Original

Morphocultural variants of *Septoria tritici* isolates

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Summary

This is a morphocultural study under *in vitro* and *in vivo* condition on some *Septoria tritici* variants mainly originated from isolates collected from cultivars bearing Bobwhite 'S' and Kavkaz germplasm. The objective was to show the variability in cultural and morphobiometrical characters of atypical isolates (variants) derived from these germplasms and discuss the relationship with the pathogen's adaptative process.
The isolates were grown on potato dextrosa agar and studies with the optical and scanning electron microscope were performed. The thallus behaviour and the vegetative structures were described. It was confirmed that these atypical structures belong the *S. tritici* thallus.
Under *in vivo* condition the isolates developed typical leaf blotch lesions with normal pycnidia. These contained cylindrical cells or pycnidiospores smaller than spores of the "wild type". Possible reasons for the origin of these variants of *S. tritici* are considered.

Key words Variants isolates, *Septoris tritici*, Selection pressure, Variability, Bobwhite'S' and Kavkaz germplasm

Variantes morfoculturales de aislamientos de *Septoria tritici*

Resumen

Un estudio morfobiométrico y cultural bajo condiciones *in vitro* e *in vivo* se condujo sobre diez aislamientos variantes de *Septoria tritici*. Estos fueron obtenidos desde cultivares de trigo con germoplasma Bobwhite 'S' y Kavkaz. El objetivo fue relacionar la frecuente aparición de estos aislamientos no tradicionales (variantes) a partir del mencionado germoplasma con el proceso adaptativo del patógeno.
Se realizaron cultivos en agar glucosado de patata y observaciones con microscópico óptico y microscopio electrónico de barrido. Se estudiaron el talo y estructuras vegetativas (conidios y células somáticas reproducidas asexualmente por gemación (yemas). Se confirmó la pertenencia de estas estructuras atípicas al talo de *Septoria tritici*.
La experimentación y observación bajo condiciones *in vivo* demostró que cada aislamiento reprodujo la mancha típica de esta enfermedad con picnidios. Estos contenían picnidiosporas más pequeñas que la de los aislamientos tradicionales o células gemantes (yemas). Se interpretan las posibles causas que dan origen a estas variantes menos patógenas.

Palabras clave Aislamientos variantes, *Septoria tritici*, Presión de selección, Variabilidad, Germoplasmas Bobwhite'S' y Kavkaz

Cultural and pathogenic variability of *Septoria tritici* Rob ex Desm. (*Mycosphaerella graminicola* Fuckel Schröeter) isolates population have been frequently analyzed in Argentina [1-3,25]. Variant isolates of *S. tritici* [4] and other *Septoria* spp. were described [5].

A high genetic variability has been demonstrated in population of this pathogen with molecular biology techniques [6,7].

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M. graminicola variation at individual RFLP loci and DNA fingerprints showed thousands of differents genotypes in individual fields of wheat [7,9].

Spontaneus mutation, sexual recombination and somatic hybridization with or without subsequent nuclear fusion and recombination (the parasexual cycle) provide new combinations of virulence generated within individual pathogen populations [8].

Many plant pathogens mutate on nutritive media and upon the host plant where the spontaneous mutations from avirulence to virulence are most frequently seen [10-15]. The new mutations will differ from the preexisting isolates solely by a change in pathogenicity at a single virulence locus [16]. Also, artificial mutants induced by toxic or mutagenic agents were obtained [17].

As a mutation, to introduce new alleles into a population, occurs at a rate of only one out of a million individuals, sexual reproduction in *M. graminicola* population is the most effective method to arrange genes into new recombinations [18].

Another mechanism that produces changes at physiological and genetic levels is adaptation [19-22]. It enables an organism to develop some physiological processes such as: the acquisition of tolerance to a toxic substance, the ability to use new substrates for growth and changes in virulence upon host plants. Adaptation can be the result of mutant selection or of the appearance of adaptive enzymes. Sometimes also the substrate (nutritive media or host plant) selects mutants or induces the formation of such enzymes [19].

The cultural aspects of some variants of several *Mycosphaerella graminicola* (anamorphous *S. tritici*) isolates in Argentina were characterized and compared with their virulences [1,2,4]. Albino and yeast-like cultural types were the least virulent upon wheat cultivars known as resistant and susceptible [4].

This study was undertaken to present new variants (no traditional *S. tritici* isolates) coming from cultivars with Bobwhite'S' and Kavkaz germplasm. Studies of morphobiometrical and cultural characters were intensified. Function of the thallus, probable origin of the variants and their relationship with the adaptive of plant pathogens are interpreted.

MATERIALS AND METHODS

The isolates of *S. tritici* were collected in the field from wheat cultivars infected naturally which inherited resistance from cross parents Bobwhite'S' or Kavkaz germplasm (table 1).

Morphological and cultural study *in vitro*. The fungus was isolated from pieces of infected tissue or by transfer of spores from one pycnidium. The cultures (six replications per isolate) were grown on 2% potato dextrose agar (PDA) under laboratory conditions (mean temperature, 20°C; diffuse light) for 21 days. Firstly, aspect of the colony, colour [24], surface, shape, margin, internal structure, size and kind of spores were determined. The colonies were described following Garassini [23]. Then, direct observation, slide cultures and micrometrical measurements of spores were performed.

To clarify the external morphology of the asexual spores and to improve the accuracy of the measurements, the scanning electron microscope (SEM) was used. A simple method was employed. The spores were taken from slant tubes, containing 5ml of sterile distilled water and two drops of ethanol, washed twice and each time centrifuged at 3900 rpm. The supernatant was discarded, the spore precipitate was spread onto a glass slide (1x1cm) and gold coated in a vacuum chamber (JFC-1100 ION SPUTTER model) before being examined under the SEM (JEOL Scanning JSH - T100 Model).

Thallus behavior. Fitzgerald and Cook's method [25] was applied to determine the germinative mechanism of each vegetative structure. This method consists of: small blocks of sporulated cultures (5mm diam.) are placed on Petri dishes (9 cm diam.) with PDA medium over sterile transparent cellulose film. The film could have the shape of circle or rectangle. The disks are placed in the centre or in four crosspoints of the dish with the upper sporulated surface facing the lid. The incubation is under laboratory conditions of alternating cycles of light (12 h L/O) and temperature (15/21°C) during 5 days. After 24 h the replicated disks can be examinated diary with the binocular magnifier, looking for new micelia and spores. After this time, the disks on cellulose film are removed and the transparent film is stained with cotton Blue in lactophenol 0.1% for 10 min (1-2 drops of the stain were placed on each centre of micelia). The film is cleaned lightly and mounted directly without cover glass for observartion. This method has the advantage of a general mountant for permanent preparation, allowing simple, daily examinations. If the stain is dryed it can be first wetted with lactophenol. This study was supplemented with a test applied to true yeast. Sexual reproduction of the organism was stimulated in slant tubes on Gorodkowa agar (GA) (1 g meat extract, 1 g meat peptone, 0.25 g dextrose, 0.5 g sodium chlorate, 20 g agar, 1 l distilled water) and acetate agar (AA) (10 g potassium acetate, 2.5 g yeast extract, 1 g dextrose, 20 g agar, 1 l distilled water).

In addition, the vegetative cells from each isolate were cultured on carrot blocks in Roux tubes or on corn meal agar (CMA) (30 g corn meal, 20 g agar, 1 l distilled water) to stimulate chlamydospore formation.

Morphological study under *in vivo* condition. Ten isolates with Bobwhite'S' and Kavkaz germplasm derived from wheat cultivars were inoculated onto the same cultivars.

The inoculation process was performing under greenhouse conditions. The isolates were obtained from leaves, which presented typical lesions with pycnidia (Table 3). Pycnidia developed spore cirrhi; from these, single spore isolates were cultivated on modified malt agar (30 g malt extract, 5 g mycological peptone, 2 g yeast extract and 1000 ml distilled water for sporulation). On day 7 a mucous mass of conidia (atypical yeast like and albino type) or filamentous of micelia developed and were used for inoculation. Spore suspension were adjusted to 1 x 10^7 sp/ml concentration in 250 ml of distilled water and inoculated onto ten pots with 5 seedlings of each variety. At the third expanded leaf stage the seedlings were sprayed with the inoculum until soaking and then placed in a humid chamber for 96 h. After that, plants were subject to greenhouse conditions. The mean temperature was 21°C and the mean relative humidity was 75%.

At 21st day, the lesions were observed. Pycnidia and spores of each isolate were described and measured under optical microscope and SEM conditions.

RESULTS

Morphological and cultural study *in vitro* condition. Monosporic colonies originated from the resistant germplasm showed differences from the "wild-type" native to Argentina (stromatic-pycnidial or yeast-like pinkish buff in colour).

The new cultural types were atypical yeast-like, Buff in colour, and albino (powdery-like and velvet "cordé type"). It continued appearing with greater frequency whenever new material from other areas with Bobwhite'S' and Kavkaz germplasm was analyzed.

The three cultural variants found were named: micelial-filamentous (P3-4 and P3-6); atypical yeast-like (P3) and albino type (P1, E9, E1, 35M, 19N, 5E and LH5m) (Table 1).

Albino type. The isolates P1, E9, 35M, 19N, 5E and LH5m are included within this group. LH5m, physiological mutant, is the result of several transfers on culture media for preservation. Albino type variants are:

- *P1 and 19N isolates* (Figures 1-4). Irregular elliptical colony of mycelial appearance raised surface, ridged edge. The centre presents spaced distribution of pseudomycelia with terminal budding cells. Budding cells acted as infective particles when inoculated upon

Bobwhite 'S' cultivars. The second reisolate from Bobwhite 'S' gave as a result a morphological mutant named P1.1 with similar characteristics to P1.

- *35M isolate* (Figures 5,6). Circular colony mycelial appearance. Coloured area gives origin to short elliptical hyaline conidia. Colony surface is plane, its edges slightly raised.

- *5E, E9 and E1 isolates*. Irregular colony, stromatic and albino, powdery or pastey in two thirds of the centro and velvet "cordé type" spotted margin. The centre of the colony presents elliptica or filiform hyaline conidia, normal secondary conidia, chlamydospores and hyphae from tortuous, closely branching septa with thick walls.

- *LH5m isolate* (Figures 7-9). Convex circular colony of mycelial appearance ridged edge. "Cordé type" mycelium is made up of pseudomycelial hyphae with cylindrical budding cells.

Mycelial filamentous type.

- *P3-4 and P3-6 isolates* (Figures 10,11). Both of them were similar and randomly chosen among 10 re-isolates from the same filial generation of P3 progeny. They produced circular colonies of mycelial aspect, plane surface with crests resembling filaments in two thirds of the centre and plane margin. The colony was covered by a light-slimy layer.

Atypical yeast-like type. Only P3 isolate (Figures 12,13). Irregular slimy colony of wet shiny aspect. Atypical yeast-like mycelium presents structures that resemble secondary conidia (long cylindrical cells) and short budding cells.

Thallus behavior. None of the isolates produced the pycnidia and pycnidiospores characteristic of the species on PDA media.

Investigations using light and scanning electron microscopy clearly indicated that the germination of the vegetative and reproductive structures varied according to the isolate. Three types of germination could be observed by Fitzgerald and Cook's (1989) technique: a) pseudomycelium with budding tips (P1, LH5m, 19N isolates); b) germtube developed from conidia (35M, P3-4, P3-6, 5E, E1, E9); c) budding cells (P3). This test did not prove the existence of a hyphal penetrating mechanism but it confirmed that the cells structures (conidia or budding cells) could multiply on leaves. Type and size of all the vegetative structures are summarized in table II.

Atypical yeast-like colonies (buff in colour) and albino type, did not produce asci and ascospores or chlamydospores, although the media (GA, CMA and carrots blocks) were stimulated these with true yeast.

Therefore, it is confirmed that these new cultural types belonged to *S. tritici* and were not true yeasts.

Morphological study under *in vivo* conditions. Ten isolates studied in this work had been reinoculated upon wheat. All of them caused typical leaf blotch lesions. Based on the SEM observations, it can be concluded that they developed normal pycnidia containing cylindrical cells or pycnidiospores, smaller than spores of the "wild type", depending of the isolate (Figures 14, 15 and 16). Size of budding cells and pycnidiospores

Table 1. Origin, cultural types and colonies colours of ten *Septoria tritici* isolates.

Isolates	Cultivar	Germplasm	Cultural type*	Colour**	Collected
P3	LPI/BW'S'	BW'S'	mucous (yeast-like)	45 Buff	Pergamino
P1	Bw/4/...Laj 3139	BW'S'	albino, mycelial, platery, "corde type"	45 Buff	Pergamino
E9	CST 169	BW'S'	stromatic, albinic, dusty	64 Honey 64 Isabellino	Uruguay
35M	CM 76751x0	BW'S'	albinic, mycelial, "corde type"	117 Pale Mouse Gray White	Barrow
P3-4	LPI/BW'S'	BW'S'	mycelial, filamentous	Vinaceous	Pergamino
P3-6	LPI/BW'S'	BW'S'	mycelial, filamentous	58 Rosy	Pergamino
19N	CM 61830	Kavkaz	albinic, powdery, plastery, mycelial	45 Buff	Necochea
5E	Millalew	Kavkaz	albinic, powdery, plastery, stromatic	61 Rosy Buff	Uruguay
E1	LI 7	BW'S'	albinic, powdery, plastery, stromatic	64 Honey	Uruguay
LH5M	Los Hornos improved line	phisiologic mutant	albinic, mycelial, "corde type"	45 Buff	Los Hornos

* Garassini (1958), ** Rayner (1970).

Table 2. Size of budding cells and conidia of ten *Septoria tritici* variants developed "*in vitro*" on PDA media (Fitzgerald and Cook method) after seven days.

Isolates	Thallus type	*In vitro* [a] length (µm)	width (µm)
P3	short budding cells[b]	2.15 (1.93-2.3)	1.25 (1.02-1.59)
	long cylindrical cells	3.66 (2.8-4.3)	1.59 (1.02-2.8)
P1	elliptical budding cells	3.92 (2.8 - 4.3)	2.02 (1.8-2.6)
E9	elliptical conidia	6.02 (4.3-7.5)	2.02 (2.9-4.3)
35M	short elliptical conidia	5.73 (4.3-6)	2.86 (1.43-4.3)
P3-4	unicellular elliptical conidia	6 (4.5-7.5)	3 (2.45-4.3)
P3-6	bicellular elliptical conidia	9.5 (6.2-11.7)	3 (2.45-4.3)
19N	secondary budding cells	7.5 (6-10.5)	3 (2.43-4.3)
	conidia	9.5 (6.2-11.7)	3 (2.43-4.3)
5E	filiform conidia	11.4 (7.15-12.9)	1.43[c] (1.02-2.8)
	chlamydospores	8.58	7.15
	secondary conidia	12 (7.15-12.9)	3 (2.43-4.3)
E1	elliptical conidia	6.02 (4.3-7.5)	3 (2.4-4.3)
LH5M	prismatic budding cells	9.5 (5.78-13.5)	3.75 (1.8-2.6)

[a] Mean of one hundred measurements; b these sizes correspond to budding cells only; c these sizes correspond to filiform conidia only.

Table 3. Size of pycnidia, budding cells and pycnidiospores of ten *Septoria tritici* variants developed on wheat plants under "*in vivo*" conditions.

Isolates	Wheat cultivar inoculated	Pycnidium (µm) lenght[a] x width	Pycnidiospores (p) or buddings cells (bc) lenght[a] x width
P3	LPI/BW'S'	106 x 38	bc 2.15 (1.93-2.3) x 1.25 (1.02-1.59)
P1	Bw/4/... LAJ 3139	110 x 35	bc 3.92 (2.08-4.3) x 2.02 (1.8-2.6)
E9	CST 169	110 x 40	p 28 (20-32.9) x 1.43 (1.02-2.8)
35M	CM 76751x0	110 x 40	p 22 (17.16-27.17) x 1.43 (1.02-2.8)
P3-4	LPI/BW'S'	110 x 40	p 25 (18.59-30) x 2 (1.8-2.6)
P3-6	LPI/BW'S'	110 x 40	p 25 (17.16-30) x 2 (1.8-2.6)
19N	CM 61830	110 x 35	bc 7.5 (6-10.5) x 3 (2.43-4.3)
5E	Millalew	110 x 35	p 25 (17.16-30) x 2 (1.8-2.6)
E1	LI 7	110 x 40	p 28 (21.45-32.9) x 2 (1.8-2.6)
LH5M	Los Hornos improved line	110 x 35	bc 9.5 (5.78-13.5) x 2.38 (1.8-2.6)

[a] Mean of one hundred measurements.

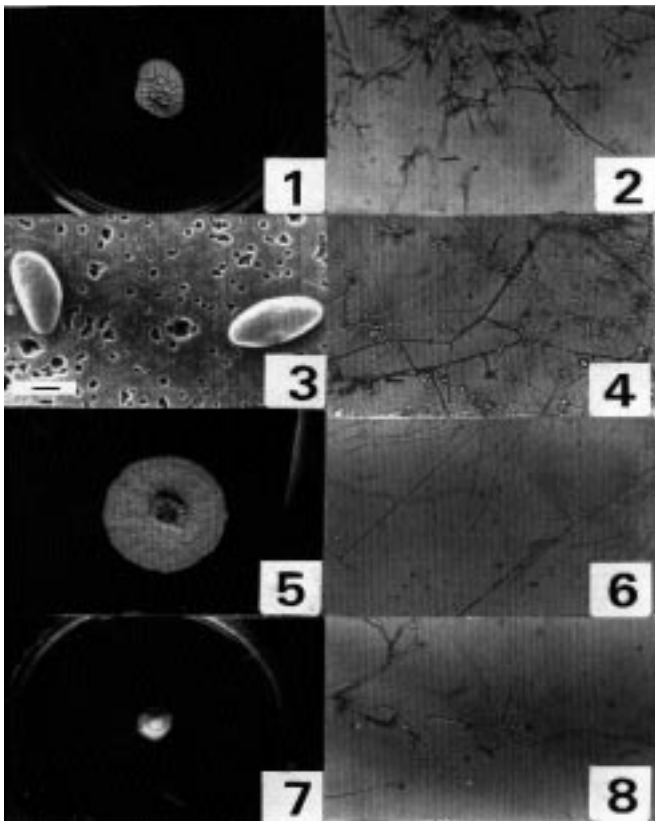

Plate 1. Figures 1-8: *Septoria tritici* light and electron micrographs. Bars: 1 µm for figures 3 and 8; 10 µm for figures 2, 4 and 5; 20 µm for figure 7. Figure 1: P1 isolate, monosporidial colony on PDA (x 0.8). Figure 2: P1 isolate, pseudomycelium with sprouting terminal cells. Figure 3: P1 isolate, sprouting terminal cells. Figure 4: 19N isolate, secondary budding cells and conidia. Figure 5: 35M isolate, monosporidial colony on PDA (x 0.8). Figure 6: 35M isolate, hyphae and shortly elliptical conidia. Figure 7: LH5m isolate, monosporidial colony on PDA (x 0.8). Figure 8: LH5m isolate, pseudomycelium hyphae with sprouting cell tips.

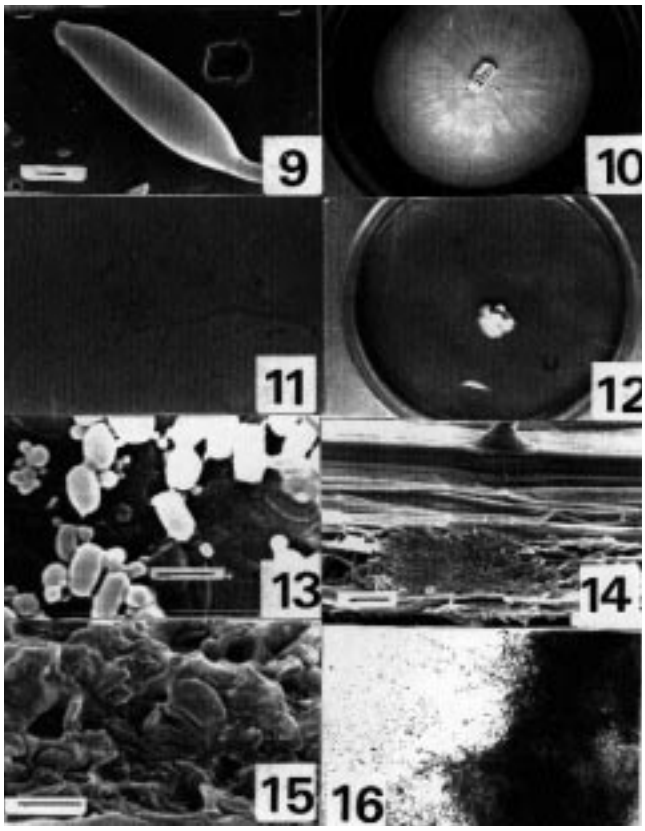

Plate 2. Figures 9-16: *Septoria tritici.* Light and electron micrographs. Bars: 1µm for figure 9; 2 µm for figures 13, 15 and 16a; 10 µm for figures 11 and 16b; 20 µm for figure 14. Figure 9: LH5m isolate, polyhedral sprouting terminal cells. Figure 10: P3-4 isolate, monosporidial colony on PDA (x 0.8); Figure 11: P3-4 isolate, mycelium hyphae and cylindrical conidia. Figure 12: P3 isolate, monosporidial yeast-like colony on PDA (x 0.8). Figure 13: P3 isolate, sprouting cylindrical cells. Figure 14: Leaf cross section of LPI/Bb'S' cv, with pycnidium. Figure 15: Pycnidium internal surface. Note sprouting cells borderline. Figure 16: P1 and LH5m isolates germinated on leaf wheat of Bobwhite 'S' germplasm.

and pycnidia of *S. tritici* variants, developed under *in vivo* condition, are shown in table 3.

DISCUSSION

With the presence of sexual reproduction, as in the argentine population of *M. graminicola* [26] new combination of genes arise into the field, from one growing season to the other.

Cultural variants were mentioned frequently on *Septoria* spp.: *Septoria lycopersici* [4], *Stagonospora nodorum* [15], *Stagonospora avenae* [11], *S. tritici* [4,3]. The existence of these variants suggests heterocaryosis occurrence. The sexual cycle and the parasexual recombination between different nucleus are the principal cause of genetic variation in spp of *Septoria* [11,15]. Moreover significant changes in genotypic density of *M. graminicola* within a year and between years into the same field were not found [27]. Sexual reproduction occurred, but the effect of it did not show diversity of genotype in the fungal population.

In this work *S. tritici* variants were isolated mostly from Bobwhite'S' (CM33203) and from Kavkaz (CM67458). Germplasm of different localities were analyzed. Both genetical sources are known to have a high level of resistance to *S. tritici*. These variants reappeared succesively during four years in infected material from different wheat areas, even when they were reisolated from the same cultivar at different seasons of the year.

The variants described in this work could have originated by recombination followed by selection. Probably, these new genotypes (atypical yeast-like and albino) coexisted with the "wild type" and a particular germplasm selected them. They have different morphological characters mainly shape, size and colour of the thallus and spores. This type of selection produced related variants with a change of pathogenicity as was mentioned [3,4]. Also their morpho and pathogenic characteristics remained after four generations through successive inoculations and reisolations on the original host, as shown in *in vivo* test. Besides, whenever any of the variants were inoculated upon wheat, they always reproduced the lesion known as septoria leaf blotch. These variants also may contribute to the pathogenic adaptation by two mechanisms:

1. Bobwhite 'S' germplasm would act as variant selecting substrate. Variants would have adapted themselves to the presence or deficit of certain metabolites in the germplasm, as the standard isolates were not affected by metabolism alterations.

2. "Adaptive enzymes" might also be a subject of discussion. By a series of gradual biochemical changes, the pathogen uses the substratum (metabolites of Bobwhite 'S' germplasm), developing an enzyme. A chain

of mutations follows, each one producing stepwise effects, developing it into an adaptive enzyme [19].

Another explanation could be the aquisition of some characteristic (pigment production, distinct cultural changes, changes in virulence) throughout a specific gene involved in variation. This gene could be present in the normal - type isolate, but inactive, and might be an imperfect copy of the one that produced mutant change [28].

Variant isolates were observed to be less virulent than morphologically normal isolates (wild type)[4]; also their physiological behaviour was different: they developed poorer growth patterns respect to the hydrocarbonate and nitrogenate source supplied [29].

The understanding of combined effects of mutation, somatic hibridization and cytological and molecular changes, on the genetic variability of the pathogen, and their epidemiological behaviour in the field, will lead to the development of more effective means of controlling of the disease. Refered that, if Bobwhite 'S' germplasm stimulate selection and if this effect was lasting, the durability of resistance in cultivars bearing such germplasm would be understood.

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References

1. Cordo CA, Lindquist JC. Análisis cualitativo de la variabilidad cultural de *Septoria tritici.* Boletín de la Sociedad Argentina de Botánica 1987; 25: 59-77.
2. Cordo CA, Arriaga HO. Variation in pathogenicity among strains of *Mycosphaerella graminicola* (anamorph *Septoria tritici*). In: Kohli MM, van Beuningen LT (Eds.) Conferencia regional sobre la septoriosis del trigo. México DF, CIMMYT 1990: 88-105.
3. Cordo CA, Perelló A, Arriaga H. Estabilidad de la virulencia sobre trigo en aislamiento de *Mycosphaerella graminicola.* Fitopatología Brasileria 1993; 18: 371-378.
4. Perelló AE, Cordo CA, Alippi HE. Características morfológicas y patogénicas de aislamientos de *Septoria tritici* Rob. ex Desm. Agronomie 1990; 10: 41-48.
5. Perelló A, Wolcan S, Alippi H. Una variante miceliar albina de *Septoria lycopersici* (speg). Turrialba 1991; 41: 190-195.
6. Mc Donald BA, Martinez JP. DNA restriction fragment lenght polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. Phytopathology 1990; 80: 1368-1373.
7. Boeger JM, Chen RS, Mc Donald BA. Gene flow between geographics population of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) detected with restiction fragment lenght polymorphism markers. Phytopathology 1993; 83: 1148-1154.
8. Burdon JJ. Genetic variation in pathogen population and its implications for adaptation to host resistance. In: Jacobs J, Parleviet E (Eds.) Durability of diseases resistancel. Wageningen, Kluwer Academic Publishers 1993: 41-46.
9. Mc Donald BA, Martinez JP. Chromosome lenght polymorphisms in *Septoria tritici* population. Cur Genetics 1991; 19: 265-271.
10. Christensen JJ. Genetics of pathogenic organisms. American Association in Advances Science 1940; 12: 77-82.
11. Hooker AL. Cultural variability in *Septoria* avenae through successive single macrospore transfer. Phytopathology 1957; 47: 460-468.
12. Johnson I. Selfing studies with physiologic races of wheat stem rust *Puccinia graminis* var. *tritici* Can J Botany 1954; 32: 228-232.
13. Luig NH, Watson IA. The effect of complex genetic resistance in wheat on the variability of *Puccinia graminis* f. sp. *tritici.* Proceedings of the Linnean Society of New South Wales 1970; 95: 23-45.
14. Haggag MEA, Samborski DS, Dyck PL. Genetics of pathogenicity in three races of leaf rust on four wheat varieties. Can J Genetics Cytol 1973; 15: 73-82.
15. Griffiths E, Ao HC. Variation in *Septoria nodorum.* Ann Rev Appl Biol 1980; 94: pp. 294-296.
16. Wellings CR, Mc Intosh RA. *Puccinia striiformis* f. sp. *tritici* in Australasia: Pathogenic changes during the first 10 years. Plant Pathol 1990; 39: 316-325.
17. Newton AC, Caten CE. Auxotrophic mutants of *Septoria nodorum* isolated by direct screening and by selection for resistance to chlorate. Trans Br Mycol Soc 1988; 90: 195-207.
18. Mc Donald BA, Mc Dermott JM. Population genetics of plant pathogenic fungi. Bio Sci 1993; 43: 311-319.
19. Buxton EW. Heterocaryosis, saltation and adpatation. In: Plant Pathology. New York, American Press, 1960: 359-405..
20. Ao HC, Griffiths E. Change in virulence of *Septoria nodorum* and *Septoria tritici* after passage through alternative host. Trans Br Mycol Soc 1976; 66: 137-140.
21. Osburn AE, Scott PR, Caten CE. The effects of host passaging on the adaptation of *Septoria nodorum* to wheat on barley. Plant Pathol 1987; 35: 135-145.
22. Osburn AE, Caten CE, Scott P.R. Adaptation of *Septoria nodorum* to wheat and barley. Plant Pathol 1987;36:565-576.
23. Garassini LA. Microbiología (1st Ed.), Caracas, Ed. Sucre, Universidad Central de Venezuela, 1958.
24. Rayner RW. A Mycological Colour Chart. Kew, The Commonwealth Mycological Institute, 1970.
25. Fitzgerald W, Cooke BM. Spore germination and pycnidial development in wheat and barley isolates of *Septoria nodorum* on cellulose film. In: III International workshop on *Septoria* species of cereals. Dublin, Department of Plant Pathology, Faculty of Agriculture,1989: 4.
26. Cordo A, Perelló A, Alippi HE, Arriaga HO. Presencia de *Mycosphaerella graminicola* (Fukel) Schroeter, telemorfo de *Septoria tritici* Rob. apud Desm. en trigos maduros de la Argentina. Revista de la Facultad de Agronomía 1990/1991; Tomo 66/67.
27. Chen RS, Boerger JM, Mc Donald BA. Genetic stability in a population of a plant pathogenic fungus over time. Mol Ecol 1994; 3: 209-218.
28. Stakman EC, Harrar GJ. The Genetics of Plant Pathology. In: Principles of Plant Pathology. New York, The Ronald Press Company, 1957: 121-127.
29. Perelló A, Cordo C. Influencia de las fuentes carbonadas y nitrogenadas en el crecimiento de *Septoria tritici.* Resúmenes del II Congreso Nacional de Trigo 1990; Pergamino, Cap. IV: 22-29.