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PHENOTYPIC AND MOLECULAR CHARACTERISATION OF THE CAUSAL AGENT OF MANGO ANTHRACNOSE DISEASE IN GHANA

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Abstract

A study was carried out to confirm the identity of the causal agent of anthracnose, a major fungal disease of mango in Ghana. Forty-five isolates of the pathogen were obtained from diseased mango plant parts, and were identified using morphological, physiological and molecular methods. The results showed that the pathogen produced short conical spores, which were rounded at the edges and showed a negative reaction in the casein hydrolysis test. The expected 480 bp product was amplified from the DNA of the isolates in polymerase chain reaction (PCR), using species specific primers. Phylogenetic studies of the rDNA-ITS region of isolates showed they were closely related to *Colletotrichum gloeosporioides* identified elsewhere. All isolates were able to induce the disease on artificially inoculated fruits. The results obtained in the study were consistent with the identification of *C. gloeosporioides* elsewhere, and confirmed the pathogen as the causal agent of the anthracnose disease of mango in Ghana.

Introduction

Mango (*Mangifera indica* L.) is one of the most important fruits in the tropics, and approximately makes up 50 per cent of tropical fruits produced in the world (Jedele *et al*., 2003). The world's largest producer of mango is India followed by China and Thailand. The largest producer of mango in Africa is Nigeria followed by Egypt while Ghana and Burkina Faso produce modest amounts (FAOSTAT, 2010).

Ghana has a comparative advantage of two production seasons per calendar year. It is believed that if the major problems related to the production of the crop are properly handled, mango has the potential of being the number one export earner of Ghana, thereby, replacing cocoa (Anon., 1996). There are many factors affecting the production of quality mango fruits in Ghana including pests and diseases. Mango anthracnose caused by *Colletotrichum gloeosporioides* (Oduro, 2000; Offei *et al*., 2008) is one of the main diseases affecting mango in Ghana. The disease has been reported as the most important fungal disease affecting the crop in the country (Oduro, 2000). The disease causes dark sunken lesions on leaves and immature fruits and can sometimes blight flowers (Agrios, 2005; Ploetz, 1998). One of the most significant effects of the disease is the blemishes caused on fruit in storage or in transit which eventually reduce the marketability of the fruits. Elsewhere, the disease is known to be favoured by high humidity or rainfall during the early fruit maturity stage

(Ploetz, 1998; Arauz, 2000). It is cultivated in drier areas or areas where rainfall does not coincide with early fruit maturity.

In Ghana, *C. gloeosporioides* has been reported as the causal agent of mango anthracnose as well as anthracnose on other fruits (Oduro, 2000; Offei *et al.*, 2008). Recent advances in techniques for the identification of pathogens have raised doubts about the identity of the pathogen causing mango anthracnose in the tropics (Phoulivong *et al*., 2010). In Ghana, descriptions of the pathogen causing mango anthracnose indicate that its identification was based on the traditional methods of species identification (Oduro, 2000; Offei *et al*., 2008). The identification relies on the use of morphology, host specialisation and mode of parasitism (Bailey *et al*., 1996; Cannon *et al*., 2000). According to Freeman & Rodriduez (1995), conidial morphology and cultural characteristics often overlap between *C. acutatum* and *C. gloeosporioides*. This makes identification based on these methods to be unreliable (Peres *et al*., 2002). It could be conjectured that the pathogen causing mango anthracnose in Ghana is not well known, raising several questions about the scientific bases for the current control practices for the disease. In view of this, a study was carried out to identify the causal agent of the disease using the traditional and molecular methods, to clear any doubts about the aetiology of the disease in Ghana.

Experimental

Isolation of causal agents

Diseased mango fruits, leaves and panicles which were collected from farmer fields in Ghana were sent to the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, Legon for isolation of the causal agents. Diseased strawberry leaves collected from farmer fields in North Carolina, USA, were sent to the Plant Pathology Laboratory of the North Carolina State University. The isolation of causal agents was first done on water agar (WA), and then potato dextrose agar (PDA) prepared at rates of 20g l^{-1} and 39 g l^{-1}, respectively. Each mixture was autoclaved at 121 ºC for 15 min, cooled and poured into clean sterilised plates to set. Pieces of the fruit tissues taken from the advancing edge of the lesion at various parts of the fruits (stem end, middle and bottom portions) were taken, surface sterilised with sodium hypochlorite for 15 sec., washed in sterile distilled water and blotted dry using a paper towel. These were plated singly on WA plates and incubated till enough growth of the pathogen was observed. The growth were then sub-cultured on PDA and incubated till sufficient growth was observed. To further purify the isolates, single spore cultures were produced and used for the study.

Identification of isolates

Cultural characteristics and growth rate

A plug of 8-day old culture of each isolate was placed on PDA and incubated under ambient temperature of 27 °C and relative humidity (R.H.) of 65 per cent on benches in the laboratory. The colony colour, and arrangement of acervuli in the mycelia were observed and recorded to aid in the identification of the isolates. Using a ruler, the diameter of the mycelia of the organisms were measured daily on the reverse side of the plate along lines ruled across the diameter of the Petri plates. Three plates of each isolate

were used for the study. The diameter of the colony was measured daily for 7 days, and growth rate was calculated as 7-day average of the mean daily growth.

Morphological characteristics

Spore suspension of each isolate was prepared by extracting and crushing few acervuli in sterile distilled water. A drop of the suspension was fixed on a slide and observed under a compound microscope (Optical Technology, Asia). The shape of the conidia was recorded. The length and breadth of 50 randomly selected conidia were measured with the aid of an eye piece graticule and recorded to aid in the identification of the organisms.

Casein hydrolysis test

The medium was prepared by combining the following reagents: 1 g of potassium hydrogen phosphate, 0.5 g of potassium chloride, 0.1 g of calcium chloride dehydrate, 0.2 g of magnesium sulphate heptahydrate, 25 g of 15 per cent skim milk, 10 g of glucose and 12 g of agar. The reagents were dissolved in 1*l* of water, autoclaved, dispensed into plates and allowed to set. A plug of the mycelia of the pathogen from mango and *C. acutatum* was placed on the media and incubated at 28 ºC for 72 h. The culture was inspected for the presence of a clear zone to indicate the hydrolysis of the medium (positive reaction).

DNA extraction and polymerase chain reactions (PCRs)

DNA extraction was performed using the Sigma's GenFlute Plant Genomic DNA Miniprep Kit (St. Louis, MO, USA), following the manufacturer's instructions. The DNA extracted from isolates obtained from Ghana were transported on dry ice to the Plant Pathology Laboratory of the North Carolina State University, and were kept frozen at -20 ºC. The PCRs were carried out using primers pairs CgInt/ITS4 (CgInt: 5'-GGGGAAGCCTCTCGCGG-3'and ITS4: TCCTCCGCTTATTGATATGC), specific to *C. gloeosporioides* and ITS1/ITS4 (ITS1: TCCGTAGGTGAACCTGCGG) to amplify the entire internal transcribed spacer region. The PCR mixture was made up of 2 µl target DNA, 5 µl of 10 × PCR buffer (Invitrogen, Carlsbad, CA), 2.5 µl of deoxynucleoside-triphosphate mix (2.5 m*M* each), 0.25 µl bovine serum albumin (20 mg/ml), 2 µl each of the forward and reverse primer, 1.8 µl of magnesium chloride (50 m*M*) and 0.2 µl of taq polymerase (Invitrogen, Carlsbad, CA) added to 34.25 µl of double distilled water. Each PCR was performed in a total reaction volume of 50 µl. The reaction was carried out in a Thermo Hybaid P × E Thermal Cycler (Thermo Electron Corporation, USA). The reaction cycles were denaturing for 2 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C and a final extension of 10 min at 72 °C. Amplification products were separated by 1.5 per cent w/v agarose gel (Invitrogen, Carlsbad, CA), stained with ethidium bromide or gel red alongside 1.0 kb marker at 100 V for about 1.5 h. Bands were observed under UV light.

Purification and sequencing of amplified products

The PCR amplified product of the ITS region of isolates were sent to ETON Bio-

science Laboratory at Raleigh in North Carolina for purification and sequencing. Ten picomole of each primer was used to sequence the product directly from both directions. Sequences were entered into the BIOEDIT software and edited.

Phylogenetic analysis

The ITS sequences of the 45 isolates from mango in Ghana and 15 sequences of isolates of different *Colletotrichum* spp. retrieved from European Molecular Biological Laboratory (EMBL) nucleotide sequence database were used in the phylogenetic studies. Among the retrieved sequences were those of *Fusarium oxysporum* which was used as the out-group. Multiple sequence alignment of the selected isolates was through ClustalW, and phylogenetic analysis was performed using MEGA5 (Tamura *et al.*, 2011). The Neighbor-Joining method (Saitou & Nei, 1987) was used to infer the evolutionary history. The percentage of replicate trees in which the associated taxa clustered together was evaluated with a bootstrap analysis with 1000 replicates. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004), and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

Pathogenicity test

Spore suspension of each isolate from Ghana was prepared by crushing acervuli in sterile distilled water, and the spore concentration adjusted to 1 × 107 spores/ml with the aid of a haemocytometer (Hawskley, England). Clean, healthy, and physiologically matured fruits of Tommy Atkins cultivar were inoculated with the spore suspension, by placing 15 ml of the suspension on filter paper discs placed on the fruit surface. The filter paper discs were used to prevent run-off of the spore suspension on the fruit surface. The isolates were used to inoculate at least three fruits. Water served as the control. Fruits were then placed in air tight containers lined with paper towels and incubated at 27 ºC. Seven days after incubation, the fruits were removed and inspected for the development of anthracnose symptoms.

Results

Cultural, growth rate and morphological characteristics of isolates

Three types of colonies were recorded for isolates sampled from mango in Ghana. These were white mycelia with or without sporulation, and white mycelia with a greyish centre. The *C. acutatum* isolates also produced white mycelia with abundant sporulation. Isolates from mangoes in Ghana possessed cylindrical conidia, which in few cases narrowed at the middle with rounded edges. The conidia were produced abundantly in disc shaped bright yellow acervuli, which were common on the surface of the naturally infected fruits collected during the survey and in culture media. Acervuli produced by isolates in culture possessed numerous setae, which were swollen at the base and tapers toward the edge. Conidial size isolates of the pathogen from mango from Ghana, averagely, was 15.9 µm

and 5.4 µm. On the other hand, isolates of *C. acutatum* (isolated from the diseased strawberry leaves) possessed fusiform spores that were pointed at the edges, and which were smaller in length and width compared to the *C. gloeosporioides* isolates. The growth rate measurements showed that isolates of the pathogen from mango in Ghana grew at a rate of 1.1 mm/day. On the other hand, *C. acutatum* isolates grew at a slower rate of 0.7 mm/day (Table 1).

Biochemical characteristics

In the casein hydrolysis test, all isolates from mango from Ghana produced a negative reaction. *C. acutatum* isolates produced a positive reaction.

Polymerase chain reaction (PCR) using specific primers

A 480 bp PCR product was amplified from DNA of isolates from mango sampled in Ghana with species specific primers CgInt and ITS4. However, there was no amplification from DNA of strawberry and bell pepper using the same primer pair (Fig. 1).

Sequences and phylogenetic studies of the ITS region

An approximately 600 bp product of the ITS region was amplified using the primer pair ITS1/ITS4 from the isolates. The assembled sequences were 535 bp long, which have been deposited in the GenBank and their accession numbers indicated (Table 2). The ITS1 region was 171 bp long. An approximately 180 bp product of the ITS1

TABLE 1

Conidial morphology, dimension, and growth rate of Colletotrichum *species*

Source of isolate	*Country of origin*	*Spore type*	*Spore length (µm)*	*Spore width (µm)*	*Growth rate (mm/day)*
Mango	Ghana	Cylindrical	15.9 ± 0.06	5.4 ± 0.08	1.10
Strawberry	U.S.A	Fusiform	12.7 ± 0.06	3.7 ± 0.04	0.70
Bell pepper	U.S.A	Fusiform	11.9 ± 0.05	3.3 ± 0.04	0.70

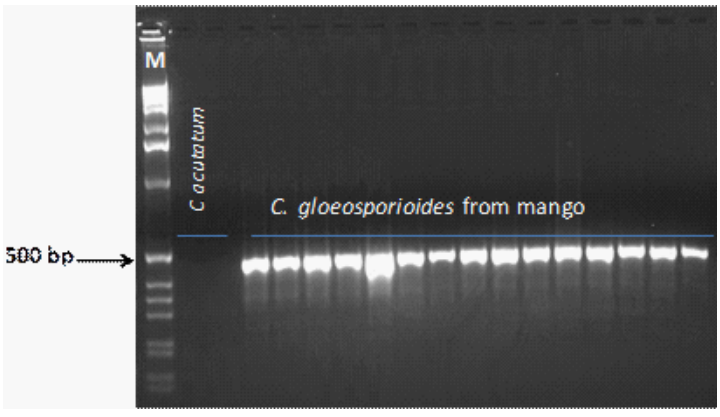

Fig. 1. Gel showing an approximately 480 bp PCR product amplified from DNA of *Colletotrichum* spp. from mango. Note the absence of the product from *C. acutatum*. M = 1 Kb marker

TABLE 2

List of isolates used in the phylogenetic studies and their GenBank accession numbers.

Isolate designation	*Host*	*GenBank accession number*	*Molecular designation*
MAN-GH1	Mango	KJ019333	*C. gloeosporioides*
MAN-GH2	Mango	KJ019334	*C. gloeosporioides*
MAN-GH3	Mango	KJ019335	*C. gloeosporioides*
MAN-GH4	Mango	KJ019336	*C. gloeosporioides*
MAN-GH5	Mango	KJ019337	*C. gloeosporioides*
MAN-GH6	Mango	KJ019338	*C. gloeosporioides*
MAN-GH7	Mango	KJ019339	*C. gloeosporioides*
MAN-GH8	Mango	KJ019340	C. gloeosporioides
MAN-GH9	Mango	KJ019341	*C. gloeosporioides*
MAN-GH10	Mango	KJ019342	*C. gloeosporioides*
MAN-GH11	Mango	KJ019343	*C. gloeosporioides*
MAN-GH12	Mango	KJ019344	*C. gloeosporioides*
MAN-GH13	Mango	KJ019345	*C. gloeosporioides*
MAN-GH14	Mango	KJ019346	*C. gloeosporioides*
MAN-GH15	Mango	KJ019347	C. gloeosporioides
MAN-GH16	Mango	KJ019348	*C. gloeosporioides*
MAN-GH17	Mango	KJ019349	*C. gloeosporioides*
MAN-GH18	Mango	KJ019350	*C. gloeosporioides*
MAN-GH19	Mango	KJ019351	*C. gloeosporioides*
MAN-GH20	Mango	KJ019352	*C. gloeosporioides*
MAN-GH21	Mango	KJ019353	*C. gloeosporioides*
MAN-GH22	Mango	KJ019354	*C. gloeosporioides*
MAN-GH23	Mango	KJ019355	*C. gloeosporioides*
MAN-GH24	Mango	KJ019356	*C. gloeosporioides*
MAN-GH25	Mango	KJ019357	*C. gloeosporioides*
MAN-GH26	Mango	KJ019358	*C. gloeosporioides*
MAN-GH27	Mango	KJ019359	*C. gloeosporioides*
MAN-GH28	Mango	KJ019360	*C. gloeosporioides*
MAN-GH29	Mango	KJ019361	*C. gloeosporioides*
MAN-GH30	Mango	KJ019362	*C. gloeosporioides*
MAN-GH31	Mango	KJ019363	*C. gloeosporioides*
MAN-GH32	Mango	KJ019364	*C. gloeosporioides*
MAN-GH33	Ghana	KJ019365	*C. gloeosporioides*
MAN-GH34	Ghana	KJ019366	*C. gloeosporioides*
MAN-GH35	Ghana	KJ019367	*C. gloeosporioides*

region from isolates collected in the study, and those retrieved from the GenBank (Table 2) were aligned and used in phylogenetic analysis. The evolutionary history was inferred using the Neighbor-Joining method and the optimal tree with the sum of branch length of 0.66984798 (Fig. 2). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985).

There were a total of 140 positions in the final dataset. Out of the 60 *Colletotrichum* spp. used in the study, 52 clustered together in the *C. gloeosporioides* complex with a high bootstrap support of more than 50 per cent (Fig. 2). Among members of the group are the reference isolate of *C. gloeosporioides* (CBS. 953.97), the selected isolates of *C. fragariae, C. kahawae,* and *C. musae,* and all the isolates of the fungi obtained from mango from Ghana. The two isolates collected from bell pepper and strawberry clustered with the reference *C. acutatum* isolate with a high bootstrap support of 97 per cent. The *C. acutatum* clade is formed far from the *C. gloeosporioides* complex clade, showing that the mango isolates of the pathogen are very different from strains of *C. acutatum*. Similarly, the other *Colletotrichum* spp. clustered away from the group containing the mango isolates, showing that none of the mango isolates belong to any of those groups.

Results obtained with the ITS1 region were confirmed by the analysis of the entire ITS region of isolates. A pairwise comparison of nucleotide sequences of the region (entire ITS) showed all isolates from mango in Ghana and other selected isolates within

TABLE 2 (Cont'd)

List of isolates used in the phylogenetic studies and their GenBank accession numbers

Isolate designation	*Place of collection*	*GenBank accession number*	*Molecular designation*
MAN-GH36	Mango	KJ019368	*C. gloeosporioides*
MAN-GH37	Mango	KJ019369	*C. gloeosporioides*
MAN-GH38	Mango	KJ019370	*C. gloeosporioides*
MAN-GH39	Mango	KJ019371	*C. gloeosporioides*
MAN-GH40	Mango	KJ019372	*C. gloeosporioides*
MAN-GH41	Mango	KJ019373	*C. gloeosporioides*
MAN-GH42	Mango	KJ019374	*C. gloeosporioides*
MAN-GH43	Mango	KJ019375	*C. gloeosporioides*
MAN-GH44	Mango	KJ019376	*C. gloeosporioides*
MAN-GH45	Mango	KJ019377	*C. gloeosporioides*
CBS 953.97	Citrus	AF090855	*C. gloeosporioides*
SAS 4	Mango	Z32953	*C. gloeosporioides*
IMI 319406	Coffee	Z32983	*C. kahawae*
FRA 63-1	Strawberry	Z32943	*C. fragariae*
MUS-UQ	Plantain	Z32997	*C. musae*
GRA-102	Sorghum	Z32974	*C. graminicola*
DEM-288810	Pink	Z32938	*C. dematium*
COC-LC	Potato	Z32931	*C. coccodes*
LIND-CLD2	Kidney beans	Z32987	*C. lindemuthianum*
ORB-172.59	Cucumber	Z33379	*C. orbiculare*
ACU-397	Strawberry	Z32915	*C. acutatum*
ACU-BP1	Bell pepper	KJ627843	*C. acutatum*
ACU-ST1	Strawberry	KJ627844	*C. acutatum*
M1/6	Mango	Z32968	*C. gloeosporioides*

the *C. gloeosporioides* complex including the type strain of *C. gloeosporioides* had high sequence homology among them. Also between these isolates and other isolates belonging to different species including *C. acutatum*, the sequence homology was lower (Table 3). This means that none of the mango isolates belong to any of the species represented by the different isolates

All isolates of the pathogen used for the pathogenicity test were able to cause the disease symptom similar to what was observed on the naturally infected fruits collected from the field. Seven days after incubation, the dark sunken lesions were observed under the filter papers on which the spore suspension was dropped on the fruits. The disease lesion was not observed under filter paper discs inoculated with distilled water. This showed that the pathogens used in the study were responsible for the disease on the field.

Discussion

Two *Colletotrichum* spp. have been recognised worldwide as etiological agents of mango anthracnose. In sub-tropical areas such as Brazil and Florida both *C. gloeosporioides* and *C. acutatum* had been reported as the causal agent of the disease (Peres *et al.*, 2002; Davis, 1999), while *C. gloeosporioides* has been reported as the major cause of the disease in the tropics (Jeffries *et al*., 1990). In Ghana, *C. gloeosporioides* is the only pathogen associated with the disease (Oduro, 2000; Offei *et al.*, 2008). Since *C. gloeosporioides* and *C. acutatum* are the only species reported on mango worldwide, distinguishing between these two species served as an important step in the identification of the causal agent of anthracnose disease in Ghana.

Growth rate and spore morphology have been commonly used to distinguish between the two species, with *C. gloeosporioides* showing a relatively faster growth rate, and possessing conical spores which are rounded at the edges, compared to the *C. acutatum* species which have fusiform-shaped conidia (Peres *et al*., 2002; Martin & Garcia-Figueres, 1999; Bernstein *et al.*, 1995). In the study, these characteristics were found

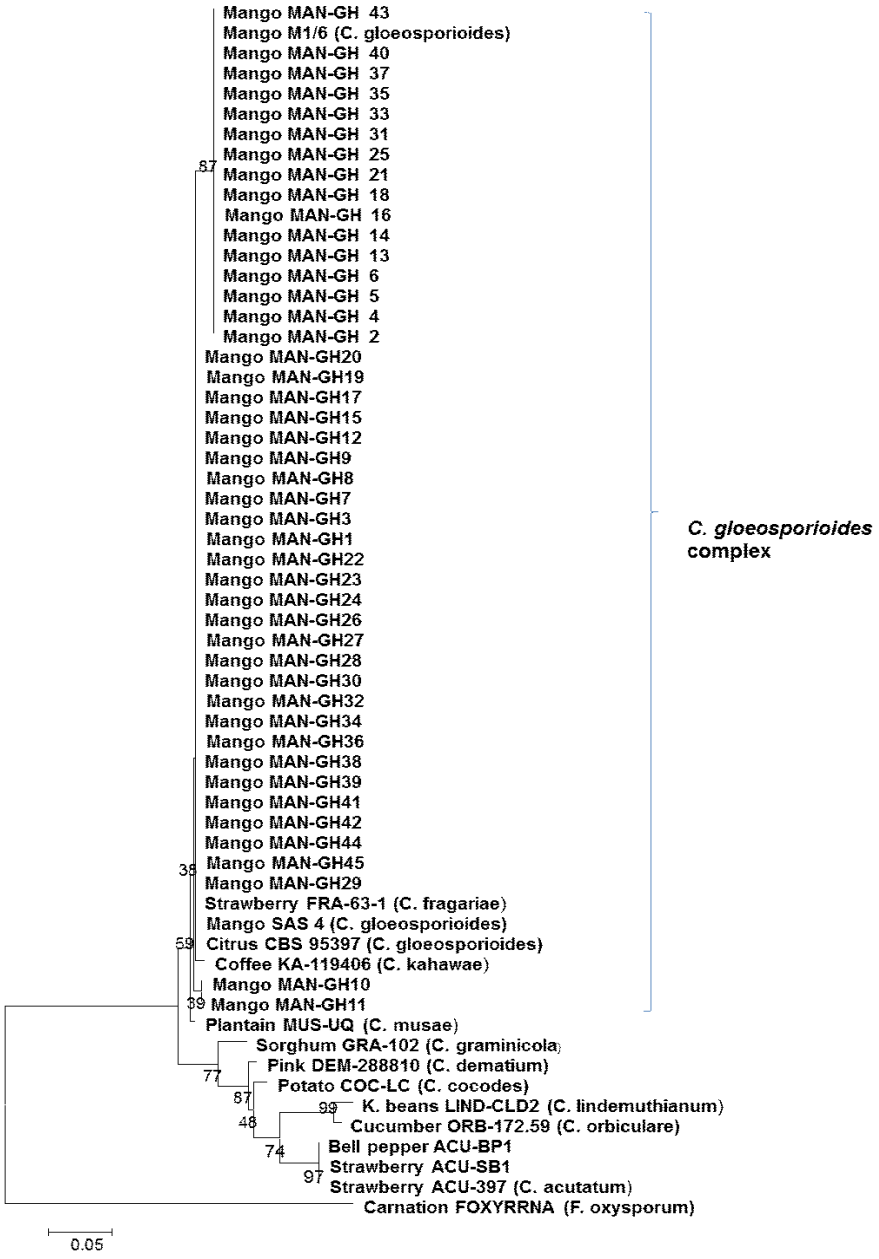

Fig. 2. A phylogram drawn with the multiple sequence alignment generated with the nucleotide sequences of rDNA-ITS1 using neighbour-joining analysis. *F. oxysporum* was used as an out group. Host name, isolate designation and species name have been listed. All isolates with designation beginning with 'MAN' were collected and their ITS regions sequenced. Sequences of all other isolates were downloaded from the EMBL database.

TABLE 3

Pairwise comparison of the entire ITS region of selected Colletotrichum *isolates used in the study*

	MAN-GH10	*MAN-GH12*	*MAN-GH21*	C. gloeo	C. dematium	C. gramini.	C. acutatum.	C. musae	F. oxy
MAN-GH10	100.0	99.3	99.0	98.6	81.9	85.6	82.9	98.1	66.7
MAN-GH12		100	99.1	98.9	82.3	85.6	82.6	97.8	66.7
MAN-GH21			100	98.6	80.9	84.7	82.2	97.6	66.5
C. gloeo sp.				100	80.2	85.6	82.1	98.6	61.5
C. dematium.					100	90.0	86.0	82.5	60.9
C. gramini.						100	88.6	86.6	66.5
C. acutatum							100	97.6	64.9
C. musae								100	66.9
F. oxy									100

F. oxy; Fusarium oxysporum.
C. gloeo sp;-C. gloeosporioides
C. gramini;-C. graminicola

to be useful in distinguishing between isolates of *Colletotrichum* spp. from mango in Ghana and *C. acutatum* from strawberry and bell pepper. The isolates obtained from mango in the study produced acervuli with setae which contained conical spores. These features of the isolates were consistent with the description of *C. gloeosporioides* on mango (Ploetz, 1998; Agrios, 2005).

Biochemical reactions have been used in distinguishing between different species of filamentous fungi (Paterson & Bridge, 1994). The casein hydrolysis test was used to distinguish between *C. gloeosporioides* and *C. acutatum* on coffee and strawberry (Martin & Garcia-figueres, 1999). In the study, the same method was used to distinguish between the isolates from mango identified as *C. gloeosporioides* using the morphological characteristics and the *C. acutatum* reference isolates. This further confirms that the two species were distinct and, hence, the mango isolates were *C. gloeosporioides* and not *C. acutatum.*

Molecular characteristics have been found to be more reliable, and some of the techniques available had been used to complement the morphological and biochemical characterisation of the pathogen (Bailey *et al.*, 1996; Cannon *et al.*, 2000; Freeman & Rodriguez, 1995; Freeman *et al*., 2000). Species-specific primers that were designed based on dissimilarities in the rDNA of putative species have been found to be very reliable in diagnosing diseases caused by either *C. gloeosporioides* or *C. acutatum* (Sreenivasaprad *et al*., 1994.). These markers have been evaluated against other diagnostic methods, and found to be very reliable, and were very robust in distinguishing between isolates of the two species infecting a wide range of plants (Liu *et al*., 2010). On mango, the method was used to complement morphological and physiological methods to correctly differentiate between isolates identified as *C. gloeosporioides* and *C. acu-*

tatum infecting the crop in Brazil (Peres *et al*., 2002). In the study, the use of the species-specific primers in the PCR gave results that were consistent with results obtained by Peres *et al*. (2002), and identified the isolates of the pathogen from only mango as *C. gloeosporioides,* thereby, separating them from the *C. acutatum* isolates from the bell pepper and strawberry.

In general, morphological species recognition (MSR), biological species recognition (BSR) and phylogenetic species recognition (PSR) are the three ways of species recognition. According to Taylor *et al*. (2000), PSR comes closer than the other two techniques for recognising species consistent with the evolutionary species concept. In the study, sequences obtained from different isolates were analysed to complement other methods for the identification of the causal agent of mango anthracnose in Ghana. The analysis showed that the mango isolates clustered more closely with several isolates identified as *C. gloeosporioides* than any other species, indicating that isolates obtained from mango in the study were *C. gloeosporioides*. Among the species that clustered together with the mango isolates were *C. kahawae*, *C. musae* and *C. fragariae*. According to Damm *et al.* (2000), these species, together with several undescribed species, possess similar spore morphology and ITS sequence as *C. gloeosporioides sensu stricto*. They, therefore, qualified to be named also as *C. gloeosporioides*. This justified their clustering with the mango isolates, which were also identified as *C. gloeosporioides*.

Pathogenicity tests using isolates of the pathogen from mango in Ghana resulted in the expression of the same disease symptoms on test crops as were recorded in the field. Subsequently, these isolates were re-isolated from the diseased lesions induced through artificial inoculation. This effectively confirmed the isolates as the causal agent of the disease in Ghana. These findings, therefore, confirm the earlier reports that *C. gloeosporioides* was the pathogen responsible for the disease in Ghana (Oduro, 2000; Offei *et al*., 2008).

The inclusion of molecular methods for the identification of the pathogen causing mango anthracnose in Ghana was very important. This is because the traditional methods of species delineation which primarily relies on morphology, and host speciation (Bailey *et al*., 1996; Cannon *et al*., 2008) may not always be correct (Bailey *et al.*, 1996; Peres *et al*., 2002). The identity of the causal agent of the disease in Ghana has, therefore, been elucidated without any doubt in the study. However, by clustering with other distinct species in the *C. gloeosporioides* complex (Damm *et al*., 2000), it needs to be determined whether the name *C. gloeosporioides* refers to the group species name (also called *C. gloeosporioides sensu lato*) or the distinct species name of the pathogen (also called *C. gloeosporioides sensu stricto*).

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References

ABANG, M. M. (2003) *Genetic diversity of* Colletotrichum gloeosporioides *Penz causing anthracnose disease of yam* (Discorea spp.) *in Nigeria*. Bibliotheca Mycologia.139 pp.

AGRIOS, G. M. (2005) *Plant Pathology* 5th edn. Academic Press, New York. 952 pp.

ARAUZ, L.F. (2000) Mango anthracnose: Economic impact and current options for integrated management. *Plant Diseases* **84** (6), 600 – 611.

BAILEY, J. A., NASH, C., MORGAN, L. W., O'CONNELL, R. J. & TEBEEST, D. O. (1996) Molecular taxonomy of *Colletotrichum* species causing anthracnose of Malvaceae. *Phytopathology* **86**, 1076 –1083.

BERNSTEIN, B., ZEHR, E. I., DEAN, R. A & SHABI, F. (1995) Characterisation of *Colletotrichum* from pear, apple, pecan and other hosts. *Plant Disease* **79**, 478 – 482.

CANNON, P. F., BRIDGE, P. D. & MONTE, E. (2000) Linking the past, present and future of *Colletotrichum* systematics. Colletotrichum: *Host specificity, Pathology and Host-pathogen interaction.* American Phytopathological Society, St Paul.

ANONYMOUS (1996) *Mango, the next cocoa of Ghana.* Graphic communications Group of Ghana, Accra, Ghana.

DAVIS, M. J. (1999) Genetic and pathological diversity of the mango anthracnose pathogen in Florida. *Proceedings, Florida State Horticultural Society.* pp. 112 – 197.

DAMM, U., BARONCELLI, R., CAI, L., KUBO, Y., O'DONNELL, R., WEIR, B., YOSHINO, K. & CANNON, P.F. (2010) *Colletotrichum*: Species, ecology and interactions. *International Mycological Association* **1**(2), 161 – 165.

FAOSTAT (2010).2002-2010.World mango production. *www.novagrim.com.pages/2000-2001_mango statistics-EN. aspx.*

FELSENSTEIN, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783 – 791.

FREEMAN, S. & RODRIGUEZ, R. J. (1995) Differentiation of *Colletotrichum* species responsible for anthracnose of strawberry by arbitrarily primed PCR. *Mycological Research* **99**, 901 – 905.

FREEMAN, S., MINZ, D., JUKERVITCH E., MAYMON, M.T. & SHABI, E. (2000) Molecular analysis of *Colletotrichum* species from almond and other fruits. *Phytopathology* **90**, 608 – 614.

JEDELE, S., HAU, M.A. & VON OPPEN, M. (2003) An analysis of the world market for mangoes and, its importance for developing countries. *Paper presented at the conference on international agricultural research for development. Gottingen, October 8 – 10.*

JEFFRIES, A., DODD, J. C., JEGER, M. J. & PLUMBLEY, R. A. (1990) The Biology and Control of *Colletotrichum* species, on tropical fruit crops. *Plant Pathology* **39**, 343 – 366.

LIU, B., LOUWS, F. J., SUTTON, T. B & CORRELL, J. (2010) A rapid qualitative molecular method for the identification of *Colletotrichum acutatum* and *Colletotrichum gloeosporioides. European Journal of Plant Pathology. DOI10-1007/s10658 – 011.*

MARTIN, M. P. & GARCIA-FIGUERES, F. (1999) *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* causing anthracnose in Olives. *European Journal of Plant Pathology* **105**, 733 – 741.

NEI, M. & KUMAR, S. (2000) *Molecular evolution*

and phylogenetics. Oxford University Press, New York.

Oduro, K. A. (2000) *Checklist of plant pests in Ghana*. *Volume 1. Diseases*. Plant Protection and Regulatory Service Directorate , Ministry of Food and Agriculture. Accra, Ghana. 105 pp.

Offei, S. K., Cornelius, E. W. & Sakyi-Dawson, O. (2008) *Crop diseases in Ghana and their management*. Smartline Publishing Ltd. 104 pp.

Paterson, R. R. M. & Bridge, P. D. (1994) Biochemical techniques for filamentous fungi. *IMI Technical Handbooks. No.1*, CAB International.123 pp.

Ploetz, R. C. (1998) *Anthracnose*. In *Compendium of tropical fruit diseases*. pp. 35 – 36. The American Phytopathological Society, Minnesota.

Phoulivong, S., Cai, L., Chen, H., Mckenzie, E. H. C., Abdulsalam, K. & Chukeatirute, E. K. D. (2010) *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Diversity* **44**, 33 – 43.

Peres, N. A. R., Kuramae, E. E., Dias, M. S. C. & De Souzza, N. L. (2002) Identification andcharacterisation of *Colletotrichum* species affecting fruit after harvest in Brazil. *Journal of Phytopathology* **150**, 128 – 134.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406 – 425.

Sreenivasaprad, S., Mills, P. R., Meehen, R. M. & Brown, A. E. (1996) Phylogeny and systematics of18 *Colletotrichum* species based on ribosomal DNA spacer sequence. *Genome* **39**, 499 – 512.

Taylor, J. W. Jacbson, D., Kroken, S., Kasuga, T., David, M., Geiser, M., David, S., Hibbett, S. & Fissher, M. (2000) Ohylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**, 21 – 32.

Tamura, K., Peterson, D., Peterson, N., Steicher, G., Nei, M. & Kumar, S. (2011) Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* **24**, 1596 – 1599.

Tamura, K., Nei, M. & Kumar, S. (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA) 101:11030-11035.*

Weir, B. S., Johnston, P. R. & Damm, U. (2012) The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* **73**, 115 – 180.

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