Vol.53, n. 4: pp.741-752, July-August 2010
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Colletotrichum Isolates Related to Anthracnose of Cashew Trees in Brazil: Morphological and Molecular Description Using LSU rDNA Sequences

Ana Maria Queijeiro Lopez[1*] and John Alexander Lucas[2,3]
[1]Instituto de Química e Biotecnologia; Universidade Federal de Alagoas; 57072-970; Maceió - AL - Brasil. [2]University of Bristol; Department of Agricultural Sciences; Bristol BS41 9AF - UK. [3]IACR - Rothamsted; Harpenden; Herts AL5 2JQ - UK

ABSTRACT

Thirty six isolates of fungi obtained from anthracnose lesions of cashew and associated host plants in Brazil, were compared by their cultural, morphological and partial sequences of the 28S ribosomal DNA characters. They showed a high degree of cultural variability. The average mycelial growth rate on all tested media ranged from 10.2-13.3 mm/day between the isolates. Most of them produced perithecia (sterile and fertile) and some produced setae (sterile and fertile). All the isolates produced acervuli with predominantly cylindrical conidia (12.4-17.7 μm X 4.8-6.0 μm in width) with round ends, which became septate on germination, and produced unlobed or slightly-lobed appressoria. Comparison of the D2 domain of the large subunit (LSU) rDNA sequences with those of other defined species of Colletotrichum *and* Glomerella *grouped 35 of the isolates with known strains of* C. gloeosporioides *from different hosts (> 98.9% homology). The one exception (LARS 921) was identical to* G. cingulata *(LARS 238) from* Vigna unguiculata.

Key words: Anthracnose; inflorescence blight; *Colletotrichum*; rDNA; cashew; *Anacardium occidentale*

INTRODUCTION

The cashew (*Anacardium occidentale* L.) crop and associated agro-industrial business are of major socio-economic impact in three states of the Northeastern Brazil (Ceará, Rio Grande do Norte, and Piauí), providing annually incomes around 230 million dollars with exports of 90% of the production of the cashew nut and shell liquid (Sindicaju, 2007). This region accounts for the third highest cashew production (FAO, 2008). The major causes of direct or indirect losses are the inflorescence blight, fruit rot and leaf anthracnose, caused by the fungus morphologically described as the anamorphic stage of *Glomerella cingulata* (Stoneman) Sp. and Schrenk, *i.e.*, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. All the commercially available clones are susceptible to infection (Freire and Cardoso, 1995). Symptoms include sunken subcircular or angular lesions that produce erumpent, mucilaginous, orange spore masses in favorable environments. In general, such symptoms are similar in all cashew hosts from different localities, but the isolates of the pathogen may vary in its degree of aggressiveness as well as in its cultural and morphological characters.

Species concepts based on morphological criteria are generally broad for the genus *Colletotrichum*, and wide variations for cultural and morphological characters, pathogenicity and host range have been

*Author for correspondence: amql@qui.ufal.br

reported among isolates of *C. gloeosporioides* (Alahakoon and Brown, 1994; Gonçalves-Vidigal et al. 2008; Jeffries et al., 1990; Skipp et al., 1995; Menezes and Hanlin, 1996a, b; Sera et al. 2007). Sequence analyses of the intergenic transcribed spacers (ITS 1, ITS 2) and Domains (D1,D2) of large subunit (LSU) ribosomal DNA (rDNA) have been valuable for delineating isolates and species from this genus (Adaskaveg and Hartin, 1997; Forseille, 2007; Freeman et al., 2001; Johnston, 2000; Johnston and Jones, 1997; Moriwaki et al., 2002; Sherriff et al., 1994; Sreenivasaprasad et al., 1996; Vinnere et al., 2002).

In a study of *C. gloeosporioides* isolates from *Stylosanthes* spp. in Mexico, morphological and molecular criteria, including amplification and restriction of a specific fragment of the internal transcribed spacer 1 (ITS1) region of rDNA, were used to cluster the isolates into three groups partially related to their host species of origin (Munaut et al., 2001). Later, Abang et al. (2002) failed to distinguish *C. coccodes* from *C. circinans* based on restriction analysis of their ITS sequence. These authors hypothesized that these species were identical and could belong to the same taxon, in spite of apparent morphological differences. In further studies, Fagbola and Abang (2004) provided evidences of this differentiation of both species after amplifying their 18S rDNA molecules (1.65 Kb long fragments), and submitting them to denaturing gradient gel electrophoresis (DGGE). Cano et al. (2004), however, demonstrated that sequencing of D1-D2 domains of the LSU rDNA gene also supported the results provided by the amplification of ITS1 in distinguishing between *Colletotrichum* spp. that were of human clinical interest. Further, Cannon et al. (2008) emphasized that the anamorphic species *C. gloeosporioides* had never been adequately typified in agreement to the modern nomenclatural practice, and there were three distinct rDNA ITS sequences of the epitype strain in GENEBANK, so it should be re-sequenced to confirm the true base-pair structure.

A better understanding on the population structure of *C. gloeosporioides* associated with anthracnose of cashew will increase the basis of recommendations for the management/control of disease, influencing in breeding programs. Since there are no good morphological markers for its subgroups, molecular markers, such as sequences of rDNA, are needed. This study aimed to determine the extent of variation in a collection of thirty six fungi isolates from anthracnose lesions in cashew and associated host plants in Brazil, combining the cultural, morphological and molecular criteria - based on D2 nucleotide sequences of the LSU rDNA, to provide their identity.

MATERIALS AND METHODS

Isolates and cultural characteristics

Thirty six isolates of *Colletotrichum* from cashew and associated host plants were obtained from various locations in NE Brazil (Table 1). All the strains were subcultured on cornmeal agar (CMA) to ensure the purity (O'Connell et al., 1985). Spores were produced by subculturing the isolates in 100 ml of *Colletotrichum* medium (CM) (Mathur et al., 1950) dispensed in flasks (250 ml), for seven days, and the spore suspensions were obtained by adding *ca.* 20 ml sterile distilled deionized water (SDW) to these cultures, and shaking. Then, single-spore cultures were produced from all the isolates prior to experimental use. The same procedure was carried out using reference strains from the Long Ashton Research Station-UK (LARS) collection, chosen as representatives of different morphological groups, with emphasis on those with straight conidia/spores that infected distinct hosts [C. capsici (LARS 141) and *G. cingulata* (LARS 238) from *Vigna unguiculata*; *C. lindemuthianum* (LARS 009) from *Phaseolus vulgaris*; *C. acutatum* (LARS 058) from *Musa nana*; *C. malvarum* (LARS 076) from *Sida spinosa*; *C. trifolii* (LARS 164) and *C. destructivum* (LARS 202) from *Medicago sativa*; *C. orbiculare* (LARS 414) from *Cucumis sativus*; and *C. gloeosporioides* from *Stylosanthes* scabra (LARS 167), S. sp (LARS 189), *Mangifera indica* (LARS 501) and *Cucumis melo* (LARS 781)].

Cultural morphology and mycelial growth rate of the studied isolates were determined by growing them on four different media [Oat meal agar OMA (Difco), PDA (Oxoid), CM (Mathur et al., 1950) and Czapek-Dox-V8 (CDV8)] dispensed in 9 cm dia. Petri dishes. CDV8 was prepared by adding 22.8 g Czapek Dox agar medium (Oxoid), 0.5g mycological peptone (Oxoid), 0.5 g yeast extract and 0.5 g casein hydrolysate to 100 ml V8 juice filtered through muslin cloth. A plug of mycelium (*ca.* 4 mm diameter), derived from single-spore

cultures, was placed in the centre of the plates and the cultures were incubated (25ºC, black light). For growth-rate studies, the colony diameter of three replicates of each isolate on different media was recorded daily during 13 days. Cultures were left for a further 10 days and then examined for the presence of stroma, setae and perithecia.

Table 1 - *Colletotrichum/Glomerella* isolates from Brazil used in this study (include LARS 009, 058, 074, 076, 141, 164, 167, 202, 238, 414, 501, 781 from different origins).

Isolates (LARS)	Origin	Infected Organ	Locality
905	*Rubus brasiliensis*	Leaf	Maceió - Alagoas [1]
906	*Spondias purpurea* L.	Leaf	Maceió - Alagoas [1]
907	*Carica papaya* L.	Leaf	Maceió – Alagoas [1]
908	*Dioscorea* sp	Leaf	Maceió - Alagoas [1]
909	*Malpighia glabra* L.	Leaf	Maceió - Alagoas [1]
910	*Anacardium occidentale* L.	Leaf	Maceió - Alagoas [1]
911	*Anacardium occidentale* L.	pseudo-fruit	Maceió – Alagoas [1]
912	*Anacardium occidentale* L.	Nut	Cruz das Almas - Bahia [1]
913	*Anacardium occidentale* L.	pseudo-fruit	Cruz das Almas - Bahia [1]
914	*Anacardium occidentale* L.	Leaf	Conceição do Almeida-Bahia [1]
915	*Anacardium occidentale* L.	Flower	Praia do Forte – Bahia [1]
916	*Anacardium occidentale* L.	Leaf	Indiaroba - Sergipe [1]
917	*Anacardium occidentale* L.	Leaf	Penedo - Alagoas [1]
918	*Anacardium occidentale* L.	Leaf	Maceió - Alagoas [1]
919	*Anacardium occidentale* L.	Leaf	Marechal Deodoro - Alagoas [1]
920	*Anacardium occidentale* L.	Leaf	Marechal Deodoro - Alagoas [1]
921	*Anacardium occidentale* L.	Leaf	Recife - Pernambuco [2]
922	*Anacardium occidentale* L.	pseudo-fruit	Belém – Pará [3]
923	*Anacardium occidentale* L.	Leaf	Fortaleza - Ceará [3]
924	*Anacardium occidentale* L.	Leaf	Pacajus - Ceará [3]
925	*Anacardium occidentale* L.	pseudo-fruit	Piracicaba - São Paulo [3]
926	*Anacardium occidentale* L.	Leaf	Cruz das Almas - Bahia [3]
927	*Anacardium occidentale* L.	pseudo-fruit	Fortaleza - Ceará [3]
928	*Anacardium occidentale* L.	pseudo-fruit	Brasília (DF) - Goiás [3]
929	*Anacardium occidentale* L.	pseudo-fruit	Pacajus - Ceará [4]
930	*Anacardium occidentale* L.	Leaf	Goiana - Pernambuco [4]
931	*Anacardium occidentale* L.	Leaf	Recife - Pernambuco [4]
932	*Anacardium occidentale* L.	Leaf	Fortaleza - Ceará [4]
933	*Anacardium occidentale* L.	Leaf	Areia - Pernambuco [4]
934	*Anacardium occidentale* L.	Leaf	Natal - Rio Grande do Norte [4]
935	*Anacardium occidentale* L.	Leaf	Recife - Pernambuco [4]
936	*Anacardium occidentale* L.	Leaf	Maceió - Alagoas [4]
937	*Anacardium occidentale* L.	Leaf	Olinda - Pernambuco [4]
938	*Anacardium occidentale* L.	Leaf	João Pessoa - Paraíba [4]
939	*Anacardium occidentale* L.	Leaf	Manaus - Amazonas [5]
940	*Anacardium occidentale* L.	Leaf	Pacajus - Ceará [5]

Isolates were provided in 1996 by : [1] Prof. Dr. Eurico Lemos, "Centro de Ciências Agrárias - UFAL", Maceió/AL; [2] Dr. M.B. Figueiredo, "Seção de Micologia - Instituto Biológico", SP; [3] Dr. E.E. Bach, "Seção de Bioquímica Vegetal - Instituto Biológico", SP; [4] Prof. Dr. M. Menezes, "Departamento de Fitossanidade - UFRPe", Recife/Pe; [5] Dr. F.P. Freire, "Centro Nacional de Pesquisa da Agroindústria Tropical - EMBRAPA", Fortaleza/Ce.

Conidial morphology, germination and formation of appressoria

Conidial suspensions (5 x 10^5 conidia/ml) of each isolate were prepared from CM flask cultures and the shape and dimensions of 30 spores were determined under a Zeiss Axiophot light microscope fitted with a graticule eyepiece. Drops (10 µl) of the conidial suspensions were placed in the wells of cavity slides that had been acid-washed (ICN-Flow, High Wycombe, Bucks, UK) and incubated in a humid chamber at 25ºC for 16-24 h in the dark. Germinating conidia were

examined by light microscopy (Zeiss Axiophot, Nomarski DIC) for septum and appressorium development, and the shape, average length and width of 30 appressoria from each isolate were measured.

DNA preparation

Conidial suspensions from CM flask cultures were used to inoculate 100 ml of Czapek Dox-V8 liquid medium in 250 ml flasks (Sherriff et al., 1994). The flasks were incubated at 25°C on an orbital shaker (126 rpm) for 48 h. To test for yeast or bacterial contamination, LB medium (Gibco BRL) was inoculated with a small aliquot of the culture fluid, just before harvest of the mycelium, and incubated for 48 h (25°C dark). The mycelium was harvested by filtration (Whatman nº 1 filter paper), using a Buchner funnel and vacuum pump, and washed with 200 ml of SDW. The liquid was removed by vacuum and the harvested mycelium was ground to powder in liquid nitrogen, with a pestle and a mortar. This was transferred to plastic tubes, freeze-dried for 24 h and stored at -20°C.

DNA was extracted from the powdered mycelium using a method adapted from Graham et al. (1994). A 2-ml screw-top Eppendorf tube was one-third filled with ground mycelium and 1.5 ml of hot (65°C) extraction buffer [TE: 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl and 2% CTAB (w/v)] was added. After mixing thoroughly, the well-soaked mycelium was incubated at 65°C for 30 min and then centrifuged at 16,000 g for 10 min. The supernatant was transferred to a new Eppendorf tube and to remove the proteins, one volume of $CHCl_3$: isoamyl-alcohol (24:1 v/v) was added and mixed by gentle inversion for 1-2 min. The suspension was then centrifuged (16,000 g, 3 min) and the upper aqueous phase collected in a 2 ml "Dolphin" microcentrifuge tube (Multi Dolphin Safe Seal Tubes, Sorenson BioScience Inc.). This was added with a 0.1 volume of 4 M sodium acetate and 2.5 volumes of ice-cold absolute EtOH. The contents of the tube were gently mixed and the DNA was precipitated at -80°C for 10 min. After centrifugation (16,000 g) for 1 min, the supernatant was discarded and the pellet was washed twice with 70% EtOH, mixing gently by inversion. The resulting DNA pellet was dried at 65°C on a heating block for 3-5 min, and dissolved in 200 µl TE buffer plus RNAase (50 µg/ml) at 37°C for 1 h. To remove the RNAase, all the previous steps from the addition of $CHCl_3$: isoamyl-alcohol (24:1 v/v) to the dried pellet DNA were repeated. The DNA was dissolved in 100 µl of TE buffer (37°C, 1 h) and stored at 4°C before amplification by PCR (Sambrook et al., 1989).

Amplification of rDNA

The concentration of DNA extracted from the mycelial samples was determined at 260 nm in a HP D-8452 spectrophotometer. Samples were diluted to 0.1 µg/µl with 1/10 TE buffer, and used in the polymerase chain reactions (PCR). Amplification of rDNA repeat sequences was carried out using the Perkin Elmer GeneAmp PCR Kit [Buffer, deoxynucleosides Triphosphate (dNTPs) and *AmpliTaq* DNA Polymerase] and Thin-walled PCR Tubes (both from Applied Biosystems), in a thermocycler (Perkin Elmer Cetus 480). The 10X PCR buffer contained 100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) and gelatin, and the dNTPs were dATP, dCTP, dGTP and dTTP.

Domain 2 within the 28S gene was amplified using the primers Pn 2 (5'-GTT CAC CAT CTT TCG GGT CC-3') and Pn 9 (5'-CTT AAG CAT ATC AAT AAG CGG AGG-3') (Guadet et al., 1989; Sherriff et al., 1994). The reaction mixture per PCR tube consisted of 10 µl of water-diluted DNA sample (0.1 µg/µl), 2 µl of each primer (50 pmol/µl, 2 µl = 1 µM of each), 2 µl of each dNTP (0.2 mM of each dATP, dCTP, dGTP and dTTP), 10 µl of PCR buffer, 0.5 µl of AmpliTaq DNA polymerase (2.5 Units) and 67.5 µl of SDW. The DNA samples plus the reaction mixture were overlaid with 50 µl PCR mineral oil (Sigma). PCR was carried out using the following thermocycler program: initial denaturation 96°C, 120 s; then, 30 cycles of 15 s at 96°C (denaturing), 30 s at 60 °C (annealing), 60 s at 72°C (elongation), followed by a final 90 s extension step at 72°C and their storage at 4°C. Samples of PCR product (8 µl plus 8 µl of 2X Orange G) were electrophoresed on 0.8% agarose gel in TBE/Eb, against 0.1 µg of DNA size marker (100 bp, 1 µg/µl).

DNA Sequencing

Sequencing reactions were carried out using the Sequenase PCR Product Sequencing Kit (USB - Amersham International plc, USA) and the protocol provided by the supplier. Primer Pn 4 (5'- CCT TGG TCC GTG TTT CAA GAC GGG-3') was diluted in sterile deionised water. Sequencing gels were prepared from SeqGel (NBL

Gene Sciences Ltd.), containing 6% acrylamide: bisacrylamide (19:1) and 7 M urea. When ready to pour, ammonium persulfate and also N,N,N',N'-tetra-methyl ethylene diamine (TEMED) was added to the gels. They were run on a vertical electrophoresis system (Base Runner Nucleic Acid Sequencer, International Biotech. Inc., USA). The developed gel was read manually, and each sequence was aligned using GCG PileUp. Data obtained from the sequencing of the D2 region of all isolates were compared with those of the D2 regions of some *Colletotrichum* species previously studied (Bailey et al., 1996; Moses et al., 1996; Sherriff et al., 1994), using the neighbour-joining method of Kumar et al. (1993), and a dendrogram depicting similarities was constructed using the MEGA (Molecular Evolutionary Genetic Analysis) software (version 1.01).

RESULTS AND DISCUSSION

Cultural morphology

The isolates examined varied in growth rate (Table 2) and cultural morphology on the four media tested. On PDA, aerial mycelium was velvety to densely floccose, with a gradual tendency from white-greyish to dark green-moss pigmentation.

On OMA, the colonies were less variable, with predominance of white-greyish floccose mycelium covering an abundant mucilaginous mass of orange acervuli and spores. CZD-V8 also induced abundant formation of acervuli in most isolates, although resulting in a slighter floccose and thinner greyish to green-moss mycelium.

Table 2 - *In vitro* growth of *Colletotrichum* isolates.

Isolates	Mean Growth Rate (mm/day)[1]					LSD 1%
LARS	CM	OMA	CZD-V8	PDA	Mean	
905	11.77 ± 1.58	12.00 ± 1.00	9.57 ± 1.25	12.33 ± 0.58	11.42 ± 1.15	3.19
906	12.33 ± 0.85	11.77 ± 0.68	12.43 ± 0.51	**10.33 ± 1.82**	11.72 ± 0.97	3.00
907	10.57 ± 2.14	9.23 ± 0.68	12.67 ± 1.52	11.10 ± 0.95	10.89 ± 1.42	3.94
908	12.33 ± 3.76	9.00 ± 1.00	11.00 ± 2.00	13.10 ± 1.85	11.36 ± 1.15	6.52
909	12.57 ± 4.05	11.90 ± 1.65	12.33 ± 1.75	12.67 ± 2.08	12.37 ± 0.34	7.07
910	13.43 ± 2.98	12.33 ± 0.58	13.67 ± 1.19	12.00 ± 1.73	12.86 ± 1.15	5.06
911	13.33 ± 0.65	12.67 ± 0.58	12.43 ± 0.51	13.33 ± 0.58	12.94 ± 0.82	1.60
912	12.33 ± 1.15	10.00 ± 1.00	12.00 ± 1.80	12.67 ± 3.79	11.75 ± 1.20	6.12
913	12.00 ± 3.46	9.67 ± 0.58	11.33 ± 0.58	12.33 ± 1.15	11.33 ± 1.18	5.12
914	**13.67 ± 3.21**	10.43 ± 0.75	11.00 ± 0	13.33 ± 1.65	12.10 ± 1.63	5.06
915	10.67 ± 0.85	9.10 ± 1.15	10.00 ± 1.00	10.90 ± 1.56	10.17 ± 0.81	3.21
916	12.67 ± 1.23	12.00 ± 1.00	**7.00 ± 1.78**	13.23 ± 0.40	11.23 ± 0.29	3.32*
917	13.00 ± 0	9.67 ± 2.08	12.67 ± 1.15	12.90 ± 0.17	12.06 ± 1.60	3.27
918	12.77 ± 2.25	**8.67 ± 1.53**	13.67 ± 0.58	12.00 ± 1.18	11.78 ± 1.15	4.15*
919	10.67 ± 4.04	12.67 ± 0.58	13.33 ± 0.58	13.90 ± 1.01	12.64 ± 1.41	5.82
920	12.67 ± 4.04	10.33 ± 0.58	13.33 ± 0.58	12.23 ± 2.14	12.14 ± 1.29	6.37
921	12.00 ± 2.00	9.01 ± 1.59	11.33 ± 1.53	13.01 ± 0.85	11.34 ± 1.70	4.25
922	12.57 ± 2.23	10.77 ± 3.61	**15.67 ± 3.79**	**10.33 ± 0.58**	12.34 ± 2.43	7.84
923	13.00 ± 2.00	11.67 ± 3.35	13.00 ± 0.89	14.33 ± 2.57	13.00 ± 1.09	6.53
924	12.33 ± 2.17	10.43 ± 3.16	12.67 ± 1.53	13.33 ± 1.42	12.19 ± 1.24	5.99
925	11.87 ± 1.69	10.00 ± 1.73	13.67 ± 3.81	11.33 ± 2.90	11.72 ± 1.37	7.36
926	11.43 ± 2.14	14.00 ± 2.65	13.33 ± 1.46	13.00 ± 1.00	12.94 ± 1.09	5.27
927	12.33 ± 2.89	13.67 ± 2.31	13.57 ± 3.19	12.00 ± 2.65	12.89 ± 0.81	7.62
928	10.33 ± 0.85	10.00 ± 1.00	11.33 ± 4.04	10.43 ± 0.74	10.52 ± 0.57	5.92
929	12.00 ± 4.63	12.33 ± 4.93	9.00 ± 3.00	12.67 ± 3.06	11.50 ± 1.69	10.98
930	10.43 ± 0.85	14.00 ± 6.33	13.67 ± 5.51	13.67 ± 6.61	12.94 ± 1.69	14.71
931	11.67 ± 4.03	**8.67 ± 1.05**	13.67 ± 2.76	12.00 ± 0.30	11.50 ± 2.08	6.87
932	13.23 ± 2.04	11.57 ± 3.35	13.23 ± 1.52	12.00 ± 1.00	12.51 ± 0.85	5.93
933	12.00 ± 2.65	12.00 ± 1.18	13.87 ± 5.34	**15.35 ± 3.32**	**13.31 ± 1.62**	9.50
934	**8.57 ± 1.25**	10.00 ± 1.00	10.33 ± 0.58	11.77 ± 1.20	**10.17 ± 1.31**	2.85*
935	13.43 ± 3.61	10.33 ± 2.65	13.00 ± 1.00	14.00 ± 5.27	12.69 ± 1.63	9.60
936	8.87 ± 1.40	10.33 ± 1.65	13.67 ± 3.79	13.00 ± 3.00	11.47 ± 2.25	7.26
937	10.77 ± 2.25	10.00 ± 0	11.67 ± 1.80	10.67 ± 1.53	10.78 ± 0.69	4.48
938	10.00 ± 2.34	11.00 ± 1.00	10.33 ± 0.58	**10.33 ± 0.55**	10.42 ± 0.42	3.67
939	12.67 ± 2.35	**14.13 ± 1.50**	12.33 ± 1.53	12.43 ± 1.50	12.89 ± 0.84	4.83
940	12.67 ± 0.58	11.00 ± 3.32	13.80 ± 1.15	12.67 ± 1.35	12.54 ± 1.15	5.22
Mean	11.92 ± 1.25	11.01± 1.56	12.27 ± 1.69	12.44 ± 1.21	11.91	0.88*
LSD 1.%	5.42	4.73*	4.91*	4.82*	2.48*	-

[1]Growth rates of the colonies between the 3rd and 6th day after inoculation on the media. Highest and lowest averages on the same tested medium are depicted in bold. LSD 1% followed by asterisk (*) indicates that significant differences were detected between 2 treatments of the same column or row.

OMA, CM and PDA induced considerable production of conidia in the aerial mycelium, and on PDA this was the predominant type of sporulation. CM induced more acervuli formation than PDA and less aerial sporulation than PDA and OMA. The reverse of the cultures was mostly greyish-white to dark green-moss. Generally, mycelial growth at 25°C was more profuse on PDA, moderate on CZD-V8 and CM, and least on OMA (Table 2). These findings were in agreement with studies of Lenné (1978), who reported that mycelial growth of numerous *Colletotrichum* species was always greater on PDA than OMA. When growth rates were evaluated on the same medium, there was no highly significant difference on CM, but some isolates showed considerable variation on the other media, for example from 7-15.7 mm per day on CZD-V8. For some isolates a highly significant variation occurred between the daily mycelial growth rate on different media. However, for most of the isolates, the growth rates were not significantly different (LSD 5% for comparing two media), even between the media, this criterion did not separate them into groups.

Several authors have reported average daily growth rates for the isolates of *C. gloeosporioides* from various host plants on PDA and other media. Overall, maximum growth rates of between 6.2 and 16.6 mm per day have been measured at optimum temperatures between 25 and 30oC (Adaskaveg and Hartin, 1997; Kuramae-Izioka et al., 1997; Lenné, 1978). Muniz et al. (1997) recorded growth of *C. gloeosporioides* isolates from cashew on malt extract agar (MEA) and showed that maximum rates (7.7-14.8 mm/day) occurred from 25 to 30°C. The isolates of C. acutatum, however, exhibited much slower growth, with a maximum at 25°C (Adaskaveg and Hartin, 1997; Agostini et al., 1992; Brown et al., 1996; Kuramae-Izioka et al., 1997; Smith and Black, 1990). Since this distinction was provided by the molecular approaches like RAPDs and sequencing of ITS, Adaskaveg and Hartin (1997) suggested that temperature relationships could be combined with the mycelial growth rates to distinguish *C. acutatum* from *C. gloeosporioides.*

Morphology of spores, germination and appressorium formation

In the present study, the spores (Table 3) of most of the isolates on PDA were generally straight with rounded apices, typical of *C. gloeosporioides* conidia, but some single-spore cultures also produced straight spores with one or two obtuse apices. Conidial morphology has also been used in taxonomic investigations of *Colletotrichum* spp. (Adaskaveg and Hartin, 1997; Lane et al., 1998; Menezes and Hanlin, 1996a, b; Moriwaki et al., 2002). According to the studies of Munaut et al. (2001), although the majority of isolates of *C. gloeosporioides* from *Stylosanthes* formed straight spores, many produced falcate ones.

Conidial length was considerably more variable than width. The ratio of the shortest (907 = 12.42 μm) to the longest (917 = 17.67 μm) isolate-conidia was 1:1.42, and of the narrowest (935 = 4.79 μm) to the broadest (928 = 5.99 μm) isolate-conidia was 1:1.25. These ratios were in agreement to the observations of Menezes and Hanlin (1996b), in which the isolates of *C. gloeosporioides* from avocado, cashew, citrus, guava, soursop and mango, the corresponding ratios were 1:1.41 and 1:1.28, respectively. The length/width (L/W) ratio of the conidia of the isolates ranged from 2.49 to 3.41, in agreement with the findings of Andrade et al. (2007) and Menezes and Hanlin (1996b).

The germination of conidia *in vitro* on glass slides, with the formation of a single septum (Table 3), followed by the production of hyaline appressoria, was observed within 6 h for all the isolates. By 24 h, the appressoria had melanized, appearing light to dark brown, thick walled with a hyaline germ-pore. Appressoria varied in morphology from globose, with a diameter of 6.3-7.5 μm, to subglobose, with lengths of 5-10 μm and widths of 5-9 μm (Table 3), similar to results for *C. gloeosporioides* reported by Baxter et al.(1983). Cox and Irwin (1988) suggested the existence of three groups within the isolates which were originally classified as *C. gloeosporioides*. The first comprised the isolates with mean conidial widths between 3 and 4.2 μm, and with either unlobed or slightly-lobed appressoria. The second had the isolates with mean conidial widths between 4.5 and 5.5 μm, and with unlobed or slightly-lobed appressoria. The third group comprised the isolates with conidial widths between 4.5 and 5.5 μm, but with obviously lobed appressoria, compatible with the definitions of *C. crassipes.* All the isolates of this work had similarities with the second group of *C. gloeosporioides* defined by Cox and Irwin (1988).

Table 3 - Morphology and dimensions of conidia and appressoria of *Colletotrichum* isolates *in vitro* (all of them produce septum only after the germination).

Isolate LARS	Conidial Shape[1]	Mean Conidial Length	Mean Conidial Width	Appressorial Shape[2]	Mean Appressorial length	Mean Appressorial Width
905	cylindrical*	14.62 ± 2.11	4.92 ± 0.32	globose ¶	6.28 ± 0.32	6.26± 0.12
906	cylindrical	14.95 ± 1.08	5.00 ± 0	subglobose¶	6.29 ± 0.29	7.39 ± 0.24
907	cylindrical*	**12.42 ± 1.27**	4.98 ± 0.09	globose¶	7.42 ± 0.24	6.43 ± 0.29
908	cylindrical	15.00 ± 0.66	5.00 ± 0	globose/ subglobose¶	7.51 ± 0.30	7.50 ± 0.15
909	cylindrical*	17.00 ± 2.40	5.00 ± 0	subglobose§	5.64 ± 0.32	7.50 ± 0
910	cylindrical*	12.67 ± 0.43	5.00 ± 0	subglobose¶	5.55 ± 0.33	7.50 ± 0
911	cylindrical*	16.55 ± 1.23	4.95 ± 0.23	subglobose¶	8.05 ± 0.27	7.35 ± 0.65
912	cylindrical*	17.20 ± 1.99	5.00 ± 0	subglobose¶	5.17 ± 0.21	7.50 ± 0
913	cylindrical*#	17.73 ± 1.94	5.00 ± 0	globose¶	7.49 ± 0.17	7.50 ± 0
914	cylindrical	15.43 ± 2.23	5.00 ± 0	subglobose¶	7.08 ± 0.55	**8.73 ± 0.13**
915	cylindrical	16.20 ± 3.48	5.08 ± 0.28	subglobose¶	**5.02 ± 0.18**	7.28 ± 0.66
916	cylindrical*	16.13 ± 1.63	5.00 ± 0	subglobose¶	9.95 ± 0.24	7.50 ± 0
917	cylindrical*	**17.67 ± 4.17**	5.17 ± 0.56	subglobose§	**9.98 ± 0.17**	7.50 ± 0
918	cylindrical*	16.01 ± 0.22	5.00 ± 0	subglobose§	8.28 ± 0.48	6.98 ± 0.96
919	cylindrical	15.07 ± 1.50	5.00 ± 0	subglobose§	6.70 ± 0.85	6.10 ± 0.60
920	cylindrical#	14.92 ± 1.03	5.00 ± 0	subglobose§	5.04 ± 0.20	7.50 ± 0
921	cylindrical	15.06 ± 0.59	5.07 ± 0.17	subglobose§	9.42 ± 1.08	6.58 ± 1.23
922	cylindrical*	14.88 ± 0.89	5.00 ± 0	subglobose§	8.21 ± 1.34	6.92 ± 0.91
923	cylindrical*	16.00 ± 4.13	5.00 ± 0	subglobose§	8.54 ± 1.64	6.58 ± 0.92
924	cylindrical	17.58 ± 2.43	5.00 ± 0	subglobose§	7.88 ± 1.23	7.38 ± 0.68
925	cylindrical	15.58 ± 2.52	5.00 ± 0	subglobose§	8.42 ± 1.31	7.17 ± 0.56
926	cylindrical	15.46 ± 1.29	5.08 ± 0.19	globose¶	8.54 ± 1.19	7.50 ± 0
927	cylindrical*	17.00 ± 4.80	5.00 ± 0	subglobose§	8.88 ± 1.06	7.33 ± 0.43
928	cylindrical	16.17 ± 3.41	**5.99 ± 1.10**	subglobose§	9.39 ± 0.47	7.33 ± 0.54
929	cylindrical	15.08 ± 1.43	5.00 ± 0	subglobose§	7.42 ± 1.47	5.83 ± 1.20
930	cylindrical	14.88 ± 1.84	5.00 ± 0	subglobose§	9.25 ± 1.02	7.50 ± 0
931	cylindrical*	16.17 ± 4.22	5.00 ± 0	subglobose§	9.71 ± 1.26	7.46 ± 0.23
932	cylindrical*#	16.08 ± 1.49	5.12 ± 0.22	globose¶	8.21 ± 1.07	7.38 ± 0.38
933	cylindrical	16.13 ± 0.95	4.95 ± 0.25	subglobose¶	8.88 ± 1.15	7.50 ± 0.33
934	cylindrical	16.13 ± 2.06	5.00 ± 0	subglobose¶ §	8.75 ± 1.23	7.38 ± 0.50
935	cylindrical	13.40 ± 1.34	**4.79 ± 0.47**	subglobose¶	7.75 ± 0.76	5.46 ± 0.70
936	cylindrical	13.75 ± 1.27	5.00 ± 0	subglobose§	9.21 ± 1.06	6.92 ± 0.63
937	cylindrical	15.04 ± 0.84	5.00 ± 0	subglobose§	8.75 ± 1.04	7.33 ± 0.43
938	cylindrical	15.75 ± 1.63	5.00 ± 0	subglobose¶	9.08 ± 1.23	**5.38 ± 0.81**
939	cylindrical*	15.54 ± 1.70	5.33 ± 0.56	subglobose¶	6.63 ± 1.51	5.54 ± 0.37
940	cylindrical*	15.42 ± 2.28	5.00 ± 0	subglobose§	8.04 ± 0.85	6.21 ± 0.70
LSD 1%	-	1.46	0.01	-	0.60	0.43

[1] Predominant shape of conidia *in vitro* (≥ 70%): cylindrical with rounded ends. Some isolates produced also: * = ovoid (one obtuse apex); # = ellipsoid (two obtuse apices) conidia.[2] Predominant shape of appressoria *in vitro* (>50%): ¶ = smooth ; §= irregular .

Morphology of perithecia and setae

Most isolates (64%) produced embedded or partially embedded perithecia, singly or in small stromata scattered over the colony surface. The majority of these embedded perithecia, when crushed for microscopic examination, were found to be sterile or producing few visible ascospores. Some isolates also produced large aggregated perithecia in embedded or prominent stromata or in tufted mycelium that were always fertile. The sterile perithecia were morphologically identical to the fertile form, with a diameter between 275 and 304 μm, while the length of the canal-lined ostiole varied from 277 to 292 μm. Irwin and Cameron (1978) reported that Type A of *C. gloeosporioides* from *Stylosanthes* produced sterile perithecia in pure cultures. Gunnel and Gubler (1992) reclassified some isolates as *C. gloeosporioides*, based on the presence of fertile as well as sterile perithecia, when they had been previously

identified as *C. fragariae* because of their production of sterile perithecia. The asci of the fertile perithecia of the different isolates were curved fusiform (60-69 μm in length) and contained eight cylindrical rounded to fusiform hyaline and aseptate ascospores (17 - 22 μm in length). The observation of the ascogenous phase in these monoconidial isolates confirmed that they were homothallic (self-fertile) in agreement with the findings of Lenné (1978) for *C. gloeosporioides.*

Molecular characterization

Comparing the aligned nucleotide sequences of the D2 region (180 nucleotides) of the studied isolates, along with data of other previously-identified *Colletotrichum* species from LARS collection (Bailey et al., 1996; Moses et al., 1996; Sherriff et al., 1994), the maximum number of differences among the aligned bases was 16, *i.e.* 8.9%. Across the set of the isolates, 21 position changes occurred, 12 of them in the last 80 sites. From the difference matrix (Table 4) and corresponding tree and bootstrap analysis (Fig. 1), except for isolate LARS 921 that was identical to *G. cingulata* (LARS 238), all the isolates from cashew and associated plants appeared to be very closely related (> 98.9 % homology) to each other and to *C. gloeosporioides* obtained from *C. melo* (LARS 781), *M. indica* (LARS 501), *A. virginica* (LARS 074), *S.scabra* (LARS 167) and *S.* sp (LARS 189).

Table 4 - Difference matrix of Domain 2 region of LSU rDNA from different *Colletotrichum/Glomerella* isolates (LARS 905-940), from cashew and associated plants, and other representative of both genera.[1]

Isolate - LARS	**058**	**141**	**202**	**167 or 189**	**909**[2]	**501**	**906**[3]	**781**	**905**[4]	**912**[5]	**009**	**238**	**921**
058 - *C.acutatum*	0.000												
141 - *C.capsici*	0.056	0.000											
202 - *C.destructivum*	0.022	0.033	0.000										
167 - *C.gloeosporioides*	0.050	0.039	0.028	0.000									
189 - *C.gloeosporioides*	0.050	0.039	0.028	0.000									
909 - *C.*sp.[2]	0.050	0.039	0.028	0.000	0.000								
501 –*C.gloeosporioides*	0.039	0.039	0.028	0.011	0.011	0.000							
906 - *C.*sp.[3]	0.039	0.033	0.033	0.011	0.011	0.000	0.000						
781 –*C.gloeosporioides*	0.044	0.044	0.028	0.006	0.006	0.006	0.006	0.000					
905- *C.*sp.[4]	0.044	0.044	0.028	0.006	0.006	0.006	0.006	0.000	0.000				
912 - *C.*sp.[5]	0.044	0.033	0.022	0.011	0.011	0.006	0.006	0.011	0.011	0.000			
009 - *C.lindemuthianum*[6]	0.089	0.078	0.072	0.056	0.056	0.067	0.067	0.061	0.061	0.067	0.000		
238 - *G. cingulata*	0.072	0.039	0.050	0.033	0.033	0.039	0.039	0.039	0.039	0.039	0.056	0.000	
921 - *C.*sp.	0.072	0.039	0.050	0.033	0.033	0.039	0.039	0.039	0.039	0.039	0.056	0.000	0.000

[1] Differences are calculated as a proportion of the sites with differences (transition and transversion only, counted as one). [2] also LARS 938 (G3); [3] also LARS 074, 907, 910, 911, 914, 915, 916, 918, 919, 920, 925, 926, 928, 929, 930, 932, 933, 934, 935, 936, 937, 939 (G2); [4] also LARS 908, 922, 923 (G1);[5] also LARS 913, 917, 924, 927, 931, 940 (G4); [6] also *C. orbiculare* (LARS 414), *C. malvarum* (LARS 076), *C. trifolii* (LARS 164).

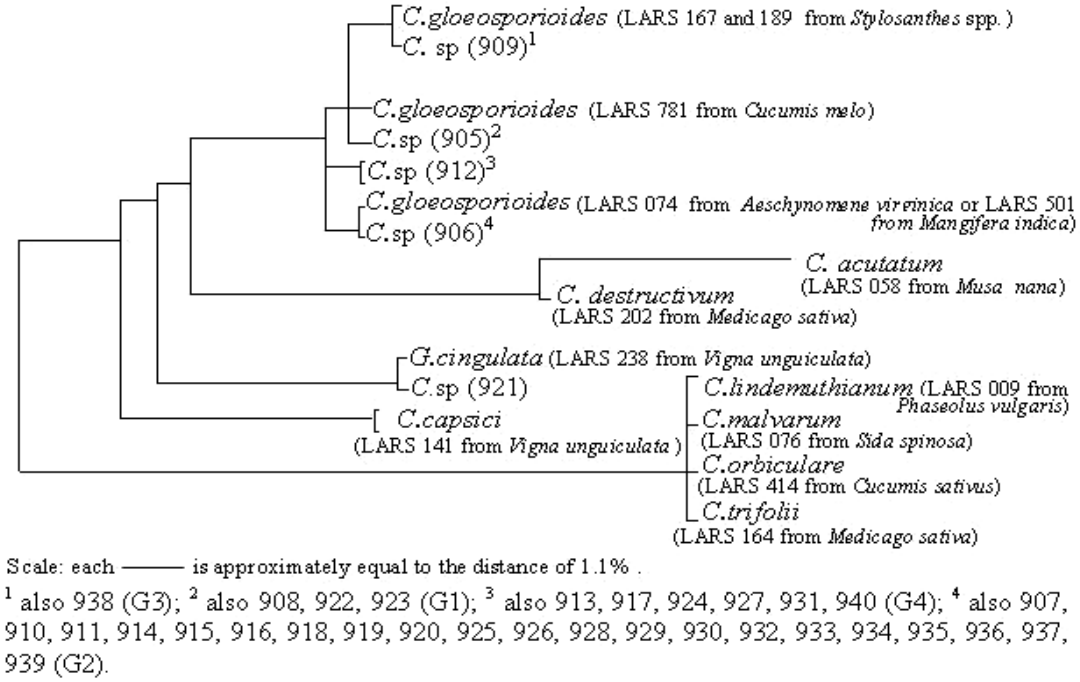

Figure 1 - Dendrogram illustrating the relationships between the *Colletotrichum/Glomerella* isolates LARS 905-940 from cashew and associated plants, and reference isolates LARS 009, 058, 074, 076, 141, 164, 167, 202, 238, 414, 501, 781.

The maximum number of differences among the aligned bases of these isolates from the ones of the references were: *C. lindemuthianum* (LARS 009), *C. trifolii* (LARS 164), *C. malvarum* (LARS 076) and *C. orbiculare* (LARS 414) - 6.7%; *C. acutatum* (LARS 058) -5.0%; *C. capsici* (LARS 141) - 4.4%; and *C. destructivum* (LARS 202) - 3.9%. Among the studied isolates LARS 905-940, four groups (G1-G4) of *C. gloeosporioides* were observed to occur. Isolates LARS 905, 908, 922 and 923 (G1) were identical to *C. gloeosporioides* from *C. melo* (LARS 781), and differed by only one nucleotide from *C. gloeosporioides* obtained from *A. virginica* (LARS 074), *Stylosanthes* spp. (LARS 167 and 189) and *M. indica* (LARS 501). Isolates LARS 906, 907, 910, 911, 914, 915, 916, 918, 919, 920, 925, 926, 928, 929, 930, 932, 933, 934, 935, 936, 937 and 939 (G2) were identical to *C. gloeosporioides* obtained from *A. virginica* or *M. indica* but differed by two nucleotides from the isolates of *Stylosanthes* spp. Isolates LARS 909 and 938 (G3) were identical to the ones obtained from *Stylosanthes* spp., and isolates from G4 (LARS 912, 913, 917, 924, 927, 931, and 940) differed by two nucleotides from those in *Stylosanthes* spp., and by only one nucleotide from those on *A. virginica*, *M. indica* or *C. melo*. Different regions (D1, D2) of the LSU rDNA and intergenic sequences (ITS1, ITS2) have more variation in their rate of evolution than do the domains of the small subunit, being useful to distinguish groups in closely related taxa, like in the genus *Colletotrichum* (Bailey et al., 1996; Kohn, 1992; Moses et al., 1996; Moriwaki et al., 2002). Sherriff et al. (1994; 1995) observed that sequence data from the D2 region was as informative as the combined D1, D2 and ITS2 regions (886 bp) in revealing the differences and relationships in *Colletotrichum*, since it has a sufficient proportion of sequence divergence to distinguish between the main species of this genus.

Moses et al. (1996) identified *C. gloeosporioides* as the causal agent of stem tip dieback of cassava (*Manihot esculenta*), based on the relatedness between their D2-rDNA sequences and the ones of other *Colletotrichum* isolates. The authors found that cassava *C. gloeosporioides* isolates differed by only one nucleotide from those of *A. virginica*, *M. indica* and *Stylosanthes* spp., exactly as for some isolates in the present study.

Sreenivasaprasad et al. (1996) reported that, for a

limited number of *Colletotrichum* species, the ITS1 region of rDNA showed a greater degree of intra and interspecific divergence (50.3% variable sites) than the ITS2 region (12.4% variable sites). Considering the isolates of *C. gloeosporioides* and *C. fragariae*, which did not diverge in ITS2 sequence but diverged (2.4%) in their ITS1 regions, Sreenivasaprasad et al. (1996) suggested that *C. fragariae* could be accommodated within the broad species concept of *C. gloeosporioides*. With regard to intraspecific variability in ITS1 of these 26 isolates of *C. gloeosporioides*, the sequences were identical in size (171 bp), but showed up to 3.6% nucleotide variability.

Abang et al. (2002), however, failed to distinguish between *C. coccodes* and *C. circinans* based on the restriction analysis of their ITS sequence, and Cano et al. (2004) demonstrated that sequencing of the domains D1-D2 of the LSU rDNA gene also supported the results provided by the amplification of ITS1 in distinguishing between the species of *Colletotrichum* of human clinical interest.

According to the results of this work, the sequence analysis of the Domain 2 region allowed to show the differences between the main species of *Colletotrichum*, especially when the morphological criteria were inconclusive. Even a small difference (3.3%) distinguished the isolate LARS 921 from the others, described in four groups of *C. gloeosporioides*. The D2-rDNA nucleotide sequence of LARS 921 was identical to *G. cingulata* from *V. unguiculata*, and although it produced perithecia in different media, another 23 isolates did the same. Hence, the presence of perithecia and host origin could not be adopted as criterion to distinguish between the species.

Pathogenicity tests performed by Lopez (1999) showed that different cashew clones were infected by the isolates of *C. gloeosporioides* obtained from cashew and other susceptible plants, cultivated in close proximity in Brazil. The present work corroborated this evidence for anthracnose cross-infection in cashew, since it showed that polymorphism of the D2 sequences of the 28S rDNAs of these isolates was not enough to clearly distinguish within the species - the four sub-groups of isolates had origins as distant as *Rubus* (LARS 905), *Spondias* (LARS 906), *Carica* (LARS 907), *Dioscorea* (LARS 908), *Cucumis melo* (G1 + LARS 781), *Mangifera* (G2 + LARS 501), and *Stylosanthes* spp. (G3 + LARS 167). This information must improve the effective measures of cashew-anthracnose control and the breeding programs. For a more detailed study of divergences among the isolates of *C. gloeosporioides* from cashew and its associated plants, a larger number of isolates and sequencing of their ITS1 rDNA region and DGGE of their 18S rDNA should be included.

RESUMO

Trinta e seis isolados de fungos obtidos de lesões de antracnose em cajueiros e outras plantas consorciadas no Brasil, foram comparados quanto a seus aspectos culturais, morfológicos e seqüências parciais do rDNA 28S. Os isolados apresentaram elevado grau de variabilidade cultural, com taxa de crescimento médio, em todos os meios testados, entre 10,2 e 13,3 mm/dia. A maioria deles produziu peritécios (estéreis e férteis), e alguns produziram setas (estéreis e férteis) nos diferentes meios. Todos apresentaram acérvulos com predominância de conídios cilíndricos (12,4-17,7 μm X 4,8-6,0 μm), de extremidades arredondadas, formando septos durante a germinação e produzindo apressórios ligeiramente lobados ou lisos. Comparando as seqüências do domínio D2 da larga subunidade (LSU) do rDNA dos isolados com aquelas já identificadas de espécies de *Colletotrichum/ Glomerella*, verificou-se que 35 deles correspondem a *C. gloeosporioides* (> 98,9% de homologia), e um deles, o isolado 921, é idêntico a *G. cingulata* (LARS 238) de *V. unguiculata*.

AKNOWLEDGEMENTS

CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Br); BBSRC (Biotechn. and Biological Sciences Research Council - UK).

REFERENCES

Abang, M.M.; Winter, S.; Green, K.R.; Hoffmann, P.; Mignouna, H.D.; Wolf, G.A. (2002), Molecular identification of *C. gloeosporioides* causing anthracnose of yam in Nigeria. *Pl. Pathol*., **51**, 63-71.

Adaskaveg, J. E.; Hartin, R. J. (1997), Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. *Phytopath.*, **87**, 979-987.

Agostini, J. P.; Timmer, L.W.; Mitchell, D. J. (1992), Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides* from citrus. *Phytopath.,* **82**, 1377-1382.

Alahakoon, P.W.; Brown, A. E. (1994), Host range of *Colletotrichum gloeosporioides* on tropical fruits in Sri Lanka. *Internat. J. Pest Manag.,* **40**, 23-26.

Andrade, E. M.; Uesugi, C. H.; Ueno, B.; Ferreira, M. A.S.V. (2007), Caracterização morfológica e cultural de *Colletotrichum gloeosporioides* patogênico ao mamoeiro. *Fitop. Bras*., **32**, 21-31.

Bailey, J.A.; Nash, C.; Morgan, L.W.; O'Connell, R.J.; Te Beest, D.O. (1996), Molecular taxonomy of *Colletotrichum* species causing anthracnose on the Malvaceae. *Phytopath.*, **86**, 1076-1083.

Baxter, A. P.; Westhuizen, G. C. A.; Eicker, A. (1983), Morphology and taxonomy of South African isolates of *Colletotrichum* . *South Af. J. Bot.*, **2**, 260-289.

Brown, A. E.; Sreenivasaprasad, S.; Timmer, L.W. (1996). Molecular characterization of slow-growing orange and lime key anthracnose strains of *Colletotrichum* from citrus and *C. acutatum. Phytopath.,* **86**, 523-527.

Cannon, P.F.; Buddie, A.G. ; Bridge, P.D. (2008), The thypification of *Colletotrichum gloeosporioides*. *Mycotaxon*, **104**, 189-204.

Cano, J.; Guarro, J.; Gene, J. (2004). Molecular and Morphological identification of *Colletotrichum* spp. of Clinical Interest. *J.Clin. Microb*., **42**, 2450-2454.

Cox, M. L and Irwin, J.A.G. (1988), Conidium and appressoria variation in Australian isolates of the *Colletotrichum gloeosporioides* group and closely related species. *Austr. System. Bot.,* **1**, 139-149.

Fagbola, O.; Abang, M.M. (2004), *Colletotrichum circinans* and *Colletotrichum coccodes* can be distinguished by DGGE analysis of PCR-amplified 18S rDNA fragments. *Af. J. Biotech*., **3** , 195-198.

FAOSTAT (2008), Food and Agriculture Organization for United Nations - Statistical Database Homepage. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567.

Forseille, L. (2007), Molecular and Pathological Differentiation of *Colletotrichum truncatum* from Scentless Chamomile and Legume Crops. Canada, Saskatchewan, Saskatoon: 122 p. Thesis (M.Sc.)-Biology Department, Univ. of Saskatchewan.

Freeman, S.; Minz, D.; Jurkevitch, E.; Maymon, M.; Shabi, E. (2000), Molecular analyses of *Colletotrichum* species from almond and other fruits. *Phytopath.*, **90**, 608-614..

Freeman, S.; Minz, D.; Maymon, M.; Zveibil, A. (2001), Genetic diversity within *Colletotrichum acutatum sensu* Simmonds. *Phytopath.,* **91**, 586-592.

Freire, F. P.; Cardoso, J. E. (1995), Doenças do Cajueiro. In*: Cajucultura: Modernas Técnicas de Produção.* Ed. Araújo, J.P.P.; Da Silva, V.V., Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil, Fortaleza, pp. 249-267.

Gonçalves-Vidigal, M.C.; Thomazella, C.; Vidigal Fº. P.; Kvitschal, M.V.; Elias, H.T. (2008). Characterization of *Colletotrichum lindemuthianum* Isolates Using Differential Cultivars of Common Bean in Santa Catarina State, Brazil. *Braz. arch. biol. technol.,* **53**, n.5, p. 883-885.

Graham, C.G.; Mayers, P.; Henry, J.R.A (1994), simplified method for the preparation of fungal genomic DNA for PCR and RAPD analysis. *Biotechnol.,* **16**, 48-50.

Guadet, J.; Julien, J.; Lafay, J. F.; Brygoo, Y. (1989), Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. *Molec. Biol. Evol*.,. **6**, 227-242.

Gunnel, P. S.; Gubler, W. D. (1992), Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. *Mycol.,* **84**, 157-165.

Irwin, H. R.; Cameron, D. F. (1978), Two diseases in *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in Australia and pathogenic specialisation within one of the causal organisms. *Austr. J. Agric. Res.,* **29**, 305-317.

Jeffries, P.; Dodd, J. C.; Jeger, M. J.; Plumbley, R. A. (1990), The biology and control of *Colletotrichum* sp. on tropical fruit crops. *Pl. Pathol.,* **39**, 343-366.

Johnston P.R. (2000), The importance of phylogeny in understanding host relationships within *Colletotrichum*. In: *Colletotrichum*: host specificity, pathogenicity, and host-pathogen interactions, Prusky, D., Dickman, M.B., Freeman, S., eds. St. Paul, Minnesota: APS Press. pp 21–28

Johnston, P.R. and Jones, D.(1997), Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. *Mycol.,* **89**, 420-430.

Kohn, L. M. (1992), Developing new characters for fungal systematic: an experimental approach for determining the rank of resolution. *Mycol.,* **84**, 139-153.

Kumar, S.; Tamura, K.; Nei, M. (1993), MEGA: Molecular Evolutionary Genetic Analysis. Ver. 1.01. USA, Pennsylvania : The Pennsylvania St. Univ.

Kuramae-Izioka, E. E.; Lopes, C. R.; Souza, N. L.; Machado, M. A. (1997), Morphological and molecular characterization of *Colletotrichum* spp. from citrus orchards affected by postbloom fruit drop in Brazil. *Europ. J. Pl. Pathol*., **103**, 323-329.

Lane, C. R.; Jeuland, H.; Hall, A.G.; Chesmore, E.D. (1998), Image analysis as an aid to discrimination between *C. acutatum* and *C. gloeosporioides*. In: *7th Internat. Congress of Plant Pathology*, Edinburgh: Scotland. Abstracts, Vol. 3, Theme 4, Abs. 4.3.2.

Lenné, J. M. (1978), *Studies on the biology and taxonomy of Colletotrichum species.* Thesis (Ph.D.) - Melbourne, Australia: Univ. of Melbourne.

Lima Fº, R.M., Oliveira, S.M.A.;Menezes, M. (2003), Caracterização enzimática e patogenicidade cruzada de *Colletotrichum* spp. associados a doenças de pós-colheita. Fitop. Bras., **28**,620-625.

Lopez, A. M. Q. (1999), The Interaction between anthracnose, *Colletotrichum gloeosporioides* Penz., and cashew, *Anacardium occidentale* (L.). Bristol-UK: 276p. Thesis (Ph.D.), LARS, Univ. of Bristol.

Mathur, R. S.; Barnett, H. L.; Lilly V. G. (1950), Sporulation of *Colletotrichum lindemuthianum* in culture. *Phytopath.*, **40**, 104-114.

Menezes, M. and Hanlin, R.J. (1996a), Morphological variability of *Colletotrichum gloeosporioides* from avocado trees from Northeastern Brazil. *Rev. Microbiol.*, **27**, 228-236.

Menezes, M. and Hanlin, R.J. (1996b), Appressoria of Brazilian isolates of *Colletotrichum gloeosporioides* (Penz.) Sacc. causal agent of anthracnose diseases. *Braz. J. Microb.*, **27**, 247-251.

Moriwaki, J.; Tsukiboshi, T.; Sato, T. (2002), Grouping of *Colletotrichum* species in Japan based on rDNA sequences. *J. Gen. Pl. Pathol*. **68**, 307–320.

Moses, E.; Nash, C.; Strange, R.N.; Bailey, J.A. (1996), *Colletotrichum gloeosporioides* as the cause of stem tip dieback of cassava. *Pl. Pathol.*, **45**, 864-871.

Munaut, F.; Hamaide, N.; Maraite, H. (2001), Molecular and morphological characterization of *Colletotrichum gloeosporioides* from native Mexican *Stylosanthes* species. *Pl. Pathol.*, **50**, 383-396.

Muniz, M. F. S.; Lemos, E. E. P.; Várzea, V. M. P.; Rodrigues, J. R.; Bessa, A. (1997), Characterization of *C. gloeosporioides* (Penz.) Sacc. isolates and resistance of cashew (*Anacardium occidentale* L.) to the pathogen. In*: International Cashew and Coconut Conference in Tanzania*. Proceedings. Reading, UK: BioHybrids International Ltd. Publisher, pp. 249-253.

O'Connell, R. J.; Bailey, J. A.; Richmond, D. V. (1985). Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. *Physiol. Pl. Pathol.*, **27**, 75-98.

Sambrook, J.; Fritsch, E.F.; Maniatis, T. (1989). *Molecular cloning: a laboratory manual*, 2nd ed., New York: Cold Spring Harbor Laboratory Press, Vols.1-3, 1659 p.

Sera, G.H.; Sera, T.; Ito, D.S.; Azevedo, J.A.; Ribeiro F, C. ; da Mata, J.S. (2007). .Partial resistance to Fruit Necrosis Associated to *Colletotrichum* spp. among Arabic Coffee Genotypes. *Brazilian Archives of Biology and Technology*, vol. 50, n.3, p. 395-402.

Sherriff, C.; Whelan, M. J.; Arnold, G. M.; Lafay, J. F.; Brygoo, Y.; Bailey, J. A. (1994), rDNA sequence analysis reveals new species groupings in the genus *Colletotrichum* . *Experim. Mycol*., **18**, 121-138.

Sherriff, C.; Whelan, M. J.; Arnold, G.M.; Lafay, J.F.; Brygoo, Y.; Bailey, J.A. (1995), rDNA sequence analysis confirms distinction between *C. graminicola* and *C. sublineolum. Mycol. Res.*, **99**, 475-478.

Sindicaju (2008), Homepage of the "Sindicato das Indústrias de Beneficiamento de Castanha de Caju e Amêndoas do Estado do Ceará – Exportação". http://www.sindicaju.org.br/site/exportacao.html.

Skipp, R. A.; Beever, R. E.; Sharrock, K. R, Rikkerink, E. H. A.; Templeton, M. D. (1995), *Colletotrichum* . In*: Pathogenesis and host specificity in plant diseases*, Kohmoto K, Singh U.S., Singh R.P., eds. Oxford, UK: Pergamon/Elsevier Sci. Ltd. Publisher, Vol. 2, pp. 119-142.

Smith, B.J.; Black, L.L.(1990), Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. *Pl. Dis.*, **4**, 69-76.

Sreenivasaprasad, S.; Mills, P. R.; Meehan, B. M.; Brown, A. E. (1996) Phylogeny and systematics of 18 *Colletotrichum* species based on ribosomal DNA spacer sequences. *Genome*, **39**, 499-512.

Vinnere, O.; Fatehi, J.; Wright, S.A.I.; Gerhardson, B. (2002), The causal agent of anthracnose of *Rhododendron* in Sweden and Latvia. *Mycol. Res.*, **106**, 60-69.

Received: January 09, 2008;
Revised: May 21, 2008;
Accepted: May 20, 2009.