length in which light is absorbed. The differences in color indicate variation between isolates. Thus, we grouped the 5 isolates into 3 different classes. The first group (Tunis and Fretissa) had a pale gray color, the second (Bizerte and Algeria) had gray color and the third one (Pont Bizerte) had gray-rose color.

The length and lhe width of 10 pycnidiospores taken at random in the inoculum suspension varied between 10.25 - 63.55 micro x 2.05 - 4.1 micro with mean of 31.82 + 11.35 x 3.17 + 0.82 (Table 2). Dimensions varying from 8 - 35 x 0.8 - 3 micro were found by Sanderson et al. (25) Shipton et al. (27) and Sprague (28).

Analysis of variance demonstrated significant differences between isolate dimensions (Table 1). Three homogeneous groups for spore length were distinguished. The first group is formed by Tunis and Algeria isolates. The second group is composed by Pont Bizerte and Fretissa isolates and the last one contains the Bizerte isolate.

The sporulation was estimated as the number of germinating spores per unit of volume of the inoculum.

Table 1: Length and width in microns of spores of Tunis, Bizerte, Pont Bizerte, Fretissa and Algeria isolates.

Dimensions of spores in microns

Isolates	Length (*)	width (*)
Tunis	26.42 a	3.12 ab
Bizerte	43.87 b	3.69 b
Pont Bizerte	34.40 ab	2.75 a
Fretissa	29.81 ab	3.16 ab
Algeria	24.56 a	3.12 ab

*: mean of 10 replications.

Table 2 presents production of spores per milliliter for each isolate. Results obtained demonstrated that there is significant differences of sporulation between isolates. In fact, we distinguish 3 isolates groups. The first group is composed of Tunis and Bizerte, the second group Pont Bizerte and Fretissa. the last group is formed by the Algeria isolate.

Table 2: Number of conidia per milliliter of suspension issued from Tunis, Bizerte, Pont Bizerte, Fretissa and Algeria isolates.

Isolates	X
Tunis	20.33 a[x]
Bizerte	19.67 a
Pont Bizerte	32.33 b
Fretissa	22.67 b
Algeria	11 c

x: Values followed by the same letter (s) are not significantly different at P=1%.

To study the virulence of the different isolates, the durum wheat cultivars were inoculated separately with the 5 septoria isolates and were successfully infected. To assess cultivar reaction to septoria isolates, we considered a cultivar susceptible when the PSA was higher than 20%. The analysis of variance revealed highly significant differences both in disease reactions of the wheat cultivars and in virulence of septoria isolates (Table 3). More important, however, was the high by significant cultivars x isolates interaction which demonstrated physiologic specialization of the pathogen.

Table 3: Mean square effects of the four evaluation methods on the eight differential durum whealt varieties.

Method of evaluation	Source of variation	Mean square
LII (1)	isolate	0.2489**
	variety	0.1116**
	iso.x var.	0.0298**
PD (2)	isolate	21.7300**
	variety	17.2900**
	iso.x var.	2.5930**
PAS (3)	isolate	0.2442**
	variety	0.1127**
	iso.x var.	0.0294**
CR (4)	isolate	0.0442*
	variety	0.0103**
	iso.x var.	0.0023**

**: significant at 1%
*: significant at 5%
(1): linear infection index (%)
(2): pycnidia density
(3): percentage of attack severity
(4): chlorophyll rate (mg chl/g dried weight)

In fact, for LII and PAS, we distinguished 3 classes of virulence types (Table 4). The first class included the Bizerte isolate, the second, Algeria isolate and the third class contains by Pont Bizerte, Tunis and Fretissa isolates.

For the PD criterion, isolates were grouped into 2 classes (Table 4). The isolates of Tunis, Fretissa and Pont Bizerte were the most virulent, those of Bizerte and Algeria were less virulent.

For the CR criterion, isolates virulence demonstrated that there were 3 classes (Table 4). In fact, Pont Bizerte and Bizerte isolates were the least virulent. The Fretissa and Algeria isolates formed the class of moderate virulence. However, Tunis isolate constituted the most virulent class.

To group the isolates into distinct races, the disease scores were divided into 3 classes for LII and PAS (Table 4) and into 2 classes for PD and CR. The fact that the PD and CR variables discriminate data in fewer classes than LII and PAS, indicate that the former provide less information (17).

Table 4: Classification of septoria isolates based on four evaluation method.

Isolates	Evaluation method			
	LII (1)	PD (2)	PAS (3)	CR (4)
Tunis	0.282 c	3.320b	28.7 c	0.035 a*
Fretissa	0.288 c	3.280 b	28.8 c	0.064 ab
Pollt Bizerte	0.254 c	3.480 b	24.9 c	0.069 b
Bizerte	0.053 a	1.460 a	5.6 a	0.071 b
Algeria	0.149 b	1.820 a	15.2 b	0.042 ab

(1) linear infection index
(2): pycnidia density
(3): percentage of attack severity
(4): chlorophyll rate (mg chl/g dried weight)
*: values followed by the same letter (s) are not significantly different at P=1%

It is noteworthy that isolate groups for morphologic and pathogenic characteristics didn't correlate. Table 3 shows a significant cultivar x isolate interaction which varied according to the evaluation method considered. Thus highly significant interactions for LII and PAS could be considered as physiologic specialization index of *Septoria tritici.* However, a significant interaction for PD and CR indicates only the difference between virulence and aggressiveness. The five isolates were classed into three groups indicating there are three races of *Septoria tritici.* The first included the races from Tunis, Fretissa and Pont Bizerte as highly virulent, the second from Algeria moderately virulent and the third group of Bizerte ligthly virulent. To complete our study, we identified the cultivars reaction on the basis of the 3 septoria races suggested here (Table 5). It is noteworthy that among the eight wheat cultivars, Amal, CHEN'S'-CD 26406-3B-5Y-OM-16Y-OB and CHEN'S'-CD 26406-1B-1Y-2Y-OM-1Y-OM reacted similarly to all isolates.

Table 5: Virulence patterns of isolates of Septoria tritici on host wheat cultivars.

	Wheat cultivars							
	BD1	BD2	BD3	BD4	BD5	BD6	BD7	BD8
Virulence groups								
I	S[x]	S	R[x]	S	S	S	R	R
II	S	R	S	R	S	S	R	R
III	R	R	R	R	R	S	R	R

R[x]: resistant, less than 20% leaf area necrotic with few pycnidia;
S[x]: susceptible, more than 20% leaf area necrotic, moderate to dense pycnidial, coverage.

BDI: Razzak; BD2: Karim 80;
BD3: BenBéchir 79; BD4: Badri;
BD5: Maghrebi 72; BD6: Amal;
BD7: CHEN'S'-CD 26406-3B+5Y-OM-16Y-OB;
BD8: CHEN'S'-CD26406-1B-1Y-2Y-OM-1Y-OM.
I: Tunis, Fretissa and Pont Bizerte isolates.
II: Algeria isolate.
III: Bizerte isolate.

Conclusion

Contrary to most reports dealing with physiological specialization of *Septoria tritici,* the present study shows clearly that the Septoria isolates interacted differentially with the durum wheat cultivars used as differentials. Isolates capable of such differential interaction with the host constitute true physiological races in the conventional sense and not more «aggressive races» (28). In fact, the latter may differ in virulence or «aggressiveness» but could not interact differentially with the host. Thus, we can distinguish three races of *Septoria tritici.* The first included the isolates of Tunis, Fretissa and Pont Bizerte as hidhly virulent, the second from Algeria moderatly virulent and the third of Bizerle highly virulent. Cultivar reaction on the basis of the three Septoria races investigated showed that among the eight wheat cultivars, Amal is susceptible against all isolates but CHEN'S'-CD 26406-3B+5YOM-16Y-OB and CHEN'S'-CD 26406-1B-1Y-2Y-OM-1Y-OM were resistant to all isolates. The occurence of distinct races of *Septoria tritici* suggests that breeding for resistance to the pathogen might not be as straightforward and requires an extensive and perpetual search for sources of resistance.

Literature

1. Arjona, C. O., Fuestes, S. y Baver, L.I. 1976. Estudio sorbre *S.tritici* Rob. ex. Desm., agente causal de la mancha de la hoja del trigo en Mexico y Guatemala. Memorias del VII congreso Nacional de fitopatologia, Soc. Mex. de Fitopatologia A. C. 1977. 8-marzo.
2. Arsenijevic, M. 1965. *Septoria tritici* (Rob. ex Desm.) as a wheat parasite in the S. R. Serbia. Zast Bilja, 16, 5-10. in Rev. Appl. Mycol., 45, 1349.
3. Ballantyne, B. 1985. Resistance to speckled leaf blotch of wheat in Southern New South Wales. pp. 31-32 in A. L. Scharen, ed. Septoria of cereals. Proc. Workshop, August 2-4, 1983, Bozeman, MT. USDA-ARS Publ. N. 12. 116 pp.
4. Brokenshire, T. 1975. The role of gramininaceous species in the epidemiology of *Septoria tritici* on wheat . Plant pathol. **24**: 33-38.
5. Caldwell, R. M. & Narvaez, I. 1960. Losses to winter wheat from infection by *Septoria tritici.* Phytopathology 50: 630 (Abstr.).
6. Cordo, C. A. y. Arriaga, H. P. 1987. Patogenicidade diferencada entre cepas argentinas de *Septoria tritici* Rob. ex. Desm. *Summa Phytopathologica* 27 (1-2).
7. Danon, T., Sacks, J. M. & Eyal, Z. 1982. The relationships among plant stature, maturity class and susceptibility to Septoria leaf blotch of wheat. Phytopathogica 27 (1-2).
8. Diaz. M. A. 1983. Variabilidad patogenica de *Septoria tritici* Rob. ex. Desm. Investigaciones Agronomicas **4**: 46-50.
9. Eyal, Z. 1972. Effect of Septoria leaf blotch on yield of spring wheat in Israel. Plant dis. Rep. 56: 983-986.
10. Eyal, Z., Amiri, Z. & Wahl, I. 1973. Physiologic specialization of *Septoria tritici.* Phytopathology **63**: 1087-1097.
11. Eyal, Z., Sharen, A. L., Huffman, M. D. M. & Prescott, J. M. 1985. Global insights into virulence frequencies of *Mycosphaerella graminicola.* Phytopathology **75**: 1456-1462.
12. Hilu, H. M. & Bever, W. M. 1957. Inoculation, oversummering and suscept-pathogen relationship of *Septoria tritici* on Triticum species. Phytopathology **47**: 474-480.
13. Morales, I. N. 1957. Studies on Septoria leaf blotch of wheat. PHD thesis. Purdue University, Lafayette IN.
14. Narvaez, I. & Caldwell, R. M. 1957. Inheritance of resistance to leaf blotch wheat caused by *Septoria tritici.* Phytopathology **47**: 529-545.
15. Paskell, R. J. 1928. Disease of cereals and forage crops in the United States in 1927. Plant dis. Rep. (Suppl.) **62**: 302-353.
16. Perello, A. E., Basinec, F. y Cordo, C. A. 1987. *Mycosphaerella graminicola* (anamorfo *Septoria tritici).* Taller de septoriosis de 1 Tripo. MAP/IPO/CIMMYT Nov. 9-14. 1987. La Estanzuela, Uruguay (proceedings en prensa).
17. Perello, A. E., Cordo, C. A., Arriaga, H. O. & Alippi, H. E. 1989. Variation in virulence in isolates of *Septoria tritici* Rob. ex Desm. on wheat. Septoria of cereals: Proceedings of the workshop held July 4-7, 1989 at Zurich, Switzerland. pp. 42-44.
18. Prestes, A. M. & Hendrix, W. J. 1977. *Septoria tritici* Rob. ex. Desm. Relacab patogena-hospeteiro, resposta varietal e influenciano sitema radicular do trigo. Suppl. ciencia e cultura 29, 23.
19. Rillo, A. O., Caldwell, R. M. & Glover. D. V. 1970. Cytogenics of resistance to wheat leaf blotch (*Septoria tritici)* in backcross derivatives of an agrotricum line Crop Sci. **10**: 223-227.
20. Rillo, A. O. & Caldwell. R. M. 1976. Inheritance of resistance to *Septoria tritici* in *Triticum aestivum* subsp. vulgare Bulgaria 88 (Abstr.). Phytopathology **56**: 897.
21. Rosielle, A. A. 1972. Sources of resistance in wheat to speckled leaf blotch caused by *Septoria tritici.* Euphytica **21**: 152-161.
22. Rosielle, A. A. & Brown, A. G. P. 1979. Inheritance, heritability and behavior of three sources of resistance to *Septoria tritici* in wheat. Euphytica **28**:385-392.
23. Saadaoui, E. M. 1987. Physiologic specialization of *Septoria tritici* in Morocco. Plant dis. **71**: 153-155.
24. Saari, E. E. & Wilcoxon, R. D. 1974. Plant disease situation of highyielding dwarf wheats in Asia and Africa Ann. Rev.Phytopathol. **12**: 49-68.
25. Sanderson, F. R., Scharen, A. L. & Scott, P. R. 1985. Sources and importance of primary infection and identities of associated propagules. In: Scharen, A. L. (Ed.), Proc. workshop on Septoria of cereals, Montana State University, Montana, U. S. A., 57-64.
26. Shaner, G. & Finney, R. E. 1976. Weather and epidemics of Septoria leaf blotch of wheat. Phytopathology **66**: 781-785.
27. Shipton, W. A., Boyd, W. J. R., Rosielle, A. A. & Shearer, B. L. 1971. The common Septoria diseases of wheat. Bot. Rev. **27**: 231-262.
28. Sprague, R. 1950. Diseases of cereals and grasses in North America. The Ronald Press Co., NY. 583 pp.
29. Tyagi, P. D., Yoshi, L. M., & Renfro, B. L. 1970. Reaction of wheat varieties to *Septoria tritici* and report of an epidemic in North-Western Punjab. India Phytopathol. **22**: 175-178.
30. Vander planck, J. E. 1968. Disease resistance in plants. Academic Press, New York. 206 pp.

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