Original Article

# VARIABILITY OF SEED-BORNE *Colletotrichum* STRAINS IN COTTON BASED ON ITS1 AND ITS2 RIBOSSOMAL GENES ANALYSIS

## *VARIABILIDADE DE ESTIRPES DE* Colletotrichum *PROVENIENTES DE SEMENTES DE ALGODÃO COM BASE NA ANÁLISE DOS GENES RIBOSSOMAIS ITS1 E ITS2*

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**ABSTRACT:** The use of DNA sequences analysis has been an important mean to distinguish and to identify populations of organisms at different levels. By molecular markers several complex organisms have been successful detected in plants for distinct aims. Ribosomal DNA (rDNA) has been used to evaluate genetic variability, microorganism phylogeny and to develop specific primers for detection of plant pathogens in plant tissues. In this study, the objective was to characterize isolates of *Colletotrichum gossypii* var. *cephalosporioides* and *Colletotrichum gossypii*, collected in different regions of Brazil, by analyzing the nucleotide sequence of rDNA regions. ITS1, ITS2, and the intervening 5.8S gene were amplified by PCR and their sequences compared to each other and to those from other species registered in the GenBank. The rDNA of isolates associated with *Gossypium* spp. showed sequence identities ranging from 96 to 100% in the ITS1 region, 98 to 100% in the 5.8S gene, and 97 to 100% in the ITS2 region. The sequences were submitted to UPGMA analysis, and according to the phylogenetic trees, the *C. gossypii* var. *cephalosporioides* and *C. gossypii* species clustered together along with isolates of *Glomerella cingulata* from mango and papaya, and thus no distinction could be made between isolates of those organisms.

**KEYWORDS:** *Colletotrichum gossypii*. *Colletotrichum gossypii* var. *cephalosporioides*. rDNA. Phylogeny. *Gossypium* spp.

## INTRODUCTION

Anthracnose and ramulosis, caused by the seed-transmitted fungi *Colletotrichum gossypii* South. and *Colletotrichum gossypii* var. *cephalosporioides* A. S. Costa, respectively, are widespread diseases of cotton with high potential to severely reduce yields (LIMA et al., 1985; TANAKA, 1995). Moreover, the amount of inoculum of both pathogens on cotton seeds influences the disease progress at variable levels of incidence and inoculum severity (ARAÚJO et al., 2006).

The species *C. gossypii*, described by Southworth in 1890 (VIÉGAS, 1946), was subsequently reported by Arx (1957) as belonging to the *C. gloeosporioides* group, with *Glomerella cingulata* as the teleomorph. In cotton plants, the *cephalosporioides* variety, the causal agent of ramulosis, reported by Costa and Fraga (1937) and described by Costa in 1946 (VIÉGAS, 1946), was first identified in the State of São Paulo, associated with the witch's broom symptoms, which were not observed in the strains that cause anthracnose.

Sutton (1992) considered *Colletotrichum gossypii* var. *cephalosporioides* a distinct group. According to the cultural, pathogenic and serological characteristics, Ottonello (1992) suggested that such group could be divided in two *forma specialis* subgroups of *C. gloeosporioides* f. sp. g*ossypii*. The first subgroup, represented by race 1, should be composed of strains not associated with dieback symptoms and, therefore, the cause of anthracnose disease. The second subgroup, represented by race 2 of *C. gloeosporioides* f. sp. *gossypii*, is composed of those strains inducing witch's broom symptoms, the causal agent of ramulosis disease.

Great variation in aggressiveness of *Colletotrichum* strains has been observed by several authors (DUDIENAS, 1990; TANAKA, 1990; IAMAMOTO, 2002; SILVA-MANN, 2002), suggesting the presence of physiological races, and according to Chitarra (1996), the strains that cause ramulosis are not clearly distinguishable from the ones that cause anthracnose. The studies of the relationship between the pathogen and the host are usually conducted with strains isolated directly from plants exhibiting the typical disease symptoms to ensure the capability of strains to induce the above-mentioned symptoms.



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The use of DNA sequences seems to be a reliable tool in defining genetically related groups of microorganisms in comparison with studies including disease symptoms (VIEIRA, 2002; VIEIRA and MACHADO, 2002). Most of the studies on genetic variability of strains from the *Colletotrichum* complex in cotton are based on polymorphism among randomly amplified DNA fragments (CHITARRA, 1996; VIEIRA, 1996; MEHTA et al., 2001; SILVA-MANN et al., 2002, 2005) and isozyme patterns (VIEIRA, 1996; CARVALHO et al., 1997). From the results of those studies, it is concluded that the primers used might be unable to distinguish members of those groups of fungi associated with cotton seeds.

Among the DNA regions used to compare species within the genus *Colletotrichum* the Internal Transcribed Spacers (ITS1 and ITS2) of the rDNA, that flank the 5.8S gene, are mostly mentioned in literature (SHERRIFF et al., 1994, 1995; BAILEY et al., 1996; SREENIVASAPRASAD et al., 1996; HSIANG and GOODWIN, 2001; DENOYES-ROTHAN et al., 2003; CANO et al., 2004). By using primers specific to the ITS2 region to characterize *Colletotrichum* spp. associated with the family Malvaceae, Bailey et al. (1996) found sequence identity of 98.5% between *C. gossypii* and *C. gossypii* var. *cephalosporioides*. They found the genetic variability between the strains from cotton is relatively low, although only one strain of *C. gossypii* and two strains of *C. gossypii* var. *cephalosporioides* strains were used. Strains of *C. gloeosporioides* from *Aeschynomene virginica*, *Stylosanthes scabra* and *Mangifera indica* grouped with the strains isolated from cotton while the strains of *C. gossypii* var. *cephalosporioides* were also considered part of a subgroup different from that of *C. gossypii* (BAILEY et al., 1996).

By using rDNA ITS2 sequences, Sherriff et al. (1995) showed that strains of *Colletotrichum graminicola* isolated from corn were different from the strains of *C. graminicola* obtained from sorghum (*Sorghum bicolour* L.) and *Rottboellia* sp., providing further evidence for the classification by Sutton (1980). Accordingly, strains from sorghum and *Rottboellia* sp. could be considered the *C. sublineolum* species. Besides, analysis of the ITS1 region confirmed the classification (SUTTON, 1980) in which strains from corn and sorghum are considered distinct species (SREENIVASAPRASAD et al., 1996).

Results based on the analysis of DNA sequences from the ITS2 region of strains of *Colletotrichum acutatum* from strawberries clearly showed the existence of two subgroups: CA-cloned, composed of strains from strawberry, and CA-variable, composed of strains from strawberry and other hosts. This suggests that the strains from strawberry from the CA-cloned subgroup have gone through a process of specialization. In that study, no correlation between genetic groups and pathogenicity was established (Denoyes-Rothan et al., 2003).

Because of the difficulties to associate *C. gossypii* var. *cephalosporioides* to different degrees of aggressiveness and expression of symptoms, efforts were directed to the genetic characterization of *Colletotrichum* spp. associated with cotton plants. To date, the results are not sufficient to overcome the problems related to diagnosis. Given the difficulties in distinguishing between the causal agents of cotton ramulosis and anthracnose, and the lack of information about their rDNA ITS1 and ITS2 regions, the objective in the present work was to carry out the phylogenetic characterization of *C. gossypii* strains, considered to be the etiological agent of anthracnose, and of *C. gossypii* var. *cephalosporioides* strains, the etiological agent of ramulosis, from different geographic origins in Brazil.

## MATERIAL AND METHODS

The work was conducted at the Seed Pathology and the Molecular Plant Virology Laboratories of the Plant Pathology Department of the Federal University of Lavras (UFLA).

Five strains of *C. gossypii* and 17 strains of *C. gossypii* var. *cephalosporioides* were analyzed. Strains were obtained from cotton seeds from Campinas, State of São Paulo, and from Lavras, State of Minas Gerais, and part of the strains was supplied by EMBRAPA/CNPA culture collection (Table 1). All strains were characterized by pathogenicity test and stored over activated silica gel at room temperature.

**Pathogenicity test.** For this test an inoculum suspension, at concentration of $10^6$ conidia × $mL^{-1}$ was sprayed onto cotton plants of the cultivar NU-15, 30 days of age. The plants were kept in a moist chamber at 25°C under 12h light/12h dark for 72 hours. The control treatment consisted of plants sprayed with distilled and sterilized water and submitted to the same conditions as those inoculated. The assessments were carried out 30 days after inoculation, following the criteria established by Cia (1977), and adapted by Silva-Mann (2002). The experiment was arranged in a randomized complete block design with 23 treatments and four replicates per treatment. The

data were submitted to variance analysis, and the means were compared by the Tukey test, at a significance level of 5%. The statistical analysis was performed using the Sisvar™ program (Ferreira, 2000).

**Table 1.** Geographic origins of isolates of *C. gossypii* and *C. gossypii* var. *cephalosporioides* used in this study.

| Strain | Cultivar | Geographic origin |
|---|---|---|
| LPS1005.*C. gossypii* | NU-15 | Lavras, MG |
| LPS1015.*C. gossypii* | NU-15 | Lavras, MG |
| LPS1016.*C. gossypii* | NU-15 | Campinas, SP |
| LPS1020.*C. gossypii* | NU-15 | Campinas, SP |
| LPS1025.*C. gossypii* | NU-15 | Campinas, SP |
| AM3F.*C. gossypii* var. *cephalosporioides* | NI* | Alto Taquari, MT |
| PF-2a.*C. gossypii* var. *cephalosporioides* | NI | Itiquira, MT |
| AM-1.*C. gossypii* var. *cephalosporioides* | Aroeira | Chapadão do Céu, GO |
| PE-1.*C. gossypii* var. *cephalosporioides* | ITA-90 | Pedra Preta, MT |
| Cg021.*C. gossypii* var. *cephalosporioides* | Ipê | Santa Helena, GO |
| Cg002.*C. gossypii* var. *cephalosporioides* | Ipê | Acreúna, GO |
| Cg012.*C. gossypii* var. *cephalosporioides* | Ipê | Acreúna, GO |
| PF-3.*C. gossypii* var. *cephalosporioides* | Cedro | Pedra Preta, MT |
| PF-1b.*C. gossypii* var. *Cephalosporioides* | Cedro | Rondonópolis, MT |
| PF-1a.*C. gossypii* var. *Cephalosporioides* | Cedro | Rondonópolis, MT |
| PD-2.*C. gossypii* var. *Cephalosporioides* | Cedro | Rondonópolis, MT |
| Cg027.*C. gossypii* var. *Cephalosporioides* | Ipê | Santa Helena, GO |
| PF-2b.*C. gossypii* var. *Cephalosporioides* | NI | Primavera do Leste, MT |
| PF-1c.*C. gossypii* var. *Cephalosporioides* | ITA-90 | Itiquira, MT |
| Cg003.*C. gossypii* var. *Cephalosporioides* | Ipê | Acreúna, GO |
| Cg015.*C. gossypii* var. *Cephalosporioides* | Ipê | Santa Helena, GO |
| Ca24.*C. gossypii* var. *cephalosporioides* | Híbrido Israelense | Novo São Joaquim, MT |

*Not informed.

**DNA extraction.** The fungal strains were cultivated in 50 mL of malt extract broth and incubated for 3 days at 20°C on a shaker (80 rpm). Latter, the mycelium was filtered using a vacuum pump and filter paper pads, and 150 mg of the mycelium mass was ground under liquid nitrogen in the presence of polyvinylpyrrolidone (PVP). Genomic DNA was extracted using the method described by Möller et al. (1992), with minor modification. Following extraction, the DNA was treated with 5 µL of bovine ribonuclease A (0.5 mg × $mL^{-1}$) at 37°C for 30 minutes. The DNA was precipitated by the addition of 10 µL of 3M sodium acetate and 275 µL of absolute ethanol, and incubation at –20°C for 30 minutes. The supernatant was removed by centrifugation at 14,000 rpm, and the DNA pellet was washed with 70% ethanol, and resuspended in 60 µL of TE buffer. The amount and quality of the DNA were assessed by electrophoresis on 0.7% agarose gels.

**Amplification of ITS1, 5.8S, and ITS2 regions.** The ITS1, 5.8S, and ITS2 regions of the nuclear ribosomal DNA were amplified by polymerase chain reaction (PCR). Each reaction was performed using 5µL of 10X PCR buffer (0.5M Tris-HCl; 0.7M KCl; 0.1M $MgCl_2$ pH 8.0), 4 µL of $MgCl_2$ (25mM), 5 µL of dNTP mix [2mM of each dNTP (100mM); 1 µL of Tris-HCl pH 8.0 (1M); 1 µL of DTT (100mM); 90 µL "ultra pure" water], 1 µL of *Taq* DNA polymerase (5 U $\mu L^{-1}$), 29.5 µL of "ultra pure" water, 2 µL (10 ρmol $\mu L^{-1}$) of the primers used to amplify the ITS1-5.8S-ITS2 region of the rDNA – ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCG TAACAAGG-3') (White et al., 1990). PCR assays were carried out for 35 cycles in a PTC-100 thermocycler (MJ Research, Inc., USA), using the following conditions: 40 seconds at 94°C, 55 seconds at 50°C, 2 minutes at 72°C, followed by a final step of 5 minutes at 72°C. PCR products were analyzed by electrophoresis on 0.7% agarose gels stained with ethidium bromide. A 1-kb DNA ladder (Life Technologies) was used as size marker. After capturing the gel image on an Image Master (Pharmacia), the PCR-amplified DNA fragment was excised from the gel, purified using the QIAquick Gel Extraction Kit (Qiagen), and

sequenced using an ABI 3100 DNA sequencer (Applied Biosystems).

**Sequence analysis.** Multiple alignments of the sequences of the amplified fragments, comprising the ITS1, 5.8S, and ITS2 regions of the ribosomal DNA, and nucleotide sequences deposited in GenBank were carried out using the ClustalW program (http://www.ebi.ac.uk/clustalw/). The BLAST program (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) was used to search for orthologous *Colletotrichum* sequences available at the NCBI database (http://www.ncbi.nlm.nih.gov/) (Table 1). Genetic distance was estimated by the UPGMA algorithm. Phylogenetic trees were constructed and visualized using the MEGA3 program (Kumar et al., 2004). Bootstrapping was performed with 1,000 replicates to test the support for each branch of the tree.

## RESULTS

**Pathogenicity test.** From that test three severity patterns of infection were observed among the *Colletotrichum* strains (Table 2) based on the disease index proposed by Cia (1977). The severity score 1 group was composed of the strains LPS 1005, 1015, 1016, 1020, and 1025, which did not induce symptoms on the plants. The severity score 2.5 group was composed of the strains Cg021, AM3F, PE-1, PF-2a, and AM-1, which induced symptoms of star-shaped spots on the top leaves and reduction in internode length by up to 40%, typical of ramulosis disease. The largest group, with mean severity scores ranging from 2.75 to 3.5, was represented by the 12 other strains, which induced more severe symptoms including star-shaped spots on the top leaves and reduction in internode length by 40 to 60%, also typical of the disease caused by *C. gossypii* var. *cephalosporioides*.

**Table 2.** Mean severity of ramulosis disease in cotton, cv. NU-15, in trial of inoculation of different *Colletotrichum gossypii* isolates under controlled conditions.

| Strain | Characterization | Severity | Symptom |
|---|---|---|---|
| LPS1005 | *C. gossypii* | 1 b | PSS |
| LPS1015 | *C. gossypii* | 1 b | PSS |
| LPS1016 | *C. gossypii* | 1 b | PSS |
| LPS1020 | *C. gossypii* | 1 b | PSS |
| LPS1025 | *C. gossypii* | 1 b | PSS |
| AM3F | *C. gossypii* var. *cephalosporioides* | 2.25 ab | PSS/RI<40% |
| PF-2a | *C. gossypii* var. *cephalosporioides* | 2.25 ab | ME/RI<40% |
| AM-1 | *C. gossypii* var. *cephalosporioides* | 2.25 ab | ME/RI<40% |
| PE-1 | *C. gossypii* var. *cephalosporioides* | 2.25 ab | PSS/ME/RI<40% |
| Cg021 | *C. gossypii* var. *cephalosporioides* | 2.25 ab | PSS/ME/RI<40% |
| Cg002 | *C. gossypii* var. *cephalosporioides* | 2.75 a | ME/RI<40% |
| Cg012 | *C. gossypii* var. *cephalosporioides* | 2.75 a | ME/RI<40% |
| PF-3 | *C. gossypii* var. *cephalosporioides* | 2.75 a | ME/RI<40% |
| PF-1b | *C. gossypii* var. *cephalosporioides* | 2.75 a | ME/RI<40% |
| PF-1a | *C. gossypii* var. *cephalosporioides* | 2.75 a | PSS/RI<40%/SB-RD40-60% |
| PD-2 | *C. gossypii* var. *cephalosporioides* | 2.75 a | ME/RI<40%/SB-RD40-60% |
| Cg027 | *C. gossypii* var. *cephalosporioides* | 3.25 a | RI<40%/SB-RD40-60% |
| PF-2b | *C. gossypii* var. *cephalosporioides* | 3.5 a | RI<40%/SB-RD40-60% |
| PF-1c | *C. gossypii* var. *cephalosporioides* | 3 a | ME/RI<40%/SB-RD40-60% |
| Cg003 | *C. gossypii* var. *cephalosporioides* | 3 a | RI<40% |
| Cg015 | *C. gossypii* var. *cephalosporioides* | 3 a | RI<40% |
| Ca24 | *C. gossypii* var. *cephalosporioides* | 3 a | ME/RI<40% |
| Test | ---------- | 1 b | PSS |

Values followed by a common letter are not statistically different ($P \leq 1\%$) as determined by the Tukey test. PSS = plant without symptom, ME = star-shaped spot, RI < 40% = reduction in internode length by up to 40%, SB-RD 40 to 60% = witch's-broom and reduction in internode length by 40 to 60%.

**Nucleotide sequence analyses.** A fragment of approximately 500-bp long comprising the complete sequences of the ITS1, 5.8S gene, and ITS2 regions of the ribosomal DNA was obtained from all *Colletotrichum* strains from cotton considered in this study by PCR assays using the primers ITS4 and ITS5. As for the individual rDNA regions sequence sizes, they were 167-168-bp, 159-bp, and 177-bp long for the ITS1, 5.8S gene, and ITS2 regions, respectively.

Multiple sequence alignment of the rDNA ITS1 region of the 22 analyzed *Colletotrichum* strains from cotton revealed sequence identities ranging from 96 to 100%. Although the strains Cg027 and AM-1 were identified as *C. gossypii* var. *cephalosporioides* by the pathogenicity test in this work, they presented the lowest sequence identity based on the ITS1 region (Figure 1). In contrast, the highest sequence identity was observed between the ITS1 regions of the *C. gossypii* strains LPS1015 and LPS1020, as well as between LPS1015 and LPS1025. The other strains showed sequence identities of 98-99%. The identity between the *C. gossypii* and *C. gossypii* var. *cephalosporioides* ITS1 sequences varied from 97 to 100%. The strains LPS1015 and LPS1025 showed 100% ITS1 sequence identity to those of 10 out of the 17 *C. gossypii* var. *cephalosporioides* strains. Among the fungal strains sequences, only the strain LPS1016 showed sequence identities of 97-99% to the strains associated with ramulosis, while the ITS1 regions of the strains LPS1015 and LPS1020 exhibited 100% identity to the regions of five of the 17 *C. gossypii* var. *cephalosporioides* strains. Among the *C. gossypii* var. *cephalosporioides* strains, PF-1b, PE-1 and PF-3 showed 100% ITS1 sequence identity to 10 strains, whereas the AM-1 strain has shown to be most divergent, sharing less than 100% ITS1 sequence identity with the other strains.

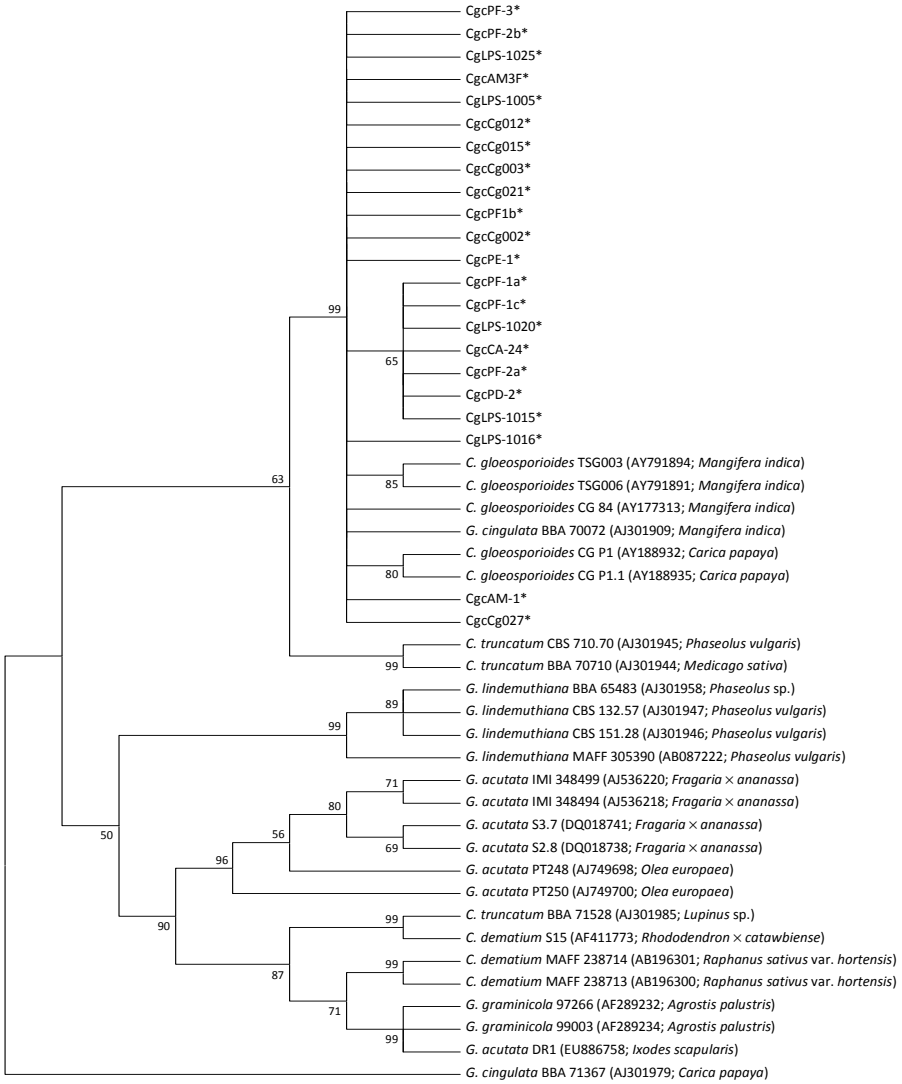


**Figure 1.** ITS1 rDNA sequence-based phylogenetic tree of *Colletotrichum* strains and orthologous species deposited in GenBank. Bootstrap values (UPGMA algorithm) were calculated using MEGA program version 3.1. Values greater than 50% (from 1,000 bootstrap replicates) are shown at nodes. GenBank accession numbers and host plants are indicated between brackets.

*Sequences from strains belonging to the cotton plant complex.

Smaller variation was found in the rDNA ITS2 region between the 22 strains, as compared to the ITS1 region (Figure 2). The majority of strains of each fungus showed 100% sequence identity, except the strains: Cg021, Cg027, PF-1b, PF-3 and AM-1.

Sequence alignment analyses of the rDNA ITS2 region of all strains including those from GenBank revealed 100% nucleotide sequence identity between the strains considered in this study and the strains BBA 70072 and CG 84 of *G. cingulata* from mango. Identity of 95% was found in sequence alignments with the other mango strains (TSG003 and TSG006), and the papaya strains CG P1 and CG P1.1.

Similarly, Bailey et al. (1996) found 96-98% nucleotide sequences identity between *C. gossypii* and *C. gossypii* var. *cephalosporioides* strains obtained in Brazil and Argentina, including the strains considered in this study, which grouped together with the strains from mango and papaya.

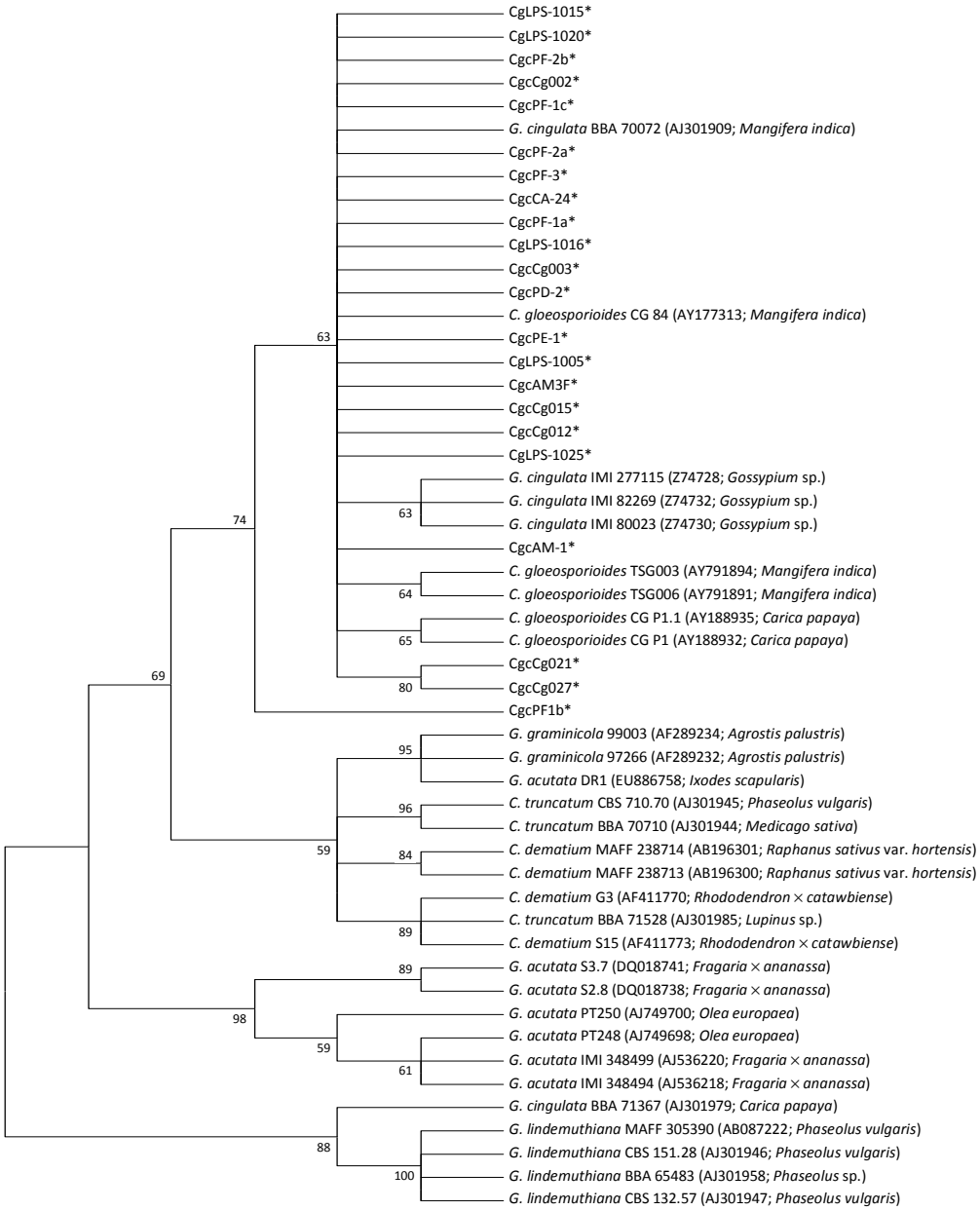


**Figure 2.** ITS2 rDNA sequence-based phylogenetic tree of *Colletotrichum* strains and orthologous species deposited in GenBank. Bootstrap values (UPGMA algorithm) were calculated using MEGA program version 3.1. Values greater than 50% (from 1,000 bootstrap replicates) are shown at nodes. GenBank accession numbers and host plants are indicated between brackets.

*Sequences from strains belonging to the cotton plant complex.

## DISCUSSION

The variability in aggressiveness among *Colletotrichum* strains has already been reported by other authors on inoculated plants of cotton cultivars, showing that the interaction between pathogen and host may be quite complex (DUDIENAS, 1990; IAMAMOTO, 2002; SILVA-MANN, 2002). In this work another evidence of that interaction was observed on some plants in that the severity score 1 group exhibited slight necrosis of leaf veins, a symptom not considered in the rating scale proposed by Cia (1997). Although this symptom has been ascribed to ramulosis, it is typical of anthracnose in other crops.

Nucleotide alignment between sequences of the strains considered in this study and the majority available at the NCBI database revealed identities ranging from 80 to 88%, showing a clear distinction of species. The highest sequence identities were shown to the strains that colonize papaya, CG P1 (94-96%) and CG P1.1 (95-97%), and to the mango strains TSG003, TSG006 and CG 84 (95-97%), and BBA 70072 (95-96%), suggesting a close relationship between them. The lowest sequence identities were found in relation to the strain BBA 71367 from papaya (72-76%). This strain also exhibited low sequence identities (varying from 75 to 85%) to the other strains in the NCBI database, including those from papaya – CG P1 (75%) and CG P1.1 (77%).

By the phylogenetic and pathogenicity analyses (Table 2, Figures. 1 and 2), *C. gossypii* and *C. gossypii* var. *cephalosporioides* strains did not differ significantly based on their ITS1 and ITS2 nucleotide sequences, which is not in agreement with the taxonomical classification as distinct fungi. These findings lead the direction of this investigation to shift to other approaches considering for example analysis of other DNA regions and proteomics analysis in addition to the use of other molecular techniques

According to the ITS1 sequence-based phylogenetic tree (Figure 1), *C. gossypii* and *C. gossypii* var. *cephalosporioides* belong to the same cluster, indicating a close relationship between them. The strains from mango and papaya, with higher sequence identity, also grouped together. The other strains represent distinct groups: *C. truncatum* strain CBS 710.70, from lupin, and BBA 70710 strain, from alfalfa, are the most closely related strains. Such specialization within and between host groups has been reported in literature (SHERIFF et al., 1995; HSIANG; GOODWIN, 2001; DENOYES-ROTHAN et al., 2003).

In relation to the alignment of the 5.8S gene sequences of the 22 fungal strains, it was revealed high nucleotide identity (98-100%) to each other. Similar results were obtained when comparing those sequences to ones available in GenBank (97-100% sequence identity). That provides further evidence for the low variability of this region which is considered to be highly conserved among fungal species (MILLER et al., 1999; SILVA-HANLIN et al., 1999; FUNGARO, 2000).

According to the ITS2 sequence-based phylogenetic tree (Fig. 2), the strains associated with anthracnose and ramulosis of cotton clustered together in a group including strains associated with mango and papaya, except the strain PF-1b of *C. gossypii* var. *cephalosporioides* which differed from the group comprised of the above-mentioned strains. In general the strains from the GenBank tended to group according to each fungal species in this work, with the exception of the strain BBA 71367 of *G. cingulata* from papaya, which clustered with *C. lindemuthianum* strains.

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**RESUMO:** O uso de sequências de fragmentos de DNA tem sido importante ferramenta para distinguir e identificar populações de organismos em diferentes níveis de variação. Por meio de marcadores moleculares alguns organismos com variações taxonômicas complexas têm sido detectados com sucesso em tecidos vegetais. O DNA ribossomal tem sido utilizado para avaliar variabilidade genética, filogenia de micro-organismo e para desenvolver oligonucleotídeos específicos visando à detecção de fitopatógenos. Nesse estudo o objetivo foi comparar isolados do complexo *Colletotrichum*, incluindo *C. gosssypii* var. *cephalosporioides* e *C. gossypii,* coletados em diferentes regiões do Brasil, todos associados às sementes de algodão, pela análise de sequências de nucleotídeos de regiões de rDNA. ITS1, ITS2 e o gene 5.8S que foram amplificados por PCR e suas sequências comparadas entre si com outras sequências

depositadas no GenBank. O rDNA de isolados associados com *Gossypium* spp. mostraram identidades de sequências na faixa de 96 to 100% na região ITS1, 98 to 100% na região de 5.8S, e 97 a 100% na região ITS2. As sequências foram submetidas a análise UPGMA, e de acordo com as árvores filogenéticas , *C. gossypii* var. *cephalosporioides* e *C. gossypii* fizeram parte de um mesmo cluster junto com isolados de *Glomerella cingulata* de manga e mamão, e assim nenhuma distinção pode ser feita entre os isolados destes organismos.

**PALAVRAS-CHAVE:** *Colletotrichum gossypii*. *Colletotrichum gossypii* var. *cephalosporioides*. rDNA, phylogeny. *Gossypium* spp.

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