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Cultural and morphological variations of *Colletotrichum* spp associated with anthracnose of various fruits in Cameroon

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Abstract— *The anthracnose of fruits due to Colletotrichum spp. is one of the principal fungal diseases which affects the production and marketing of fruits in Cameroon. Isolates of Colletotrichum were collected from various fruits and characterised for cultural and morphological variations. The results show that Colletotrichum colonies varied in the appearance of their culture ranging from fibrous, compact and cottony colonies. The colour of colonies ranged between whitish to greyish, pinkish and greyish green. AVIS1, BAIS2, MAIS1, PAIS1 and PLIS1 had an intermediate growth varying between 13.02 to 13.61 mm/day, whitish to greyish mycelium and fusiform conidia of size ranging between 19.98 x 4.17 and 21.29 x 5.14 µm. BAIS2 had the fastest growth (17.19 mm/day) with a pinkish fibrous mycelium and cylindrical or spindle-shaped conidia of 25.62 x 6.04 µm in size and a sporulation rate of 8.69 x 10^4. These results highlight some variations in morphocultural characteristics of Colletotrichum species, however molecular analyses are still going on for adequate differentiation among those isolatesfrom different fruits.*

Keywords—Anthracnose, Colletotrichum spp., fruits, morphocultural, variations.

I. INTRODUCTION

Anthracnose of fruits caused by the *Colletotrichum* species is one of the most important postharvest diseases of fruits. Symptoms of anthracnose include black and sunken lesions with spore masses or acervuli in the lesion. Infection on fruits usually starts during the development of the fruit but remains quiescent until the fruit ripens; symptoms often manifest during storage and marketing (Prusky and Plumbley 1992). Anthracnose becomes severe when the fruits are wounded by scratches during handling and transportation, making the fruit unmarketable. Two types of symptoms are found on fruits. The commonest is a dark-brown lesion which is slightly sunken with raised rims (Bailey *et al.*, 1992; Agrios, 2005). This can be found on very young fruits or matured fruits in storage or transit. The lesions can enlarge on the fruit surface and eventually penetrate the fruit and infected young fruits usually drop (Nelson, 2008). The black necrotic lesions may or may not be accompanied by bright orange acervuli which are the fruiting bodies of the pathogen (Agrios, 2005). The second type of symptom is commonly referred to as tear strain symptom in which are linear necrotic regions on the fruit that may or may not be associated with superficial cracking of the fruit epidermis causing an alligator skin effect on the fruit surface (Nelson, 2008). According to Dodd *et al.*, (1992) anthracnose causes premature fruit drop and direct reduction in quality of ripe fruits and shortening storage life time.

The disease may develop on fruits belonging to extremely varied families. For a good number of fruits, it represents the principal post-harvest disease of fungal origin as it is the case in mango, plums, pawpaw, banana and avocado (Sangeetha and Rawal, 2009; Hala and Coulibaly, 2006; COLEACP, 2011). Contamination on fruits causes necrosis and in long term, putrefaction (Prusky *et al.*, 2000). This degrades the quality of fruit and post-harvest losses of up to 100% of the production can be recorded (COLEACP, 2008). The pathogen can infect young fruits at fruit initiation (Arauz, 2000; COLEACP, 2008) or on already developed fruits. The most significant damages on the fruits are generally expressed after harvest (Sanders and Korsten, 2003).

Differentiation between *Colletotrichum* species based on host range or host of origin may not be a reliable criterion for fungi of this genus, since taxa such as *C. gloeosporioides*, *C. dematium*, *C. acutatum*, and others infect a broad range of host plants. Some taxa appear to be restricted to host families, genus or species within those families, or even cultivars, whereas others have more extensive host ranges (Freeman *et al.*, 1998). Identification of *Colletotrichum* spp. is therefore a fundamental criterion in the development of more control measures. Traditional identification and characterisation of *Colletotrichum* species has relied primarily on differences in morphological features such as colony colour, size and shape of conidia and appressoria, growth rate, presence or absence of

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setae, and existence of the *Glomerella* teleomorph (Smith and Black, 1990; Gunnell and Gubler, 1992; Sutton, 1992). Studies of these features on *Colletotrichum* species have not yet been conducted in Cameroon. The present preliminary are aimed at investigating on the morphocultural variation of the genus *Colletotrichum* isolated from five postharvest fruits sold in market in Cameroon.

II. MATERIALS AND METHODS

Isolation of *Colletotrichum* species from infected fruits

Pulp fragment from each fruit (avocado, banana, mango, pawpaw and plum) showing typical anthracnose symptom were thoroughly washed in tap water and separately cut into small pieces at about half a centimeter in size, showing half healthy and half diseased tissue, with the help of previously sterilized blade. The pieces were surface sterilized with 5 % sodium hypochlorite solution for 5 minutes, followed by 3 changes with washings with sterilized distilled water. The surface sterilized diseased pieces were then aseptically transferred separately to Petri dishes containing 20 ml Potato Dextrose Agar (PDA) medium amended with Chloramphenicol (1 g/l) to prevent bacterial contamination and then incubated at 24 ± 2° C. After 2 to 3 days of incubation, the growing mycelium was sub-cultured on fresh PDA medium until pure cultures. In this way, the cultures of different isolates were obtained and maintained in a refrigerator at 4° C.

Morphological identification of *Colletotrichum* isolates was carried out based on the cultural characteristics and with the help of identification keys of mycology (Barnet and Hunter, 1972; Cannon *et al.*, 2008; Prihastuti *et al.*, 2009; Phoulivong *et al.*, 2010; Su *et al.*, 2011). A summary of *Colletotrichum* isolates used in this study are listed in Table 1.

Table.1: Origin of Colletotrichum spp isolates used for the study.

Isolate code	Host fruit	Scientific Names of fruit
AVIS1	Avocado	*Persea americana*
AVIS2	Avocado	*Persea americana*
BAIS1	Banana	*Musa sapientum*
BAIS2	Banana	*Musa sapientum*
MAIS1	Mango	*Mangifera indica*
MAIS2	Mango	*Mangifera indica*
PAIS1	Pawpaw	*Carica papaya*
PLIS1	Plum	*Dacryodes edulis*

Cultural characteristics

Mycelial discs (6 mm) of 7 day old culture of *Colletotrichum* isolates were transferred aseptically to the center of PDA plates and incubated at 24 ± 2° C. Culture characteristics such as colony aspect and color were observed and recorded after 7 days of incubation. Colony diameter was measured in two perpendicular directions on the reverse side Petri dishesevery two days after incubation and growth rate was calculated on day 7 followed the formula of Sofi *et al.* (2013).

$$\text{Growth rate} = \frac{\text{Growth observed on a particular day (mm)} - \text{Growth on previous observation (mm)}}{2}$$

This experiment was repeated four times.

Morphological characteristics

For each isolate, a conidial suspension was prepared by carefully brushing 10 days old cultures into 20 ml of sterilized distilled waterin a 90 mm Petri dish and a drop of Tween 20 was added to each plate to homogenise the suspension. Conidial suspensions obtained were filtered through a double layer of cheesecloth to remove leaf debris. Then a drop of conidial suspension from each isolate of *Colletotrichum* from different fruits was mounted and quantified using a haematocymeter. Afterwards, the sizes of conidia were determined by measuring 50 random conidia with a calibrated microscope (Olympus brand) at magnification 400X. The shapes of these conidia were also recorded. The experiment was repeated fourtimes.

Experimental Design and Statistical Analysis

All the experiments were conducted following a completely randomized design (CRD), and data on radial growth, growth rate and sporulation rate were analyzed using an analysis of variance (ANOVA) in SPSS software version 20.0 and mean separated with Duncan's Multiple Range test (DMR) at a 5 % probability level.

III. RESULTS

Cultural characteristics

On the basis culturalcharacteristics, isolates from fruits showed different colony aspect and colourafter 7 days (Fig 1). Colonies

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produced by AVIS1, MAIS1, PAIS1 and PLIS1 collected respectively from avocado, mango, papaw and plums fruits varied from whitish to greyish with cottony aerial mycelium and a few bright orange masses near the inoculum point. BAIS1 and MAIS2 isolates from banana and mango producedgreyish green colonies with compact mycelium. MAIS2 presented sparse white fluffy aerial mycelium. Colonies produced by BAIS2 from banana with pinkish colouration had showed fibrous mycelia. AVIS2 isolates from avocado produced greyish colonies with fibrous mycelia.

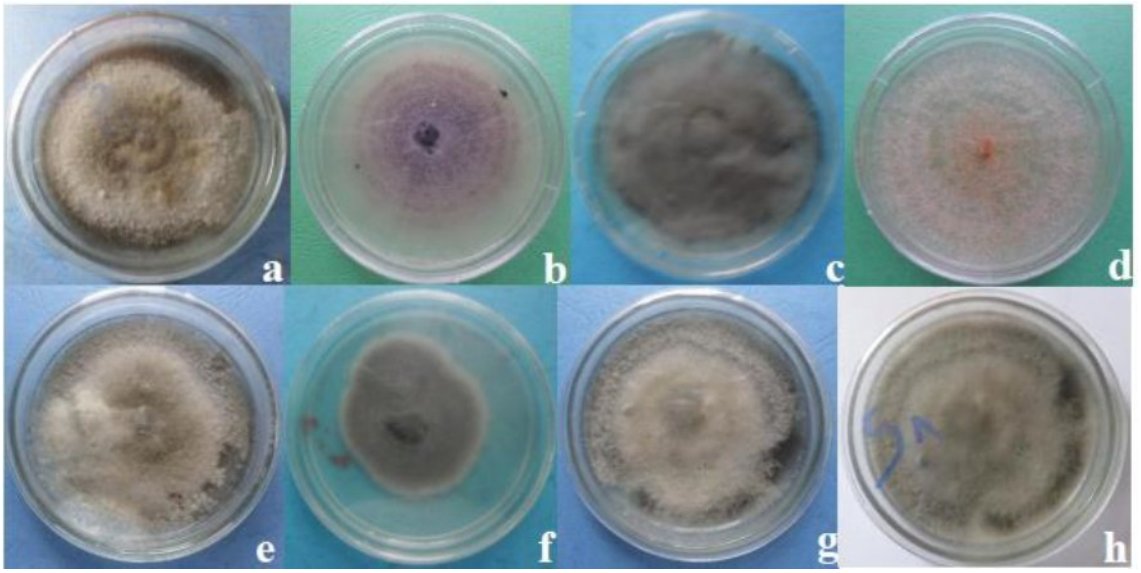

Fig.1: Colonies of Colletotrichum species on PDA. (a) pure culture of AVIS1; (b) pure culture of AVIS2; (c) pure culture of BAIS1; (d) pure culture of BAIS2; (e) pure culture of MAIS1; (f) pure culture of MAIS2; (g) pure culture of PAIS1 and (h) pure culture of PLIS1.

BAIS2 isolate obtained from banana, had a highest radial growth of 79.32 mm, followed by AVIS1, BAIS1, MAIS1, PAIS1 and PLIS1 that had average radial growths ranging from 54.05 to 65.27 mm while AVIS2 and MAIS2 had lowest radial growth of 38.47 to 40.06 mm (Table 2). Also, the rate of growth of isolates ranged from 7.37 to 17.19 mm/day. The rate of growth of isolate BAIS2 (17.19 mm/day) was the fastest, followed by AVIS1, BAIS1, MAIS1, PAIS1 and PLIS1 (13.41 to 13.61 mm/day). The slowest growing culture was isolated MAI2 and AVIS2 (7.37 and 7. 62 mm/day).

Table.2: Cultural characteristics of Colletotrichum isolates from different fruits

Isolates code	Aspect of colony	Colour of colony	Radial growth on the 7th day (mm)	Growth rate day 7 (mm/day)
AVIS1	Cottony	Whitish to greyish	61.54 ± 2.25b	13.02 ± 0.89b
AVIS2	Fibrous	Greyish	38.47 ± 3.15c	7.62 ± 0.74c
BAIS1	Compact	Greyish green	63.81 ± 2.57b	13.61 ± 0.72b
BAIS2	Fibrous	Pinkish	79.32 ± 1.70a	17.19 ± 0.54a
MAIS1	Cottony	Whitish to greyish	60.44 ± 4.63b	13.47 ± 0.89b
MAIS2	Compact	Greyish green	40.06 ± 4.17c	7.37 ± 0.72c
PAIS1	Cottony	Whitish to greyish	65.27 ± 3.84b	13.41 ± 0.77b
PLIS1	Cottony	Whitish to greyish	54.05 ± 3.51b	13.14 ± 0.60b

**Means in columns followed by the same letter are not significantly different by Duncan's Multiple Range test at a 5% probability level.*

Morphological characteristics

Conidia produced by isolates AVIS1, BAIS1, MAIS1, PAIS1 and PLIS1 varied from fusiform with obtuse to slightly rounded ends to sometimes oblong. BAIS2 produced cylindrical conidia with obtuse to slightly rounded ends. AVIS2 and MAIS2 produced fusiform conidia with obtuse ends (oblong) with narrowing at the centre (Fig 2)

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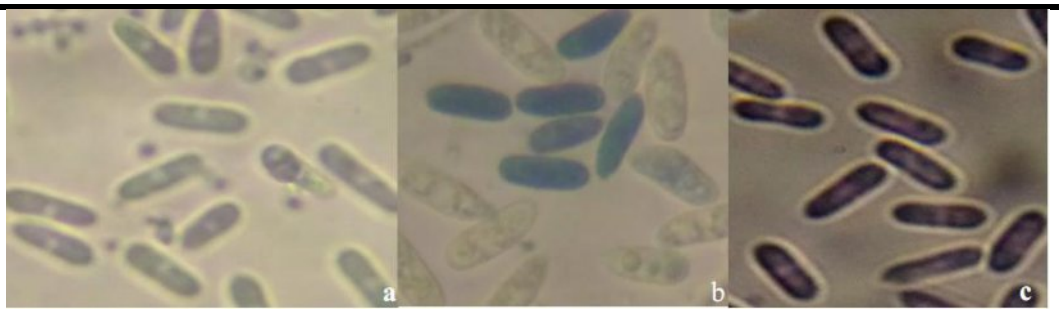

Fig.2: Conidia morphology of Colletotricum spp. isolated from various fruits. (a) AVIS1, BAIS1, MAIS1, PAIS1 and PLIS1; (b) BAIS2; (c) AVIS2 and MAIS2.

Conidia produced by BAIS2 had biggest sizes (25.62 x 6.04 µm) and had the most abundant sporulation rate on the 10th days (13.19 x 10^6 conidia/ml). AVIS1, BAIS1, MAIS1, PAIS1 and PLIS1 produced conidia which and measured 19.98 – 21.29 x 4.17 – 5.14 µmwith sporulation rates ranging from 7.7 x 10^6 to 9.42 x 10^6 conidia/ml.AVIS2 and MAIS2 produced have cylindrical conidia of smaller sizes which vary from 15.72 x 3.14 to 16.43 x 3.52 µm as well as sporulation rates which varied from 7.21 x 10^6 to 7.26 x 10^6 conidia/ml (Table 3).

Table.3: Conidia shape, size and sporulation rate of Colletotrichum isolated from different fruits.

Isolate	Conidial shape	Size of conidia (μm)	Sporulation rate on day 10 (x 10^6 conidia/ml)
AVIS1	Fusiform	20.71 x 4.65	9.42 ± 0.43b
BAIS1	Fusiform	21.29 x 5.14	8.27 ± 0.62b
MAIS1	Fusiform	19.91 x 4.17	7.50 ± 0.61bc
PAIS1	Fusiform	19.98 x 4.89	7.81 ± 0.97bc
PLIS1	Fusiform	20.43 x 4.77	7.70 ± 0.80bc
BAIS2	Cylindrical	25.62 x 6.04	13.19 ± 0.47a
AVIS2	Fusiform	15.72 x 3.14	7.21 ± 0.74c
MAIS2	Fusiform	16.43 x 3.52	7.26 ± 0.94c

**Means in columns followed by the same letter are not significantly different by Duncan's Multiple Range test 5% at a probability level.*

Dendrogram resulting from the hierarchical cluster analysis of mycelial growth rate, diameter of colonies and size of conidia of *Colletotrichum* spp isolates from various fruits showed three differents groups; Group I (MAIS1, PAIS1, PLIS1, BAIS1 and AVIS1), group II (AVIS2 et MAIS2) and group III (BAIS2) (Fig 3).

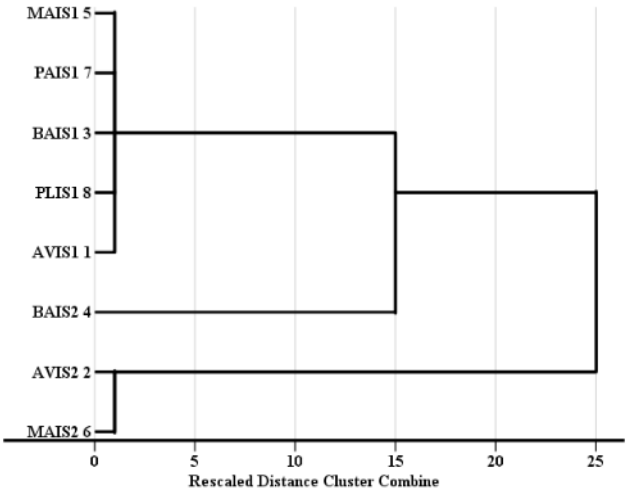

Fig.3: Dendrogram resulting from the hierarchical cluster analysis showing the groups formed according to the variables; mycelial growth rate, diameter of colonies and size of conidia after seven days cultivation of Colletotrichum spp from various fruits.

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IV. DISCUSSION

The results of the study indicate that *Colletotrichum* species isolated from fruits of avocado, banana, mango, pawpaw and plums show some variations in cultural and morphological characters. Based on these characteristics, *Colletotrichum* isolates were ranked into three groups; the first group made up of AVIS1, MAIS1, BAIS1, PAIS1 and PLIS1 isolates with whitish to greyish colonies and fusiform conidia, the 2nd group made up AVIS2 and MAIS2 with respectively fibrous and compact greyish green mycelia colonies and cylindrical conidia and the 3rd group (BAIS2) with pinkish mycelia and cylindrical conidia. The difference in coloration and the aspect of the isolates would be related to the fruit host, the nature of the *Colletotrichum* isolate and the environmental conditions. Identification of *Colletotrichum* species was reported to be mostly based on morphological and cultural criteria, coupled with knowledge of the host origin of the pathogen. However, many isolates of *Colletotrichum* show extensive variation in culture (Sutton, 1992; Bailey *et al.*, 1995). Several cultural and morphological types have been observed on *Colletotrichum* species isolated from fruits of mango (Sanders and Korsten, 2003; N'Guettia *et al.*, 2013), bananas (Cannon *et al.*, 2008; Prihastuti *et al.*, 2009; Su *et al.*, 2011), pawpaw (Rampersad, 2011) and avocados (Keuete, 2014).

The mycelial growth differentiated three groups of isolates 7 days after culture. The 1st group (BAIS2) recorded a fast growth (17.19 mm/day), 2nd group (AVIS1, BAIS1, MAIS1, PAIS1 and PLIS1) recorded an average growth of 13.02 to 13.62 mm/day and the 3rd group (AVIS2 and MAIS2) recorded a slow growth (7.37 to 7.62 mm/day). The mycelial growth has been reported to be a criterion that helps to differentiate the species of *Colletotrichum* (Waller *et al.*, 1993; Crouch *et al.*, 2009; Liu *et al.*, 2012). However, according to Serra *et al.* (2006).Although it is not a stable criterion of differentiation of *Colletotrichum* species, but it plays a significant role in variability within the species. Bailey *et al.* (1995) argued that many isolates of *Colletotrichum* often show extensive variation in culture and furthermore, the culture conditions , including the media,the age of culture and the temperatures used, cannot be standard betweenlaboratories (Sutton 1992).

Conidial morphology has always been emphasized over other taxonomic criteria in taxonomic investigations of the genus *Colletotrichum.* Baxter et *al.* (1983) used conidial shape and size as the main criteria to distinguish a number of species. According to Sutton (1992) and Bailey *et al.* (1995), identification of *Colletotrichum* species had been mostly based on these criteria, coupled with knowledge of the host origin of the pathogen, however, extensive variation of many isolates of *Colletotrichum* had been shown in culture.

On the basis of conidia size and shape, *Colletotrichum* isolates were divided into three groups. The first also producing fusiform shapes conidia whose sizes varied from 19.98 x 4.17 to 21.29 x 5.14 µm. These values fall within the interval described by Rivera *et al.* (2006) for conidia of *C. gloeosporioides. C. gloeosporioides* had been considered to be a group species or species complex found on a wide variety of fruits, such as apple, avocado, citrus, papaya, peach, mango and strawberry (Freeman 2000). The variation may also be attributed to the adaptation of the species to a non-specific, broad host range (Freeman *et al.* 1998). For *Colletotrichum* species, it is common for single hosts to become infected by a single species or for multiple hosts to be infected by a single species of the pathogen (Freeman 2000). Infection of multiple hosts by *C. musae* has been reported by Su *et al.* (2011).

The 2nd group consistingof AVIS2 and MAIS2 isolates was characterised by fusiform conidia with the sizes lying between 15.72 x 3.14 and 16.43 x 3.52 µm.Freeman (2002), Than *et al.* (2008), Peres *et al.* (2008) and Damm *et al.* (2012) observed similar shape and size of conidia on *C. acutatum*. The 3rd group made up of BAIS2 isolates had spindle-shaped conidia with the sizes of 25.62 x 6.04 µm. The grouping of *Colletotrichum* isolates into three subclades suggest that the isolates may represent a sub-population of the pathogens with distinct genetic characters. Similar results were reported by Prihastuti *et al.* (2009) and Waller *et al.* (1993) in which *C. gloeosporioides* on coffee berries showed several distinct genetic and phenotypic species. However, it is not yet known, whether these isolates are distinct or not.

V. CONCLUSION

The study highlights some variation in morphocultural characterisrics of *Colletotrichum* species from various fruits, but sequence analysis are still to be carried on to confirm the existence of more than one distinct isolates since *Colletotrichum* has wide host range.

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