PATHOGENIC VARIABILITY IN MONOCONIDIAL ISOLATES OF THE SORGHUM ANTHRACNOSE FUNGUS *COLLETOTRICHUM GRAMINICOLA* FROM SINGLE LESIONS AND FROM MONOCONIDIAL CULTURES*

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ABSTRACT

Pathogenicity of monoconidial cultures from a discrete lesion and from monoconidial isolates of *Colletotrichum graminicola* obtained from the sorghum custivars Tx623 and SC748-5 was evaluated on 5 differential sorghum cultvars. Monoconidial isolates from a single lesion and from monoconidial cultures and sub-cultures were separated into different virulence phenotypes, indicating the occurrence of pathogenic instability in some isolates of this pathogen. Reversion from a virulent to an avirulent state was observed for some isolates.

Key words: Sorghum anthracnose, *Colletotrichum graminicola*, pathogenic variability.

RESUMO

Variabilidade patogênica em isolamentos monospóricos de *Colletotrichum graminicola*, agente causal da antracnose do sorgo, originados de uma única lesão e de culturas monospóricas.

A patogenidade de culturas e sub-culturas monospóricas de *Colletotrichum graminicola* obtidas de uma única lesão e de isolamentos monospóricos das cultivares de sorgo Tx623 e SC748-5 foi avaliada em 5 cultivares diferenciadoras. Isolados foram separados em diferentes fenótipos de virulência, indicando a ocorrência de instabilidade patogenica em isolados deste patógeno. Reversão de um estado de virulência para um de avirulência foi observada para alguns isolados.

INTRODUCTION

Sorghum anthracnose, caused by *Colletotrichum graminicola* (Cesati) Wilson (Syn. *Colletotrichum sublineolum* P. Henn), is one of the mos import diseases affecting the sorghum primarily in the major tropical and sub-tropical growing regions, characterized by high humidity and rainfall (Frederiksen, 1984; Ali and Warren, 1988). Yield reductions are estimaned to exceed 50% in susceptible cultivars under severe epidemics (Harris and Cunfer, 1976). Losses are greater when alternate dry and wet conditions occur during periods of high temperatures (Warren, 1986). Anthracnose is the most serious disease of sorghum in Brazil, and is endemic to all of the nation's sorghum growing areas.

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C. graminicola is a highly variable organism. Harris and Johnson (1967) suggested the occurrence of physiological races of this pathogen in the United States of America. Frederiksen and Rosenow (1971) observed specific differences in reactions of some sorghum cultivars to anthraconose in Texas, Mississipi and Georgia. Results from the International Sorghum Anthracnose Virulence Nursey (ISAVN) suggested the occurrence of physiological races of *C. graminicola* in nature. Cultivrs MN960 and TAM428 were highly susceptible in Nigeria and resistant in the USA, Venezuela, and Puerto Rico, whereas cultivars PB846 and Tx398 were resistant in Nigeria but susceptible in Venezuela, USA, and Puerto Rico (King and Frederiksen, 1976).

The existence of physiologycal races of *C.graminicola* was first reported in Brazil by Nakamura (1982). Five races reported based on the reaction of five differential cultivars to monoconidial isolates from infected sorghum plants from different regions of Brazil. Seven physiological races of *C. graminicola* were later identified in Brazil among seven isolates of the pathogen, based on the reaction of twelve sorghum differential cultivars (Ferreira and Casela, 1986). Thirteen additional races were identified among monoconidal isolates obtained from diseased plants throughout the country, by using a differential set formed by nine sorghum cultivars (Casela and Ferreira, 1987).

Races of *C. graminicola* have been reported in the United States. Ali and Warren (1987) identified three races of this pathogen based on the pathogenicity of nine isolates from the USA and from Puerto Rico, on six differential sorghum cultivars. The authors reported the occurence of races even among isolates from the same geographical area. Cardwell *et al.* (1989) were able to characterize eight pathotypes out of twelve isolates from four areas (Central and Southern Texas; Griffin, GA; and Isabella, Puerto Rico), using eight differential sorghum cultivars.

Recently Pande *et al.* (1991) characterized the pathogenicity of nine sorghum isolates of *C. graminicola* from different locations in India on 30 sorghum cultivars by concluding that the nine isolates were distinct physiological races.

Preliminary observations under greenhouse conditions indicated that monoconidial isolates of *C. graminicola* derived from a single lesion sometimes show variability in pathogenicity when tested on a group of differential cultivars. A similar pattern of variability has been observed in the rice blast fungus *Magnaporthe grisea* (Herbert) Barr (anamorph *Pyricularia oryzae* Cav.) (Ou and Ayad, 1968); Ou *et al.* 1970; Giatgong and Frederiksen, 1969). The main objective of this study was to investigate the variability and/or stability of monoconidial isolates of *C. graminicola* from sigle lesions and from monoconidial subcultures obtained from monoconidial isolates of the pathogen.

MATERIAL AND METHODS

Two anthracnose lesions, one from the previously resistant genotype SC748-5 and one from the susceptible cultivar Tx623, were obtained, respectively, from the EMGOPA (Empresa Goiana de Pesquisa Agropecuária) nurseries in Goiás, Brazil, and from the Georgia Experiment Station nurseries in Griffin, GA, USA. Lesions were collected, respectively, in March and October 1990.

From an infected leaf of Tx623, a discrete lesion was isolated, washed in running tap water, washed three times in sterile distilled water, placed in moistened filter paper in a petri dish, and incubated under continuous fluorescent light at room temperature until the production of abundant spore. The sporulating lesion was placed in 10 ml of sterile distelled water and stirred several times to dislodge the conidia. One ml of this spore suspension was transferred to a petri dish containing a thin layer of 2% water-agar and incubated at room temperature under continuous light for 12 hours to induce spore germination. Individual germined spores were outlined on the medium by a wire loop fastened below the low power objective of a microscope. The outlined area was examined for the presence of other spores, and if none was observed, the selected spore was transferred to **OMA** culture tubes. After development, cultures were flooded with sterile mineral oil for maintenance.

Monoconidial isolation from a sigle lesion of the cultivar SC748-5 were conducted in the Laboratory of Pathology of EMBRAPA, Centro Nacional de Pesquisa de Milho e Sorgo in Sete Lagoas, MG, Brazil, following the same procedures. These isolates were introduced from Brazil under permit and subseqsuent sub-cultures were done in the United States.

From each lesion, a total of 20 monoconidial isolates were obtained, followed by the isolation of 20 monoconidial sub-cultures from one isolate of each set of 20, plus an additional generation of 20 monoconidial sub-subcultures from one isolate of each sub-set, totalling 120 mononidial isolates. All isolates were stored in mineral oil as previously described.

Fifteen isolates per lesion, per generation, totalling 90 monoconidial cultures, were included in this study. The experimental design was a split-plot arrangement where the 15 isolates were whole plots, and 5 differential cultivars were sub-plots. Three replications of each block of 15 monoconidial isolates plus one control and 5 differential cultivars were inoculated at different times, due to limitations of space in the greenhouse.

Inoculum was produced by growing the monoconidial isolates on OMA for 7-days at room temperature, under continuous fluorescent light to induce sporulation. After incubation, individual plates were flooded with 10 ml of sterile distilled water and scraped with a scalpel to dislodge the conidia. The spore suspension was passed throught a double layer of cheesecloth to retain fragments of mycellia and culture media. Before use in inoculation the spore suspension of each isolate was adjusted to 10^6 conidia/ml. For the adjustament of the spore concentration, 16 fields 0.1 mm^3 were counted per isolate on an improved Neubauer Hy Life Hemacytometer (Chausser Scientific). Tween 20 (3 drops/1000 ml) was added to the spore suspension as a wetting agent.

Thyrty days after planting, differentials were inoculated by applying approximately 20ml of the spore suspension on both leaf surfaces, with a hand sprayer. Control plants of each differential were sprayed with sterile distilled water.

Plants were placed in a dark Percival Dew deposition chamber for 18-20 hours at the average air temperature of 40C and a wall temperature of 13C, and 100% relative humidity. The divisions inside the dew chamber allowed for the separation among isolates preventing cross contamination. After the incubation period, plants were returned to the greenhouse where they remained until evaluation.

Plants were classified for infection type 12 days after inoculation, based on a 1 to 5 scale, according to criteria described below:

1. Presence of chriorotic flecks.
2. Red spots on the leaf lamina.
3. Necrotic lesions, sometimes elongated, but no acervuli (sporulation) formed.
4. Necrotic lesions formed with acervuli present in the center.
5. Necrotic lesions, sometimes coalescing, with abundant formation of acervuli.

Symptoms were separated into two groups: R= resistant (includind the infection types 1,2 and 3) and S= susceptible (including the infection types 4 and 5). After evaluation, all inoculated plants were destroyed by steam sterilization and discarded.

Virulence phenotypes for monocodial isolates were determined on the sorghum genotypes Tx378 (BR008), SC326-6 (BR005), SC414-12E, Tx2536, and SC748-5. These sorghum accessions are a subset of the International Sorghum Anthracnose Virulence Nursery (ISAVN).

RESULTS

Variability during three succesive generations from two monocodial cultures was obtained. Altogether the 45 monocodial cultures and sub-cultures from the cultivar Tx623 were separated into 8, whereas those from the cultivar SC748-5 were divided into 3 virulence phenotypes (Tables 1 and 2).

From the 15 monocodial cultures from a single lesion of the genotypes Tx623 and SC748-5, 6 and 2 diferent virulence phenotypes, respectively, were identified by the reaction on the 5 diferential sorghum cultivars (Tables 1 and 2). Monoconidial isolates Tx623.11 and Tx623.11.10 were used as the parental isolates for the second and third generations of cultures. Out of 15 monoconidial cultures tested in each of these generations, 2 and 5 virulence phenotypes were detected (Table 1), whereas from the second and third generations of monoconidial isolates, from the isolates source SC748-5.09 and SC748-5.09.13,3 virulence phenotypes in each were identified (Table 2).

Isolates showing virulence exclusively to SC326-6 and/ or to SC414-12E were only observed in the monoconidial isolates from the single lesion of Tx623. Virulence to SC326-6, SC414-12-E, and Tx2536 were the predominant type throughout the study of monoconidial isolates from Tx623, totaling 24 out of the total number of 45 isolates. This was the virulence phenotype of the parental isolates from which the generations of monoconidial isolates were obtanined. The same trend was observed in relation to the second generation of monoconidial isolates obtained from the isolate SC748-5.09, virulent to SC326-6, SC414-12E, Tx2536, and SC728-5. Less variation in virulence phenotypes occured in monoconidial lines from SC748-5 than from the cultivar Tx623. Complex virulence phenotypes were isolated from the single lesion of the highly susceptible cultivar Tx623 together with isolates showing virulence to only one differential. No virulence to SC748-5 was observed among isolates from Tx623. Loss of virulence was observed in relation to the sorghum cultivar SC326-6 and SC414-12E, in the third generation of monoconidial lines of Tx623.

The majority of isolates in the second generation of monoconidial cultures from SC748-5, were of the same virulence phenotype as the parental isolate, 4 lost their virulence to SC326-6, and 1, a new pathogenic type, was

TABLE 1 - Reaction of 5 differential sorghum cultivars to monoconidial cultures of *Colletotrichum graminicola*, in three succesive generations, from a single lesion of the cultivar Tx623, from Griffin, GA.

Differential Cultivar	Reaction Class[a]/Generation[b]												
	1						2		3				
Tx378	R[c]	R	R	R	R	R	R	S[b]	R	R	R	S	R
Tx2536	S	S	R	S	R	R	S	S	S	S	S	S	S
SC326-6	S	R	S	R	S	R	S	S	S	R	R	S	S
SC414-12E	S	S	S	R	R	S	S	S	S	S	R	S	R
SC748-5	R	R	R	R	R	R	R	R	R	R	R	R	R
Frequency	5	3	2	2	2	1	13	2	6	4	2	2	1

[a] R = Resistant S = Susceptible.
[b] Each generation represents 15 monoconidial isolates
[c] Parental isolate for the next generation.

TABLE 2 - Reaction of 5 differential sorghum cultivars to monoconidial cultures of *Colletotrichum graminicola*, in three successive generations, from a single lesion of the cultivar SC748-5. Goiânia, Goiás, Brazil.

Differential Cultivar	Reaction Class[a]/Generation[b]							
	1		2			3		
Tx378	R	R	R	R	R	R	R	R
Tx2536	S	S	S	S	S	S	S	S
SC326-6	S	R	S	R	R	R	R	S
SC414-12E	S	S	S	S	R	R	S	S
SC748-5	S	S	S	S	R	S	S	S
Frequency	10	5	10	4	1	6	6	3

[a] R = Resistant S = Susceptible.
[b] Each generation represents 15 monoconidial isolates.
[c] Parental isolate for the next generation.

only virulent to Tx2536 and to SC748-5. This isolate was the source to the second generation of monoconidial cultures and, although 6 isolates in this generation were similar to the parental type, 6 had virulence to SC414-12E, and 3 regained virulence to SC326-6 and to SC414-12E.

DISCUSSION

Results from this study confirm previous observation that there is pathogenic instability in monoconidial cultures of C.*graminicola* derived from single lesions and from monoconidial cultures. Repeatability of the results was observed throughout the 3 replications at different times, for all monoconidial isolates from one source. Thus, the reported variation was not determined by environmental fluctuations or plant conditions, one of the main reasons given by Laterell (1975) for the high rate of pathogenic variability in *M. grisea*, reported by Ou and Ayad (1968), Ou *et al.* (1970), and Giatgong and Frederiksen (1969). Instability of monoconidial cultures of *M. grisea* was recently reported by Wu (1990). It appears that, for this organism, stability for host specificity is variable, depending on the specific strain and genes involved (Valent and Chumley, 1991).

This is the first report of pathogenic variability in monoconidial isolates of C.*graminicola*. Even though is possible that some of the reported variability within discrete lesions resulted from infection by more than one genotype, these data are helpful in explain at least part of the variability in pathogenicity observed in natural populations of this organism, as changes in cultivar host specificity occur very frequently. Several mechanisms have been proposed to explain the variation observed in *M. grisea*. These include heterokaryosis, aneuploidy, recombination, mutations, and recently the possible involvement of transposable elements (Suzuky, 1965; Ou and Ayad, 1968; Giatgong and Frederiksen, 1969; Genovesi and Magill, 1976; Ou, 1980; and Wu, 1990).

The primary objective of this study was to demonstrate that a pattern of variability, in some way similar to what has been observed in the rice blast pathogen *M. grisea*, could also be found in C.*graminicola*. As mentioned above, several mechanisms have been proposed to explain the pathogenic variation in that fungus since it was reported for the first time by Ou and Ayad (1968), and the recent results obtained by Wu (1990) led to the conclusion that transposablity may be involved in the phenomenon, although it still demands further studies to be confirmed. Since this is the first observation of this type of variability in the sorghum anthracnose fungus C. *graminicola*, some of the above mentioned explanations will be considered as possible contributors to the pathogenic variation of this pathogen.

Panaccioni *et al.* (1989) reported the production of oval-shaped conidia by isolates of C.*graminicola* from corn, johnsongrass, and sorghum. Oval conidia with 2 nuclei were common and represented 30% of the population, an oval conidia with 3 and 4 nuclei, were observed in levels always lower than 10% in the population. Oval-shaped conidia are produced below the surface conidia. A possible role for oval cells could be the specific propagation of hyphae that form acervul and bear falcate conidia. Although heterokaryosis has not been observed in nature, the production of multinucleate conidia indicates that heterokaryosis as a mechanism contribuing to pathogenic variation in C.*graminicola* should be under consideration. The uninucleate state of falcate conidia indicates, however that heterokaryosis could not be a primary mechanism for pathogenic variation originating from monoconidial cultures.

No information is avaliable in the literature on variation in chromossome number in conidia of C. *graminicola*, but considering that some possible "revertants" from virulent to an avirulent state were observed, the same arguments discussed by Wu (1990) in relation to M. grisea apply here, since reversion would require the recovery of "lost" chromosomes in the case of aneuploidy, alleles for avirulence in the case of parasexual recombination.

Some isolates in the third generation of monoconidial lines had the same virulence phenotype of isolates obtained from a single lesion of both cultivars, similar to what was observed by Giatgong and Frederiksen (1971) and Wu (1990). The similarities between these two organisms in terms of pathogenic variability, can lead to the idea of transposability as another possible explanations for what was observed. Additional observations are of course necessary, for a clearer indication that transposability could be involved. For example, tests with a larger number of lines could be useful in comparative studies such as in the observation of chromosomal rearrangements and in RFLP or RAPD changes.

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