Morphological and Molecular Diversity of *Colletotrichum gloeosporioides* Causing Yam (*Dioscorea alata* L.) Anthracnose Disease in Côte d'Ivoire

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ABSTRACT

Yam is a plant of great importance for the Ivorian people in terms of food, nutrition, economy, and socio-cultural value. However, its production is threatened by several biotic factors, including anthracnose, one of the most destructive diseases affecting yams. The objective of this study was to assess the morphological and molecular diversity of *Colletotrichum gloeosporioides* isolates responsible for anthracnose in yam (*Dioscorea alata*) in Côte d'Ivoire. Twenty-seven previously isolated strains were collected from Plant Health Laboratory of Nangui ABROGOUA University and analyzed. The study examined the cultural (coloration, growth mode, texture, and colony diameter), microscopic (conidial shape, size, and spore concentration), and molecular characteristics of these isolates. Significant morphological diversity was observed among the isolates. Macroscopically, three major groups were identified based on colony coloration (whitish, orange, and grayish). Colony textures varied, presenting cottony, flaky, downy, or filamentous appearances, with growth patterns ranging from concentric and radiating to regular rings. Colony diameters ranged from 37 mm to 82 mm. Microscopically, sporulation varied significantly among isolates, ranging from 1.1 × 10□ to 85.4 × 10□ spores, as did conidial size, which ranged from 10.54 to 19.29 µm in length and 3.93 to 6.43 µm in diameter. Molecular analysis through comparison with GenBank nucleotide sequences revealed homology rates of 99-100% and varying proximities between the rDNA sequences of the isolates. In conclusion, the characterization of these isolates provides valuable insights for improving disease control strategies for yam anthracnose.

Keywords: Anthracnose, Colletotrichum gloeosporioides, Morphological diversity, Molecular diversity, Dioscorea alata

1 INTRODUCTION

Yam is a staple food of many rural populations in central and eastern Côte d'Ivoire. In urban areas, yam tubers are the most consumed food in households after rice [1]. In Côte d'Ivoire, it is a widely consumed product in both rural and urban areas for the Ivorian population. The tubers are eaten in various processed food, like pounded, boiled, braised, fried and stewed form by the households [2]. With its many varieties belonging to several species with one or two harvests and its best storing quality than other tubers, yam contributes to food security of Ivorian populations and could replace relatively expensive imported food products [3]. Similarly, certain species of yams which have high organoleptic quality are subject of strong transactions from production areas to urban areas. Those transactions generate income for all actors in yam sector. In addition, yams contribute to poverty reduction among Ivorian population [4]. However, diseases caused by biotic agents, are one of the major constraints in yam production. Among those diseases, anthracnose caused by fungi belonging to genus *Colletotrichum* has been associated with severe loss of the yam plants in the fields. This disease is the most destructive fungal disease especially on *Dioscorea alata* species anywhere in the world [5]. Symptoms induced by anthracnose cause alteration of yam aerial organs. The peculiar symptom of the disease includes irregular small brown or black spots on the leaves that enlarge and eventually cause entire leaves necrosis.

With heavy rainstorms and high temperatures conditions, propagules (spores and conidia) of the fungi infect stems and gradually destroy entire aerial part of the plant. The disease caused huge production losses ranging from 50 to 90 % or even 100 % in the West Indies [6] and 30 to 90 % in Nigeria [7]. In Ghana, fields have been

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abandoned due to high incidence of yam anthracnose [8]. Similarly, prevalence of yam anthracnose disease ranging from 70 % to total destruction of yam fields have been observed in the forest zone of Côte d'Ivoire [2]. The impact of anthracnose has been an increasing pressure on yam cultivation in Côte d'Ivoire and there is no any curative methods to control this disease. This situation has created a negative impact on food security of population where yam is a staple food and also a socio-culturally and economically important crop. A precise identification of pathogen could help to find a sustainable solution to this disease. Best identification of pathogen responsible for yam anthracnose at morphological and molecular level will be instrumental in anthracnose disease management.

2 MATERIALS AND METHODS

2.1 Collection of *Colletotrichum* Isolates

Twenty-seven (27) isolates of *Colletotrichum* collected from symptomatic leaves and stems of yam plants available at the Plant Health Laboratory of Nangui ABROGOUA University were used for this study.

2.2 Morphological Characterization

2.2.1 Cultural Characteristics

A total of 27 *Colletotrichum* isolates were sub cultured on sterile potato dextrose agar (PDA) medium. 5 mm diameter inoculum was removed from 10-day-old pure cultures using a sterile cookie cutter and transferred at the center of Petri dish containing solidified PDA medium. Petri dish center was marked by the intersection of two perpendicular lines drawn on reverse side. Each seeded Petri dish was sealed and was stored in dark at room temperature (27 ± 2 °C).

Cultural characteristics of isolates were described by mycelia color, growth pattern and growth rate in plates.

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For each isolate, five Petri dishes (replicates) were prepared. Diameter of mycelial growth of each colony was measured daily along the two lines, until complete colonization of colony on PDA medium in Petri dish. Experiment was repeated three times. Colony growth diameter (D) of each isolate was calculated for each Petri dish. Mean colony growth was determined according to following Eqn. 1:

$$D(mm) = \frac{1}{n}\sum_{n=1}^{5} \frac{d1 + d2}{2}$$

Eqn. 1

Where **D:** Mean diameter; **d1:** diameter on axis 1; **d2:** diameter on axis 2; **n:** number of Petri dishes per isolate

2.2.2 Microscopic Characteristics

Conidia of *Colletotrichum* isolates from fifteen to twenty-one old days culture were observed under light microscope (ZEISS brand). Then microscopic characteristics viz. conidia shape, size (length and diameter) and spore concentration for each isolate were observed and measured.

2.2.2.1 Conidial Shape

Mycelial colony or conidia mass sample were taken and observed under light microscope at 400 magnification (G x 400). For each isolate, shape of 100 conidia selected randomly were described at the rate of 20 conidia per Petri dish. Fungal isolates were then divided according to their shape into different classes according to [9] as follows:

- ✓ Class 0: conidia with both ends rounded (cylindrical conidia);
- ✓ Class 1: conidia with one end rounded and other pointed (cylindrical conidia);
- ✓ Class 2: conidia with both ends pointed (fusiform or elliptical conidia).

2.2.2.2 Conidial Size

Size (length and diameter) of 100 conidia per isolate whose shapes were described above, was determined

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using a micrometer incorporated in eyepiece of microscope (G x 400). Mean length (L) and mean diameter (d) of conidia were then calculated using the following Eqn. 2 and 3:

$$L_i = \frac{\text{Sum of conidia lengths in a Petri dish}}{20}$$

Eqn. 2

$$d_i = \frac{\text{Sum of conidia diameters in a Petri dish}}{20}$$

Eqn. 3

2.2.2.3 Conidial Concentration

Number of conidia produced per isolate was also determined as number of conidia per milliliter of suspension according to method used by [10]. Suspension preparation was done with five inocula, each having 5 mm in diameter, taken from 15 to 21-day-old pure colonies and placed in a test tube containing 10 ml of sterile distilled water. Contents of this tube were first shaken by hand for 30 s and vortexed for 1 min to detach conidia from their conidiophores. Resulting suspension was then filtered through sterile muslin cloth to remove mycelial fragments. This conidia suspension was finally recovered in a test tube. For each fungal isolate, five test tubes were prepared, with one test tube per Petri dish. 10 µl of each suspension was spread on Malassez slide and observed under light microscope (G x 400), for conidia counting. Fifty (50) counts were made at rate of 10 counts per test tube for each isolate. Each count was performed on 10 gridded rectangles (consisting of 20 small squares) distributed over entire surface of Malassez slide. Mean number of conidia per milliliter of suspension (N) was calculated using the following Eqn. 4 and 5:

$$N = \frac{1}{50} \sum ni \times 100\,000$$

Eqn. 4

$$ni = \sum ni1 - 10$$

Eqn. 5

N: Mean number of conidia per milliliter of suspension per fungal isolate; **ni :** Mean number of conidia in rectangles; **ni1-10:** Number of conidia in each of the 10 rectangles; **100 000:** Factor which reduces volume of a rectangle (1 µL or 0.01 mm3) to a milliliter

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2.3 Molecular Characterization

2.3.1 DNA Extraction

The 7-day-old isolates obtained from a single-spore culture were used for DNA extraction following method used by [11]. Mycelial colonies were harvested and crushed separately with 1 ml of CTAB. Resulting crushing's were then collected in 2 ml sterile micro centrifuge tubes and placed in water bath at 65 °C for 30 min. After water bath, tubes were left for 2 min at laboratory room temperature (25 ± 2 °C) and then 800 µl of Chloroform isoamyl alcohol (24:1) was added to each tube, vortexed and centrifuged at 13,000 rpm for 10 min. After centrifugation, 650 µl of supernatant from each tube was removed and put into clean microcentrifuge tubes separately. Then 650 µl of ice-cold (-20 °C) isopropanol was also added to supernatant in each tube and incubated overnight in freezer at overnight to precipitate DNA. After incubation in freezer, tubes were centrifuged at 13,000 rpm for 10 min. After centrifugation, supernatant was decade and pellet was conserved. In each microfuge tube, 500 µl of 70 % ethanol was added and tubes were centrifuged at 13,000 rpm for 5 min. Ethanol was decaded in each tube after centrifugation, and DNA pellet was dried at room temperature. 100 µl of TE buffer was added into micro-tubes to dissolve DNA and tubes were stored at -20° C for future analysis.

2.3.2 PCR Amplification and Sequencing

Polymerase Chain Reaction (PCR) was performed with *C. gloeosporioides* specific primer pair ITS4/CgInt according to method of [11]. PCR reaction was performed in 10 µl reaction volume containing 0.3 µl of ITS4 primer (17 ρmol), 0.28 µl of CgInt primer (18 ρmol), 0.04 µl of Taq polymerase enzyme (5 units/µL), 0.6 µl of MgCl2 (25 mM), 1 µl of dNTP (2 mM), 1 µl of 10X kit buffer, 4.78 µl of pure water, and 2 µl of DNA from each *C. gloeosporioides*. Three other *C. gloeosporioides* strains already known on GenBank were taken as positive control.

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Amplification was performed in a thermocycler (Bio-Rad's Thermal Cycler) according to following program (Mention the protocol name if it is followed from other protocol): an initial denaturation, one cycle (at 95° C for 5 min) followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min 15s, extension at 72°C for 2 min and final extension at 72°C for 5 min. PCR products were separated on 1 % agarose gel incorporated with ethidium bromide in 0.5 X TBE buffer (90 mM Tris-Borate, 1 mM EDTA) by electrophoresis. Amplified PCR products on a target fragment were sequenced at Eurofins Genomic (Les Ulis, France). Sequences obtained were compared to those of 11 *C. gloeosporioides* strains sequences from National Center for Biotechnology Information (NCBI). All nucleotide sequences obtained were then aligned with MEGA version 5 software and phylogenetic tree was constructed with Figtree version 3.2 software

2.4 Data Analysis

All collected data were subjected to analysis of variance with Statistica Version 7.1 software. A one-way analysis of variance (ANOVA 1) was performed to compare colony growth, conidia lengths and width, conidia concentration in sporale concentrations, for the twenty-seven *Colletotrichum isolates*. In case of significant difference at 5% level, Fisher's least significant Difference (LSD) test was used to determine homogeneous groups.

2 RESULTS

3.1 Cultural characteristics of *Colletotrichum* isolates

The twenty-seven (27) isolates of *Colletotrichum* mycelial color varied between whitish, orange and greyish. Several color were observed for each coloration group. Colonies showed cottony, flaky, downy or filamentous structures; with concentric, radiating or regular ring growths. Cultural characteristics of these isolates were respectively associated with their 21 days old conidia (Fig. 1).

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Colony mycelial growth diameter of *Colletotrichum* isolates were varied from one isolate to another isolate as presented in Table 1. Isolate Cg27 had the smallest mean growth diameter (37 mm) while isolate Cg17 had the largest mean growth (82 mm). A significant difference (P < 0.05) was observed statisticaly between their colony mean growth diameter.

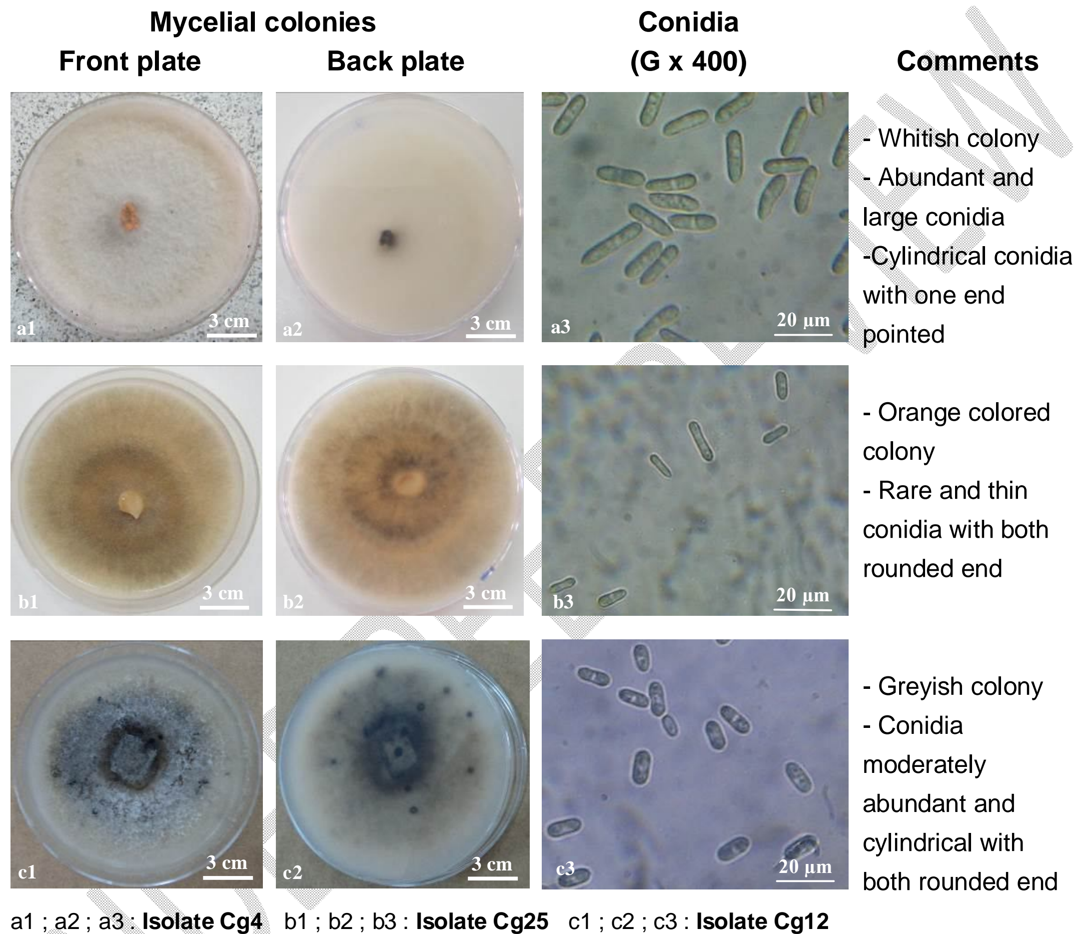

Fig. 1: Colonies and conidia of 21 days old culture of three *Colletotrichum* isolates on PDA medium

3.2 Microscopic characteristics of Colletotrichum isolates

Conidia of *Colletotrichum* isolates were straight cylindrical with rounded end (Table 1) except for those of isolates Cg4 and Cg7 for which one end was pointed and other rounded respectively. In contrast, conidia from other 25 isolates had both rounded end. Sporulation of *Colletotrichum* isolates showed a wide range of mean

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number of conidia per isolates. The mean number of conidia per milliliter differed significantly ($P < 0.05$) among the 27 isolates. A lowest mean number of conidia was noted with isolate Cg3 (1.10 x 105 conidia/ml) whereas isolate Cg25 isolate, had the highest mean number of conidia per milliliter (8.54×10^6 conidia/ml) (Table 1). Conidia size of the 27 *Colletotrichum* isolates varied significantly at 5 % level (Table 1). Mean length of conidia also varied statistically from 10.54 µm to 19.29 µm (Table 1). Isolate Cg27 produced the shortest conidia (10.54 µm) while isolate Cg4 produced the longest conidia (19.29 µm). Conidia width of isolates ranged from 3.93 µm to 6.43 µm. The smallest mean width was observed on conidia of isolate Cg24 while those produced by isolate Cg9 were the largest (Table 1). Conidia mean width of all *Colletotrichum* isolates were statistically different ($P < 0.05$)

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Table 1: Morphological characteristics of *Colletotrichum gloeosporioides* isolates causing yam anthracnose in Côte d'Ivoire

Fungi isolate	Cultural characteristics				Microscopic characteristics			
	Colonies color	Colony appearance	Colonies growth mode	Mean colony diameter (mm)	Conidia shape	Concentration (10^5conidia/ml)	Conidia length (µm)	Conidia diameter (µm)
Cg1	Whitish	Flaky	Concentric rings	80,67 ±1,45^{ab}	Cylindrical	1,50 ± 0,22^{jk}	17,86 ± 2,14^{ab}	5,54 ± 0,37^{abcd}
Cg2	Yellow-white	Cottony	Concentric rings	77,33 ± 0,88^{bcd}	Cylindrical	24,50 ± 1,51^{d}	15,36 ± 2,13^{abcdef}	6,25 ± 0,55^{ab}
Cg3	Grey-dark	Fluffy	Concentric rings	76,00 ± 1,15^{cde}	Cylindrical	1,10 ± 0,10^{k}	13,93 ± 1,11^{bcdefg}	4,29 ± 0,25^{ef}
Cg4	Grayish	Flaky	Concentric rings	73,33 ± 1,45^{defg}	Cylindrical	50,00 ± 2,25^{c}	19,29 ± 2,16^{a}	4,46 ± 0,25^{def}
Cg5	Light-gray	Cottony	Concentric rings	56,67 ± 2,40^{k}	Cylindrical	17,10 ± 1,00^{ef}	15,54 ± 2,00^{abcdef}	5,18 ± 0,64^{bcde}
Cg6	Light-gray	Cottony	Concentric rings	50,33 ± 1,45^{lm}	Cylindrical	14,40 ± 1,39^{fg}	13,39 ± 1,18^{bcdefg}	4,64 ± 0,23^{cdef}
Cg7	Grayish	Flaky	Concentric rings	71,67 ± 0,67^{fg}	Cylindrical	27,00 ± 1,43^{d}	17,50 ± 2,78^{abc}	4,82 ± 0,33^{cdef}
Cg8	Whitish	Cottony	Regular	67,00 ± 1,15^{hi}	Cylindrical	10,60 ± 0,69^{gh}	16,07 ± 0,92^{abcde}	4,64 ± 0,23^{cdef}
Cg9	Grey-white	Fluffy	Concentric rings	63,67 ± 0,67^{ij}	Cylindrical	16,30 ± 0,73^{ef}	15,00 ± 0,94^{abcdefg}	6,43 ±0,51^{a}
Cg10	Light-gray-orange	Cottony	Regular	61,33 ± 1,45^{j}	Cylindrical	15,30 ± 1,01^{ef}	16,96 ± 0,94^{abcd}	4,11 ± 0,36^{ef}
Cg11	Brown-yellow	Flaky	Concentric rings	72,67 ± 2,91^{efg}	Cylindrical	14,80 ± 1,05^{ef}	14,75 ± 1,46^{abcdefg}	4,46 ± 0,25^{def}
Cg12	Dark grey-orange	Cottony	Concentric rings	73,67 ± 1,45^{defg}	Cylindrical	24,30 ± 0,98^{d}	12,86 ± 1,27^{defg}	5,71 ± 0,54^{abc}
Cg13	Black-white	Cottony	Regular	74,33 ± 2,03^{defg}	Cylindrical	67,10 ± 3,21^{b}	15,36 ± 0,65^{abcdef}	4,82 ± 0,33^{cdef}
Cg14	Whitish	Cottony	Regular	76,33 ± 0,88^{cde}	Cylindrical	65,50 ± 4,11^{b}	13,93 ± 1,50^{bcdefg}	4,29 ± 0,46^{ef}
Cg15	Dark-gray	Cottony	Concentric rings	65,00 ± 1,73^{ij}	Cylindrical	18,80 ± 1,57^{e}	14,29 ± 0,90^{bcdefg}	4,64 ± 0,23^{cdef}
Cg16	Wooly white	Flaky	Regular	74,67 ± 1,76^{def}	Cylindrical	17,20 ± 1,34^{ef}	16,43 ± 2,24^{abcd}	4,46 ± 0,37^{def}
Cg17	Whitish	Flaky	Concentric rings	82,00 ± 1,15^{a}	Cylindrical	16,00 ± 1,07^{ef}	16,43 ± 2,37^{abcd}	4,11 ± 0,45^{ef}
Cg18	Ochre-yellow	Cottony	Concentric rings	70,33 ± 0,88^{gh}	Cylindrical	6,10 ± 0,64^{i}	11,61 ± 0,71^{efg}	6,25 ± 0,47^{ab}
Cg19	Dark-gray-orange	Cottony	Concentric rings	80,00 ± 1,15^{abc}	Cylindrical	1,30 ± 0,21^{jk}	17,14 ± 1,84^{abcd}	4,64 ± 0,23^{cdef}
Cg20	White-dark-gray	Flaky	Concentric rings	71,00 ± 1,15^{fgh}	Cylindrical	7,70 ± 0,87^{hi}	13,75 ± 1,12^{bcdefg}	4,64 ± 0,36^{cdef}
Cg21	Dark brown-yellow	Flaky	Concentric rings	73,33 ± 1,76^{defg}	Cylindrical	27,00 ± 1,07^{d}	14,29 ± 1,79^{bcdefg}	5,18 ± 0,51^{bcde}
Cg22	White-orange	Cottony	Concentric rings	51,33 ± 0,88^{l}	Cylindrical	5,30 ± 0,37^{ij}	11,43 ± 1,07^{fg}	5,18 ± 0,33^{bcde}
Cg23	Blackish	Filamentous	Radiant	62,00 ± 1,73^{j}	Cylindrical	6,20 ± 0,65^{i}	13,93 ± 2,49^{bcdefg}	5,00 ± 0,67^{cdef}
Cg24	Dark-gray	Cottony	Regular	46,33 ± 0,88^{m}	Cylindrical	7,00 ± 0,65^{hi}	11,25 ± 0,94^{fg}	3,93 ± 0,33^{f}
Cg25	Light-brown-yellow	Fluffy	Concentric rings	37,33 ± 2,03^{n}	Cylindrical	85,40 ± 1,54^{a}	13,75 ± 1,61^{bcdefg}	4,82 ± 0,51^{cdef}
Cg26	Dark-gray	Cottony	Concentric rings	61,00 ± 1,15^{j}	Cylindrical	6,50 ± 0,62h^{i}	13,21 ± 1,18^{cdefg}	5,54 ± 0,46^{abcd}
Cg27	White-light-gray	Cottony	Concentric rings	37,00 ± 1,15^{n}	Cylindrical	63,30 ± 2,45^{b}	10,54 ±1,72^{g}	4,46 ± 0,54^{def}

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3.3 Molecular characteristics of *Colletotrichum* isolates

All the 27 *Colletotrichum* isolates analyzed were successfully amplified by *Colletotrichum gloeosporioides* specific ITS4/CgInt primer pair (Fig. 2). PCRs from DNA from these isolates migrated at molecular weight of 450 base pairs (bp) like those of control. Comparison of sequences of the 27 *Colletotrichum* isolates from yam plants grown in Côte d'Ivoire with *C. gloeosporioides* strains present on NCBI GenBank (https://blast.ncbi.nlm.nih.gov), using BLAST revealed homology rates ranging from 99 to 100%. Sequences of isolates Cg1, Cg15, Cg18 and Cg20 were 100% homologous with *C. gloeosporioides* sequences of accession numbers KT390195, KM269466, KT390189 and KF773859 respectively. Sequences of other isolates were 99% homologues with those used in Genbank (Table 2). Analysis of all sequences belonging to each isolates confirmed that isolates from yam plants grown in Côte d'Ivoire were *Colletotrichum gloeosporioides* species. Sequence accession numbers for ten isolates from yams in Côte d'Ivoire are also given in Table 2. Phylogenetic tree analysis revealed variable proximities between corresponding regions of rDNA sequences of 10 *C. gloeosporioides* isolates from yam varieties grown in Côte d'Ivoire and 11 *C. gloeosporioides* sequences taken from GenBank. All these isolates can be classified into two main groups (group 1 and 2). Each group was also divided into two subgroups (Fig. 3). In subgroup 1.1, isolates Cg1 and Cg15 from yam plant of experimental site of Nangui ABROGOUA University (UNA) were close to strain KT224835 identified in China. Isolates Cg27, Cg6, Cg20, Cg10 and Cg4 belonging to subgroup 1.2 originating from yam plants of two experimental sites (UNA and Soubré) were genetically closer to strain KX401434 identified in Malaysia. In subgroup 1.2, isolates Cg19 and Cg8 from yam plants of Soubré experimental site (Sou) were more closely related to strain KM203611 identified in India. Isolate Cg18 from subgroup 2.2, identified on bètè-bètè yam variety grown in Soubré, is closer to its counterpart also identified in Côte d'Ivoire.

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Colletotrichum gloeosporioides isolates on yam from Côte d'Ivoire were most closely of *Colletotrichum gloeosporioides* from south-east Asia than those from West Africa.

M : Molecular marker (500 bp); **1 - 27 :** *C. gloeosporioides* isolates; **T1, T2, T3 :** *C. gloeosporioides* (Control)

Fig. 2: Electrophoretic profile of gene amplification products from *Colletotrichum gloeosporioides* isolates with specific primers ITS4/CgInt

Table 2: Similarity between rDNA nucleotide sequences of 10 Colletotrichum gloeosporioides isolates from yams in Côte d'Ivoire and those of the GenBank database (NCBI)

Isolate code	Isolate accession numbers	Yam varieties / Cultivation site	Similarity (%)	*C. gloeosporioides* sequence accession numbers on GenBank
TMY_Cg1	OR354323	bètè-bètè / UNA Test site	100	KT390195
TMY_Cg4	OR354324	bètè-bètè / UNA Test site	99	KX447642
TMY_Cg6	OR354325	bètè-bètè / UNA Test site	99	KX401434
TMY_Cg8	OR354326	TDa / Soubré Test site	99	KU527801
TMY_Cg10	OR354327	C18 / Soubré Test site	99	KT582197
TMY_Cg15	OR354328	bètè-bètè / UNA Test site	100	KM269466
TMY_Cg18	OR354329	bètè-bètè / Soubré Test site	100	KT390189
TMY_Cg19	OR354330	bètè-bètè / Soubré Test site	99	KM203611
TMY_Cg20	OR354331	bètè-bètè / Soubré Test site	100	KF773859
TMY_Cg27	OR354332	C18 / Soubré Test site	99	KX401434

UNA: University of Nangui ABROGOUA

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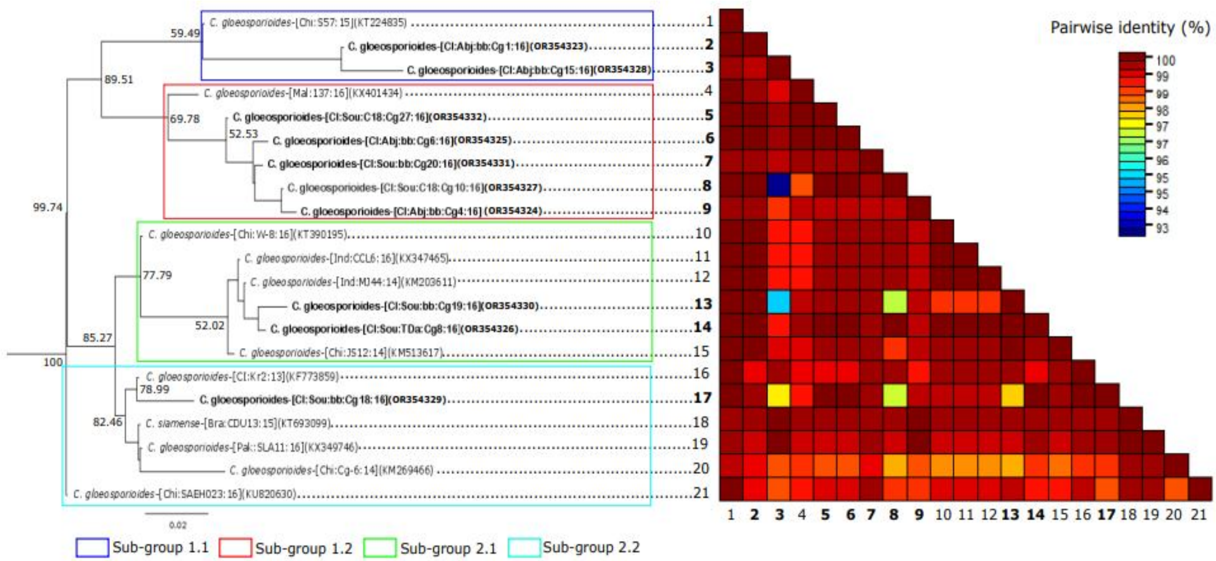

Fungi name-[Country: City: yam variety: Strain: Date] (Accession number) ; **Chi**: China; **CI**: Côte d'Ivoire; **Mal**: Malaysia; **Ind**: India; **Bra**: Brazilia; **Pak**: Pakistan; **Abj**: Abidjan; **Sou**: Soubré

Yam varieties: bb = bètè-bètè; C18; TDa = TDa00/90

Fig. 3: Phylogenetic tree of *Colletotrichum gloeosporioides* showing nucleotide sequence relationships

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4 DISCUSSION

Diversity of isolates of *Colletotrichum gloeosporioides* responsible for yam anthracnose in Côte d'Ivoire was observed. This variability has been observed at different levels on cultural characteristics (coloration, mycelial appearance and growth diameter) and also on microscopic characteristics (shape, concentration and size of conidia). These fungal characteristics are mainly dependent on morphology (color and shape) of stems and leaves of host yam varieties. Indeed, color of aerial organs of yam varieties sampled was green, brown, violet or red in stems, and light to dark green with some yellowish veins in leaves [12]. These leaves also had soft or leathery texture and were either small, medium or large in size. These morphological features in aerial organs of yams were also observed by [12].

This great diversity of organs infected by *C. gloeosporioides* could be origin of morphological diversity of this pathogenic fungus. Coloration of *C. gloeosporioides* isolates could be a function of colors of yam varieties from which fungus was isolated. [13] have shown that morphological and anatomical diversity of yam leaves and stems are at the root of great variability of symptoms caused by *C. gloeosporioides* on various yam species and varieties under cultivation. Variability in mycelial colony growth, conidia size and concentration of these 27 *C. gloeosporioides* isolates was noted. These variabilities could be due to resistance reactions of yam varieties when attacked by pathogenic fungus. Indeed, leathery leaves of yam varieties would react differently to infection than the softer and wider ones, as opposed to the smaller ones. Similar variability in *C. gloeosporioides* isolated from yams was also reported by [14] during his study of biology, pathogenicity and control of *C. gloeosporioides* in India. He observed conidia size variation from 11-18.5 µm to 3.7-6.4 µm just as in our study, from 10.5-19.3 µm to 3.9-6.4 µm for conidia lengths and width (diameters) respectively. Those characteristics were influenced by cultivation environment and presence of intermediate forms of *Colletotrichum* species and they were not entirely reliable enough to differentiate species of the genus *Colletotrichum*. At genetic level, variable homology

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rates were noted between rDNA nucleotide sequences of *C. gloeosporioides* isolates. Similarly, phylogenetic tree revealed variable proximities between fungal isolates. Some isolates identified on yams grown at University and Soubré sites were distant from each other, while others were closer to *C. gloeosporioides* strains isolated in China, Malaysia and India. This genetic diversity could be due to mutations and/or gene recombination in genotype of the isolates. This would explain constant progression of yam anthracnose in fields. [15] have also shown that climatic conditions of humid tropics favor rapid evolution through mutation and recombination of bacteria and fungi. These environmental conditions confer pathogens with a strong capacity to adapt to changes in environment, to pesticides used and to resistance genes of host plants. Morphological and genetic diversity of *C. gloeosporioides* isolates responsible for yam anthracnose in Côte d'Ivoire would explain the variability of symptoms of this disease in field.

5 CONCLUSION

In this study several morphological and genetically different isolates of *Colletotrichum gloeosporioides* were responsible for yam anthracnose in Côte d'Ivoire. Diversity of these isolates was observed through variability of cultural, microscopic and genetic characteristics. This diagnosis leads to better understanding of this pathogenic fungus and opens up new avenues of thought on how to control yam anthracnose, with aim of improving yam yields in Côte d'Ivoire.

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