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IDENTIFICATION OF *BIPOLARIS BICOLOR* AND *BIPOLARIS SOROKINIANA* ON WHEAT SEEDS (*TRITICUM AESTIVUM* L.) IN BRAZIL

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ABSTRACT

Diseases caused in wheat by *Helminthosporium* spp. have led to considerable yield and production losses. Different species in this genus are associated with wheat seeds. In Brazil, spot blotch in wheat is caused by *Bipolaris sorokiniana* (Sacc) Schoem, and another fungus *Bipolaris bicolor* (Mitra) Shoem that has been also isolated from wheat seeds. The current study was undertaken to identify the most frequent fungus species that normaly infects wheat seeds and compared them with *B. sorokiniana*. The fungus *Bipolaris bicolor* (Mitra) Shoem., isolated from wheat seeds cultivar IAPAR, was identified by taxonomic methods and compared with the fungus *Bipolaris sorokiniana* (Sacc.) Shoem., in relation to growth characteristics on the seeds, as well as to growth characteristics in PDA and morphology of the structures. Type of colony observed on the seeds is important for the differentiation between the fungus species. *Bipolaris sorokiniana* presented black colonies, which were well-adherent to the seeds, whereas *B. bicolor* presented grayish, aerial, cotton-like colonies. The size of the conidia also differed in length and width, and *B. bicolor* presented the smallest dimensions. In relation to septa, *B. bicolor* conidia presented deep ones, with dark color bases, but seldom presented dark apex. *Bipolaris sorokiniana* presented homogenous color.

Key words: wheat, *Bipolaris bicolor, Bipolaris sorokiniana*

INTRODUCTION

Helminthosporiosis causes many losses in wheat cultures. The disease is caused by *Helminthosporium* spp, and different species in this genus are associated with wheat seeds. *Helminthosporium* differs from *Bipolaris, Drechslera* and *Exserohilum* by forming parallel-walled, erect conidiophores and *Bipolaris* presents bipolar germination of conidia. In Brazil, spot blotch in wheat is caused by *Bipolaris sorokiniana* (Sacc) Schoem. Another fungi has been isolated from wheat seeds as *Bipolaris bicolor* (Mitra) Shoem that causes as well spot blotch but with minor lesions and can be the most deleterious disease for the producers (7,8,10). Morphological characteristics such conidia (size, shape, and septation) are used as the primary characters for the practical and working identification of *Bipolaris* genus (1). The present work was conducted to study morphological and cultural characters from wheat seed isolates such as *B. bicolor* and compared with *B. sorokiniana.*

MATERIALS AND METHODS

Sanitary analysis of the wheat seeds cultivar IAPAR was performed using the filter paper method and freezing, procedure recommended by the Standard Methods for Seed Analysis (4). After incubation, the identification of the fungi from the seeds was performed by means of mycelial growth, using a Wild M400 stereomicroscope (Wild mod WMS02 and with Wild MPS 55 automatic camera) and conidia, conidiophores by light microscopy (Carl Zeiss, SV-11).

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Conidia of each isolates were transferred to a Petri dish containing solid medium PDA (potato dextrose agar), incubated at 25º to 28ºC, under alternating light (12h white fluorescent light / 12h dark). Six plates of PDA were produced for each species.

On the 7th day of incubation, conidia were removed from the substrate using distilled water and placed on glass slides in order to assess the origin of the hilum and to determine the average size of 100 conidia and conidiophores, using a Carl Zeiss light microscope (x400 to x800). Colonies were also described in relation to their growth on the culture medium, in order to draw a comparison between the species studied. The key proposed by Ellis (6) and the report by Chidambaram (5) were used in the identification of the species.

RESULTS AND DISCUSSION

Bipolaris was observed in wheat seeds originated from different places under microscopy and then obtained 10 isolates from *B. sorokiniana* and six isolates from *B. bicolor.* After 7 days in medium PDA were observed the aspect of development in medium.

Macroscopic morphology

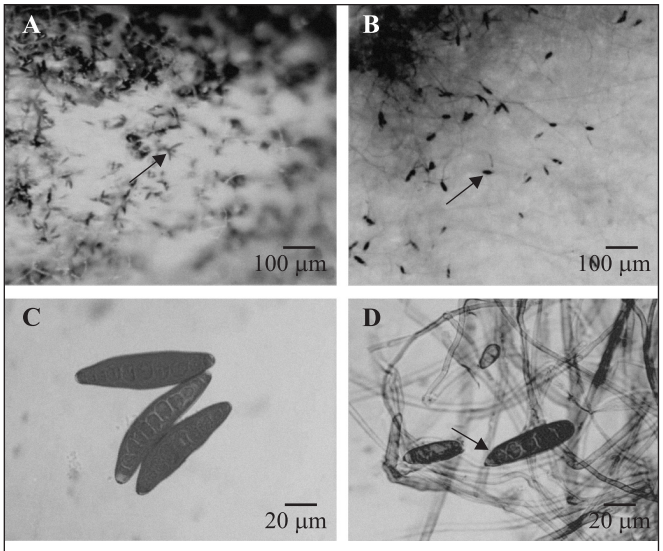
B. sorokiniana and *B. bicolor* colonies were differentiated on wheat seeds (Fig. 1 A, B) and the following characteristics were used: *B. sorokiniana* – black, velvet-like colonies, mycelium practically absent, and close to the seed, short conidiophores, black and shiny conidia. *B. bicolor* – gray, cotton-like colonies, abundant aerial mycelium, sparse conidia on the mycelium.

Bipolaris colonies grow rapidly in PDA media, reaching a diameter of 3 to 9 cm following incubation at 25ºC for 7 days on potato dextrose agar. The colony becomes mature within 5 days.

Observations on petri plates were:

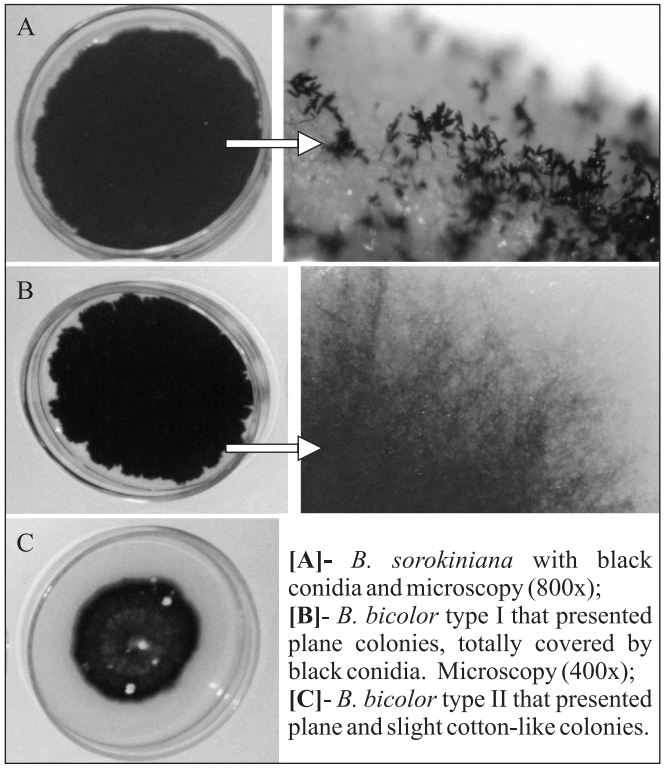
Bipolaris sorokiniana – colonies were velvet-like, dark olive; plane, totally covered by short conidiophores, with black conidia in their apex. Conidia reflected the light and gave the colony a shiny appearance. The reverse side of the colony was dark olive (Fig. 2 A).

Bipolaris bicolor presented two types of colony. The type I - velvet-like, dark olive, plane colonies, totally covered by conidia. Mycelium was absent. Colonies were very similar to what is generally described for *B. sorokiniana.* Reverse side presents different zones, with alternation of dark olive and light brown sections (Fig. 2 B), and the type II – dark olive and slight cotton-like colonies. The reverse side was the same color, and presents a grayish dark olive halo involving the central area (Fig. 2 C). Colonies in PDA presented some differences. However, the characteristic of the colony could not be used as a



[A]- *B. sorokiniana* and arrow indicating the conidia on the seeds with short conidiophores, black and shiny conidia;
[B]- *B. bicolor* and arrow indicating the conidia on the seeds with abundant aerial mycelium and sparse conidia on the mycelium;
[C]- *B. sorokiniana,* aspect of conidia;
[D]- *B. bicolor*, aspect of conidia.

Figure 1. Observation of conidia in wheat seeds under microscopy.



[A]- *B. sorokiniana* with black conidia and microscopy (800x);
[B]- *B. bicolor* type I that presented plane colonies, totally covered by black conidia. Microscopy (400x);
[C]- *B. bicolor* type II that presented plane and slight cotton-like colonies.

Figure 2. Development of *B. sorokiniana* and *B. bicolo*r in solid medium PDA after 7 days from inoculation.

measurable constant standard, once appearance and color intensity may vary with the isolate, due to the heterokaryotic condition of fungi in nature.

Microscopic morphology

B. sorokiniana conidia were longer than 75µ and less than 20µ wide. Pseudosepta were present at any age (Fig. 1C).

B. bicolor conidia size ranged from 40 to 78µ long and from 12.1 to 17.3µ wide. It presented 5 to 9 pseudosepta, generally six. They were straight, ellipsoid, with round edges, occasionally similar to an inverted club, rarely curve, from medium brown to dark brown in color. In more advanced developmental stages, cells from the edges tend to become more hyaline and pseudosepta in these cells seemed to be thicker, whereas the central part became almost black and made pseudosepta impossible to count (Fig. 1D). Results of the observations on *B. bicolor* are in accordance with the description by Morejón (9), who reported the same characteristics of the fungus causing leaf spot in peachpalm.

Although *B. bicolor* and *B. sorokiniana* conidia may present some similarities in relation to shape and color, when microscopically examined for the first time, conidia of the second species are larger and longer. However, this characteristic may sometimes be confusing, due to superposition in the variations among isolates.

Other characteristics that aided the identification for both were: *B. bicolor* and *B. sorokiniana* conidiophores were long, and reached 400µ long and 5.5 - 10µ wide. They were as well flexible, smooth, septated, and medium to dark brown. They were repeatedly geniculated in the terminal edge, presenting pores or scars at short intervals, which indicated the sites conidiophores were produced. They presented conidiogenous polytretic, integrated, terminal, intercalary and cicatrized cells.

Bipolaris sorokiniana (teleomorph *Cochliobolus sativus*) is the causal agent of common root rot, leaf spot disease, seedling blight, head blight, and black point of wheat and barley. The fungus is one of the most serious foliar disease constraints for both crops in warmer growing areas and causes significant yield losses.

However, we have isolated in wheat seeds *B. bicolor* which was considered to be pathogenic in Cuba (9). In that country, it has been isolated from leaf lesions in sugar cane, occuring under climatic conditions similar to those reported by Mehta (8) and Reis (10,11), which are the most propitious conditions for the incidence of helmintosporiosis in wheat. According to Chidambaram (5), *B. bicolor* is knowingly pathogenic in some countries, and may produce coleoptile rot and darkening of the roots in wheat. Bach *et al.* (2) demonstrated that five isolates from *B. bicolor*, when at 10^5 conidia suspension was used in wheat plants in a greenhouse, spot bloth on leaves was observed. Bach and Kimati (3) also demonstrated that *Bipolaris bicolor* from wheat was differentiated from others *Bipolaris* with esterase electrophoretic analysis. So, the isolates of *B. sorokiniana* was the most serious disease in wheat plants in this country but, isolates of *B. bicolor* can also presented pathogenicity when in Brazil the climatic conditions was ideal for the outbreak of the disease and we have a problem in a once more pathogenic disease.

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RESUMO

Identificação de *Bipolaris bicolor* e *Bipolaris sorokiniana* sobre sementes de trigo (*Triticum aestivum* L.) no Brasil

Doenças causadas por *Helminthosporium* spp. em trigo, causam consideráveis perdas na produção. Diferentes espécies do gênero do fungo podem ser encontradas em sementes. No caso do Brasil, a mancha foliar do trigo tem sido causada por *Bipolaris sorokiniana* (Sacc) Schoem, entretanto, outro fungo como *Bipolaris bicolor* (Mitra) Shoem tem sido isolado de sementes do trigo. O objetivo do presente trabalho foi identificar as espécies de fungo que normalmente infectam sementes de trigo e comparar com a mais comum *B. sorokiniana*. O fungo *Bipolaris bicolor* (Mitra) Shoem., isolado de sementes de trigo var. IAPAR, foi identificado por métodos taxonômicos e comparado com o fungo *Bipolaris sorokiniana* (Sacc.) Shoem. em relação ao crescimento sobre as sementes bem como às características culturais no meio BDA e morfologia das estruturas. O tipo de colônia observada sobre as sementes é importante para diferenciar as espécies entre si. O fungo *B. sorokiniana* apresentou colônias de cor preta sendo bem aderidas às sementes, enquanto *B. bicolor* apresentou colônias aéreas acinzentadas e com aspecto cotonoso. O tamanho dos conídios também diferenciaram no comprimento e largura onde os valores menores corresponderam a *B. bicolor*. Em relação aos septos, os conídios de *B. bicolor* apresentavam septo "profundo", com coloração escura na base e, raras vezes no ápice. Os conídios de *B. sorokiniana* apresentaram coloração homogênea.

Palavras-chave: trigo, *Bipolaris bicolor*, *Bipolaris sorokiniana*

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