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Molecular Characterization of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) Causing Mango Anthracnose Disease

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mango production in Tamil Nadu is known to be adversely affected by *C. gloeosporioides* in both field and post harvest stage of crop and drastically reduces market and edible value of fruit. To manage the disease it is essential to learn about the nature of pathogen and its way of interaction with host and its survival ability in environment. Hence a study has been carried out on Isolation of fungal pathogen was done using PDA medium in order to identify the disease's causative agent and its variation in morphology and colony characters. A total of twenty-four *C. gloeosporioides* isolates were obtained from various Tamil Nadu mango cultiavting areas of Tamil Nadu. The isolates were identified morphologically and molecularly. The microscopic features such as conidia shape and size were used to identify *C. gloeosporioides*. The CTAB technique was employed to extract the DNA from the isolates of *C. gloeosporioides*. A total of 24 isolates were subjected to ITS

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(Inter Transcribed Spacer) of 18s ribosomal RNA amplification; all of these isolates exhibited amplified products with a size range of 560 bp, indicating that they were fungal pathogen. The MkCgF and MkCgR PCR primer combination was unique to the ITS region of *C. gloeosporioides* species. It has been confirmed that all 24 of the *C. gloeosporioides* isolates are really C. gloeosporioides by amplifying the predicted size of 380 bp.

Keywords: Mango; C. gloeosporioides; ITS; MkCgF/MkCgR.

1. INTRODUCTION

Mango anthracnose is a worldwide disease that is extremely destructive to mangoes before and after harvest. The economic and scientific importance of post-harvest damage from mango anthracnose disease is that it lowers fruit quality and shelf life, thereby influencing export quality standards [1]. The *Colletotrichum* species complex is responsible for the fungal disease known as MAD. Weir et al. [2] used morphological and molecular techniques to determine that roughly twenty-two species and one subspecies make up the *C. gloeosporioides* complex. However, several *C. gloeosporioides* isolates in various locations worldwide are characterized by characteristic black, expanding lesions on mango plant parts, including fruits, leaves, flowers, petioles, twigs, and stems [3].

The most serious fungal disease limiting the production and export of mango fruits worldwide is mango anthracnose disease. The causal organism of mango anthracnose disease, C. gloeosporioides, is one of the most economically significant agents that might impact the way mango fruits are sorted, packaged, shipped, stored, and sold [4]. Mango losses due to anthracnose disease range from 30% to 60% annually, with 100% destruction possible under the right circumstances [5].

Oval or irregular dark to deep brown sunken point-sized flecks of varied sizes are the disease's symptoms, which are prominent on young leaves but can also appear on older leaves. These spots are distributed throughout the leaf surface. These dots grow into bigger lesions with an irregular or circular form and a crimson halo surrounding them as the leaf ages [6]. Although it can happen at any point in the fruit's life cycle, mango anthracnose disease is most frequently found on immature fruits and during transportation and storage. The infection on bigger fruits could remain latent or inactive until the fruit ripens, at which point black, deep necrotic lesions emerge on the fruit peel and quickly enlarge. The younger fruits are either aborted or mummified [7]. Certain morphological characteristics, including mycelial growth, conidia size, colony color, texture, and the presence or absence of setae, have historically been used to diagnose mango anthracnose disease [8]

Molecular techniques are sensitive, fast, precise, and accurate in identifying the disease's causative organisms, which aids in the comprehension of the mechanisms behind disease progression as well as management [9]. According to Cannon et al. [10], the ITS gene sequence is useful for identifying *Colletotrichum* spp., but it is unable to differentiate between species that are closely related.

Unfortunately, species categorization sometimes requires time-consuming and complex identification due to character overlap. Adopting efficient agricultural techniques as soon as feasible requires accurate identification. Since ITS has limitations, researchers have also looked at the differences between *Colletotrichum* species using additional genes, including actin, β-tubulin, chitin synthase [11] glyceraldehyde-3-phosphate dehydrogenase, calmodulin, and glutamine synthetase [12]. various approaches have to be developed in order to recognize and distinguish between various fungus species. Plant samples that contain *Collectrotrichum* spp. may be effectively detected using species-specific molecular primers [13-15]. The PCR approach offers a quick, easy, and dependable substitute for traditional techniques for identifying common fungal isolates. The use of molecular approaches to identify *C. gloeosporioides* specific ITS region is the study's goal.

2. MATERIALS AND METHODS

2.1 Survey and Assessment

During 2019 to 2021, a market survey was conducted across several regions in Tamil Nadu to determine the geographical distribution and severity of the anthracnose disease in mango (Fig.1). Mango fruits exhibiting brown to black sunken patches have been assessed according

to standard grading by Prabakar et al. [16] to determine the Percent Disease Index.

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of all rating } \times 100}{\text{Total number of fruits observed } \times \text{ maximum grade used}}$$

2.2 Isolation and Identification of *Colletotrichum gloeosporioides*

Twenty-four *C. gloeosporioides* isolates were obtained from fruits infected with anthracnose. Small tissue sections were separated from both healthy and diseased fruit tissue. The tissue pieces are surface sterilized with 70% ethanol, then exposed to 1% sodium hypochlorite for 60 seconds. The tissue pieces are allowed to air dry while being kept in a laminar hood made of sterilized tissue paper. The sterile tissue fragments were placed on plates that were filled with Potato Dextrose Agar medium to facilitate the development of mycelium. The tips of the mycelia hyphae were then transferred, together with an agar plug, to Petri plates that were filled with PDA and left to incubate. Twelve days following inoculation, the plates are covered by *C. gloeosporioides* mycelium [17].

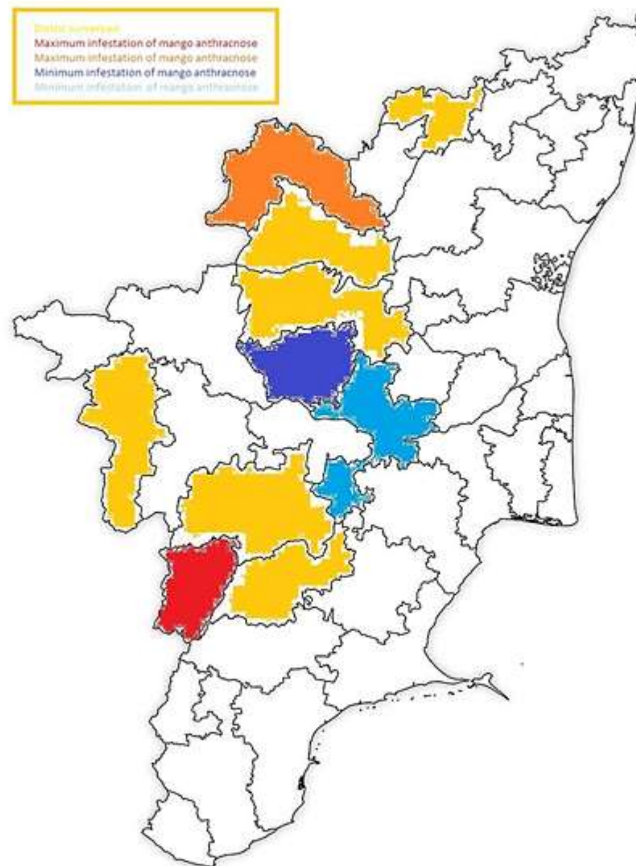

Fig. 1. Geographical representation on distribution of Mango Anthracnose disease in various districts of Tamil Nadu

List 1. Description of Grade and category value

Description of Grade	Category value
No lesion	0
Small restricted lesions covering 1 to 25 % of fruit surface	1
Moderate Lesions covering 25.1 to 50 % of fruit surface	2
Coalescence of small lesions covering 50.1 to 75 % of fruit surface	3
Lesions covering more than 75 %	4

List 2. Molecular characterization

ITS 1	5′-TCCGTAGGTGAACCTGCGG-3′	White *et al.,* 1990
ITS 4	5’-TCCTCCGCTTATTGATATGC-3’	White *et al.,* 1990
MKCgF	5’-TTGCTTCGGCGGGTAGGGTC-3’	Kamle *et al*., 2013
MkCgR	5’- ACGCAAAGGAGGCTCCGGGA-3’	Kamle *et al*., 2013

Morphological characters of the isolates: Twenty-four isolates of *C. gloeosporioides* were grown individually on PDA medium for ten days in order to study the variation in cultural and morphological characters. Three replications were maintained for each isolate. Various cultural characteristics such as colour, diameter, zonation, pigmentation, sporulation and margin of colony on PDA medium were observed. The shape of the conidia, length and width of 10 spores were measured for each isolate using a Phase contrast microscope under 40 X magnifications.

Molecular characterization of *C. gloeosporioides* isolates: Genomic DNA was extracted from the mycelial mat of *C. gloeosporioides* isolates by Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Knapp and Chandlee [18].

3. RESULTS

3.1 Market Survey

A Market survey was conducted to assess the status of anthracnose disease incidence in major markets 10 districts of Tamil Nadu (Table 1.). The mean level of disease incidence was calculated based on the occurrence of anthracnose disease on locally available varieties of Alphonso, Banglora, Banganapalli, Mulgoa, Neelum, Nadusalai, Imampasand and Senthuram. From the survey it is inferred that maximum mean level of anthracnose disease incidence was recorded in a Theni district (65.5 PDI) followed by Krishnagiri district (62.08 PDI). Lowest mean level of disease incidence was recorded in Namakkal district (16.2) followed by Trichy district (20.8) (Fig.1.).

Morphological diversity of *C. gloeosporioides*: Twenty-four strains of *C. gloeosporioides* were collected from mango fruits of various kinds infected with anthracnose. A comparison was made between the morphological characteristics and colony development rate of twenty-four isolates in order to determine the differences in pathogenic fungi that cause anthracnose disease (Table 2, Fig. 2). Regardless of the variety from which they were acquired, distinctness in those features led to the formation of ten morphological groups, and those groups were connected with isolates of *C. gloeosporioides.* (Table 3, Fig. 3).

Fig. 2. Cultural characteristics of *Colletotrichum gloeosporioides* in PDA medium

Table 1. Distribution of mango anthracnose in different regions of Tamil Nadu

Place/ Variety	Neelum	Bangalora	Banganapalli	Imampasand	Nadusalai	Sendhuram	Alphonsa	Malkova
Salem	54.5^{d} (47.58)	25.2^{c} (30.13)	40.2^{ab} (39.342)	-^{d}	25.6^{c} (30.36)	23.6 ^{e} (29.06)	25.9^{e} (30.59)	22.1^{e} (28.04)
Coimbatore	45.2^{f} (42.44)	29.6^{b} (32.95)	35.3^{c} (36.45)	25.5^{c} (30.32)	22.1^{de} (28.04)	21.8^{e} (27.8)	33.8^{c} (35.54)	33.5^{bc} (35.35)
Namakkal	48.2^{ef} (43.96)	16.2^{e} (23.72)	33.3^{cd} (35.23)	-^{d}	21.9^{ef} (27.89)	^{f}	-^{g}	31.5^{c} (34.13)
Trichy	49.2^{e} (44.54)	20.8^{d} (27.12)	30^{e} (33.20)	-^{d}	19.6^{g} (26.27)	^{f}	-^{g}	33.3^{bc} (35.23)
Dindigul	56.1^{cd} (48.50)	25.9^{c} (30.59)	25.7^{f} (30.46)	-^{d}	23.8^{cd} (29.19)	^{f}	29.9^{d} (33.14)	35.6^{b} (36.62)
Madurai	59.5^{bc} (50.49)	30.5^{b} (33.51)	21.6^{g} (27.68)	-^{d}	33.3^{b} (35.24)	35.5^{c} (36.56)	22.5^{f} (28.31)	32.15^{c} (34.53)
Theni	65.5^{a} (54.04)	38.4^{a} (38.28)	42.1^{a} (40.45)	-^{d}	36.5^{a} (37.16)	41.2^{a} (39.92)	42^{a} (40.83)	46.52^{a} (43.00)
Dharmapuri	49.7^{e} (44.82)	31.5^{b} (34.13)	32d^{e} (39.34)	33.6^{b} (35.42)	31.8^{b} (34.32)	38.5^{b} (38.3)	36.5^{b} (37.16)	32.58^{c} (34.8)
Vellore	41.5^{g} (40.10)	25.2^{c} (30.13)	21.05^{g} (36.44)	-^{d}	20^{fg} (26.55)	33.1^{d} (35.11)	-^{f}	27.3^{d} (31.49)
Krishnagiri	62.8^{ab} (52.41)	39.6^{a} (38.98)	39.5^{b} (35.24)	41.2^{a} (39.92)	35.9^{a} (36.8)	40.5^{a} (39.52)	40.9^{a} (39.11)	48.5^{a} (44.13)

Mean of three replicaton

In a a column, means followed by a common letter are not significantly different at 5% level by DMRT

Values in paranthesis are arc sin transformed values

Table 2. Colony characters of *Colletotrichum gloeosporioides* isolates

Isolates	Colony colour	Texture	Pigmentation	Colony Diameter	Sporulation
Cgman1	Greyish White	Fluffy	—	8.2(16.63)^{b}	+
Cgman2	Greyish White	Fluffy	—	8.8(17.25)^{a}	+
Cgman3	Grey	Sparse	—	8(16.43)^{bc}	++
Cgman4	Grey	Fluffy	—	7.4(15.78)^{cdef}	+
Cgman5	White	Fluffy	—	8.9(17.3)^{a}	+
Cgman6	Salmon	Sparse	—	6.9(15.23)^{fgh}	++
Cgman7	Salmon	Sparse	—	6.8(15.11)^{fgh}	++
Cgman8	Brownish Black	Scanty	Black	6.3(14.53)^{hi}	+++
Cgman9	Brownish Black	Scanty	Black	5.9(14.05)^{i}	+++
Cgman10	White	Fluffy	Grey	9(17.46)^{a}	+
Cgman11	Salmon	Sparse	—	7.3(15.67)^{def}	++
Cgman12	Greyish Black	Scanty	Black	6.5(14.76)^{ghi}	+++
Cgman13	Salmon Grey	Fluffy	Black	8.9(17.35)^{a}	+
Cgman14	Greyish Brown	Scanty	Black	6.4(14.64)^{hi}	+++
Cgman15	Greyish White	Sparse	—	8.9(17.35)^{a}	++
Cgman16	Salmon	Sparse	—	7.4(15.78)^{cdef}	++
Cgman17	Black	Dense	Black	7.1(15.44)^{efg}	+
Cgman18	Grey	Fluffy	Black	7.8(16.21)^{bcd}	+
Cgman19	White	Fluffy	—	8.9(17.35)^{a}	+
Cgman20	Grey	Fluffy	—	7.8(16.21)^{bcd}	+
Cgman21	Greyish Brown	Scanty	Black	6.9(15.22)^{fgh}	+++
Cgman22	White	Dense	—	8.2(16.63)^{b}	+
Cgman23	Salmon Grey	Scanty	Black	5.9(14.05)^{i}	+++
Cgman 24	Black	Dense	Black	7.7(16.11)^{bcde}	+

Mean of three replicaton
In a a column, means followed by a common letter are not significantly different at 5% level by DMRT
SEd=0.2894 CD=0.7

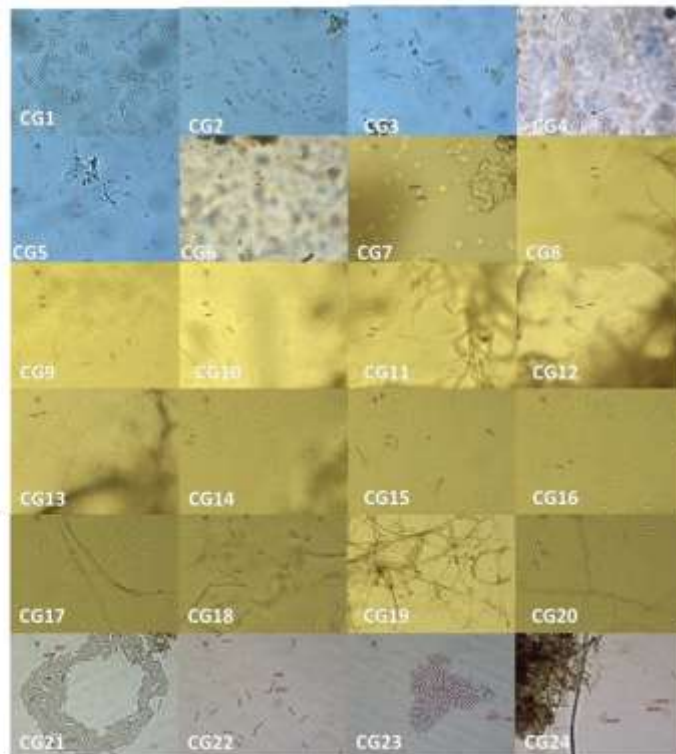

Fig. 3. Morphology characters of *Colletotrichum gloeosporioides* isolates

Table 3. Morphology characters of *Colletotrichum gloeosporioides* isolates

Isolates	Mycelium	Colour	Conida	Length	Width	Oil globules	Acervuli	Setae	Colour
Cgman1	Septate	Hyaline	Cylindrical	14.8^{ghihk} (22.62)	3.6d (10.93)	Present	Present	Absent	Hyaline
Cgman2	Septate	Hyaline	Cylindrical	16.2^{def} (23.73)	5.5a (13.56)	Present	Present	Absent	Hyaline
Cgman3	Septate	Hyaline	Cylindrical	13^{m} (21.13)	2.9e (9.8)	Present	Present	Absent	Hyaline
Cgman4	Septate	Hyaline	Cylindrical	13.8^{jklm} (21.80)	4.4c (12.10)	Present	Present	Absent	Hyaline
Cgman5	Septate	Hyaline	Cylindrical	19.8^{a} (26.42)	5.5a (13.55)	Present	Present	Absent	Hyaline
Cgman6	Septate	Hyaline	Cylindrical	13.7^{klm} (21.71)	4.5c (12.24)	Present	Present	Absent	Hyaline
Cgman7	Septate	Hyaline	Cylindrical	17.8^{bc} (24.94)	2.8e (9.633)	Present	Present	Absent	Hyaline
Cgman8	Septate	Hyaline	Cylindrical	$13.8j^{klm}$ (21.8)	4.4c (12.10)	Present	Present	Absent	Hyaline
Cgman9	Septate	Hyaline	Cylindrical	14.6^{hijkl} (22.45)	4.5c (12.24)	Present	Present	Absent	Hyaline
Cgman10	Septate	Hyaline	Cylindrical	$13.4l^{m}$ (21.46)	3.6d (10.93)	Present	Present	Absent	Hyaline
Cgman11	Septate	Hyaline	Cylindrical	16.0^{efg} (23.57)	4.4d (12.10)	Present	Present	Absent	Hyaline
Cgman12	Septate	Hyaline	Cylindrical	12.8^{m} (20.96)	4.5c (12.24)	Present	Present	Absent	Hyaline
Cgman13	Septate	Hyaline	Cylindrical	15.2^{fghi} (22.94)	5.6a (13.68)	Present	Present	Absent	Hyaline
Cgman14	Septate	Hyaline	Cylindrical	15.7^{efgh} (23.34)	4.4d (12.10)	Present	Present	Absent	Hyaline
Cgman15	Septate	Hyaline	Cylindrical	15.0^{fghij} (22.78)	4.7bc (12.51)	Present	Present	Absent	Hyaline
Cgman16	Septate	Hyaline	Cylindrical	18.1^{b} (25.17)	4.6bc (12.38)	Present	Present	Absent	Hyaline

Isolates	Mycelium	Colour	Conida	Length	Width	Oil globules	Acervuli	Setae	Colour
Cgman17	Septate	Hyaline	Cylindrical	16.7[cde] (24.11)	3.0ab (9.97)	Present	Present	Absent	Hyaline
Cgman18	Septate	Hyaline	Cylindrical	17.7[bc] (24.87)	3.6d (10.93)	Present	Present	Absent	Hyaline
Cgman19	Septate	Hyaline	Cylindrical	15.6[efghi] (23.25)	4.3c (11.96)	Present	Present	Absent	Hyaline
Cgman20	Septate	Hyaline	Cylindrical	15.2[fghi] (22.93)	3.3de (10.46)	Present	Present	Absent	Hyaline
Cgman21	Septate	Hyaline	Cylindrical	19.6[a] (26.27)	4.4c (12.10)	Present	Present	Absent	Hyaline
Cgman22	Septate	Hyaline	Cylindrical	16.6[cde] (24.03)	4.6bc (12.38)	Present	Present	Absent	Hyaline
Cgman23	Septate	Hyaline	Cylindrical	17.3[bcd] (24.56)	5.1ab (13.0)	Present	Present	Absent	Hyaline
Cgman24	Septate	Hyaline	Cylindrical	14.4[cde] (22.295)	4.5c (12.24)	Present	Present	Absent	Hyaline

Mean of three replicaton

In a a column, means followed by a common letter are not significantly different at 5% level by DMRT

SEd=0.2669 CD=0.7

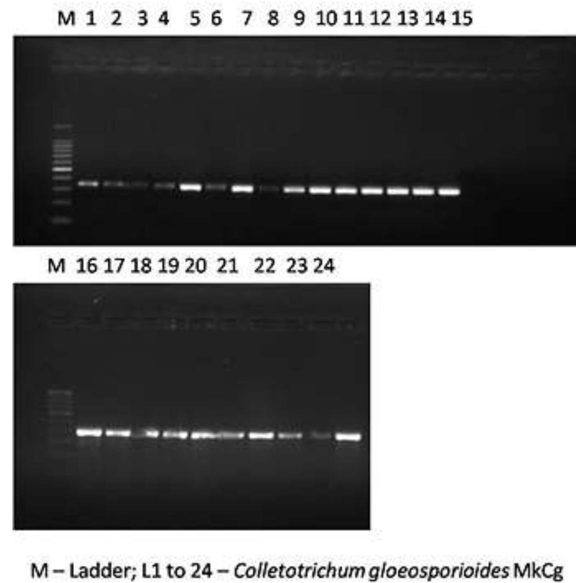

Fig. 4. Gel Electrophoresis result of Species – specific primer MKCgF and MkCgR

~ 380bp Amplicaons was viewed in UV illumination facilitated Gel Analyzer cum documentation unit

3.2 DNA Isolation and Molecular Characterization

Although the 24 isolates were morphologically identified as *Colletotrichum gloeosporioides* to ensure the accuracy it is further subjected to molecular characterization through universal and species-specific oligomers. The agarose gel analysis with ITS 1 and ITS 4 and *C.gloeosporioides* Species – specific ITS primer resulted as follows, all the isolates produce amplicons at 560 bp and to confirm the species it is again involved in PCR with *C.gloeosporioides* ITS species-specific oligomers and it yield amplicons at 380 bp. Since all the isolates are confirmed as *Colletotrichum gloeosporioides* in order to substantiate the confirmation, it is sequenced and analyzed in NCBI Blast Tool in which it shows the isolates has 95% similarity identity with *Colletotrichum gloeosporioides* NCBI database with an accession number ON799265 and OR685106.

4. DISCUSSION

Post-harvest losses of mango might be due to various factors, in that fruit losses are severe due to fungal diseases, especially the anthracnose which contributed a considerable concern. Dofuor et al., [19] reported that postharvest losses could go up to 100% depending on cultivars, locations, cultural practices and environment. Thus, complete survey is needed to assess the post-harvest losses in fruits. In the present study, a systematic market survey was conducted in ten different places of Tamil Nadu to record the severity of anthracnose disease of mango from the locally available varieties. From the results, the disease incidence was more in Theni, accounting the mean Per cent disease index of 65.5 , followed by Krishnagiri (62. 8 %). Considering the varieties, Neelum variety possesses higher disease index of (65.5%) followed by Malkowa (48 %) and lowest severity was recorded in Nadusalai (20.5) in vellore district and followed by banganapalli in Madurai distrct (21.65 %).

The results are consistent with Maymon et al. [20] findings, which showed that *Lasiodiplodia theobromae* was 1% common for anthracnose and stem end rot diseases caused by *C. gloeosporioides* among the five different domestic mango markets, while *Alternaria alternata* and *Aspergillus niger* were 80% common in Punjab. According to Sutton et al. [21], morphological analysis of these *C. gloeosporioides* isolates showed a significant degree of variation. Diverse PDA medium variances were noted among the 10 groups that emerged from this investigation, according to the colony color Grey white, black, brownish black, grey, greysh black, grayish brown, grayish white, salmon, salmon grey, white. Across 24 isolates, 4 types of texture regular fluffy (9 isolates), dense (3 isolates), scanty (6 isolates) and sparse

(6 isolates) were observed, regardless of the color of the colony with the pigmentation black and grey. The majority of these isolates produced acervuli that were black in color and sporulated at varying speeds. When cultivated on PDA, all isolates maintained the same basic growth pattern and colony type. However, there was some variation in the colony development, color, mycelium pigmentation, conidia size, and sporulation rate among the groups that were characterized. All twenty-four isolates had hyaline conidia with oil globules, ranging in size from µm in length and 2.8 to 5.50 µm in width. Previous research on *Colletotrichum's* taxonomy of most species was conducted using the differences in conidial size, shape, appressoria, and colony characteristics.

The dynamics of disease and, by implication, the effectiveness of disease management techniques, such as the creation of disease-resistant cultivars, are significantly influenced by the diversity of pathogens. Based on mycelial growth, these 24 *C. gloeosporioides* isolates exhibit variability as well.

The fungus *C. gloeosporioides* is known to be highly variable in nature. Many morphological traits of the genus *Colletotrichum* are extremely plastic and variable and depend mostly on cultural and environmental conditions, which are rarely standardized [22]. Molecular systematics has been successfully used in studies of this problematic genus and has resulted in well-defined delineations of species [10]. The traditional method of relying on cultural and morphological grounds for identification is not dependable as the identification based on morphology and cultural grounds in many cases misleads since it is subjective in nature. The existence of various pathotypes in this fungus further makes it complicated for identification. Molecular genetic studies have provided useful data for clarifying the systematics of the genus *Colletotrichum* [23]. The polymerase chain reaction [24] offers the opportunity to characterize fungal symbionts by amplification of specific sequences and can provide very accurate quantitative data required for control and quarantine decisions. Hence, the present study was resorted into exploitation of ITS and Mkcg derived primers for identification. The internal transcribed spacer (ITS) region contains two variable non coding regions that are nested within the rDNA repeat between the highly conserved small subunit, 5.8S and large subunit rRNA genes [25]. The internal transcribed spacer regions (ITS1 and ITS4) within the nuclear ribosomal gene clusters are particularly attractive loci of PCR-based detection assays since they are readily accessible using universal primers. In fungi, the entire ITS region is often relies between 600 and 800 bp and can be amplified with universal primers that are complementary to sequences within the rRNA genes [26]. In the present study, ITS 1 and ITS 4 primers amplified a fragment of 560 bp corresponding to the region of 18S rDNA sequence in twenty four isolates of *Colletotrichum* and the result confirmed that all the twenty four isolates pertain to the group of *Colletotrichum.* The results confirmed the findings of Kamle et al. [5] where in they reported amplification of ITS region of *C. gloeosporioides* from mango yielded 560 bp amplicon. Similarly, many workers confirmed the pathogen by amplifying ITS region of *C. gloeosporioides* [27,28,29]. The findings of present study are supported by the statement by Sherriff et al. [30] whereby the ITS region should provide opportunities for more rapid progress in taxonomic studies of the genus *Colletotrichum*. The ITS regions of nuclear rDNA have been good targets for the identification, differentiation and phylogenetic analysis of fungi using molecular techniques [31]. 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 [32]. The development of species-specific primers has provided a powerful tool for the detection of plant pathogens. In the present investigation, a sensitive PCR-based diagnostic assay was developed with the aim to detect *C. gloeosporioides* of mango anthracnose pathogen in infected fruit tissue using species-specific designed primer pair of Mkcg f and Mkcg r which amplified the DNA at 380 bp. The size of the amplification product (450 bp) obtained with species-specific primers was similar to previous reports of *C. gloeosporioides* [32].

5. CONCLUSION

In conclusion, the morphological characteristics and virulence index of each of the twenty-four *C. gloeosporioides* isolates vary. An efficient method for resolving the issue of fungal species identification and characterization is the combination of phenotypic and molecular features. We demonstrated that reliable identification of the *Colletotrichum* species causing anthracnose disease in mangos may be accomplished through genetic analysis through ITS and Species-specific primer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kankam F, Larbi-Koranteng S, Adomako J, Kwodaga JK, Akpatsu IB, Danso Y, Sowley ENK. Anthracnose disease of mango: Epidemiology, impact and management options. In Current and Emerging Challenges in the Diseases of Trees. IntechOpen; 2022.
2. Weir BS, Johnston PR, Damm U. The *Colletotrichum gloeosporioides* species complex. Stud. Mycol. 2012;73:115–180. DOI:10.3114/sim0011.
3. Tovar-Pedraza JM, Mora-Aguilera JA, Nava-Diaz C, Lima NB, Michereff SJ, Sandoval-Islas J. S, Leyva-Mir SG. Distribution and pathogenicity of Colletotrichum species associated with mango anthracnose in Mexico. Plant Disease. 2020;104(1):137-146.
4. Bhagwat RG, Mehta BP, Patil VA, Sharma H. Screening of cultivars/varieties against mango anthracnose caused by *Colletotrichum gloeosporioides*. Int. J. Environ. Agric. Res. 2015;1:21-23.
5. Kamle M, Kumar P. *Colletotrichum gloeosporioides*: Pathogen of anthracnose disease in mango (Mangifera indica L.). Curr. Trends Plant Dis. Diagn. Manag. Pract. 2016;207–219. DOI:10.1007/978-3-319-27312-9_9
6. Qin LP, Zhang Y, Su Q, Chen YL, Nong Q, Xie L, et al. First report of anthracnose of Mangifera indica caused by Colletotrichum scovillei in China. Plant Dis. 2019;103: 1043. DOI:10.1094/PDIS-11-18-1980-PDN
7. De Souza A, Delphino Carboni RC, Wickert E, de Macedo Lemos EG, de Goes A. Lack of host specificity of Colletotrichum spp. isolates associated with anthracnose symptoms on mango in Brazil. Plant Pathol. 2013;62:1038–1047. DOI:10.1111/ppa.12021
8. Ashraful A, Sanjoy KA, Mahtalat A. Morphological characterization of *Colletotrichum gloeosporioides* identified from anthracnose of Mangifera indica L. Asian J. Plant. Pathol. 2017;11:102–117. DOI:10.3923/ajppaj.2017.102.117
9. Kamle M, Pandey BK, Kumar P, Kumar M. A species-specific PCR based assay for rapid detection of mango anthracnose pathogen *Colletotrichum gloeosporioides* Penz. and Sacc. J Plant Pathol Microb. 2013 Jun 10;4(184):2.
10. Damm U, Cannon PF, Woudenberg JHC, Crous PW. The Colletotrichum acutatum species complex. Stud. Mycol. 2012;73: 37–113. DOI:10.3114/sim0010
11. Dela Cueva FM, Laurel NR, Dalisay TU, Sison MLJ. Identification and characterisation of Colletotrichum fructicola, C. tropicale and C. theobromicola causing mango anthracnose in the Philippines. Arch. Phytopathol. 2021; 54:1989–2006. DOI:10.1080/03235408.2021.1968234
12. Lima NB, De Batista MVA, De Morais MA, Barbosa MA, Michereff SJ, Hyde KD, et al. Five Colletotrichum species are responsible for mango anthracnose in northeastern Brazil. Fungal Divers. 2013a;61:75–88. DOI:10.1007/s13225-013-0237-6
13. Akem CN. Mango anthracnose disease: present status and future research priorities. Plant Pathol. J. 2006;5:266–273. DOI:10.3923/ppj.2006.266.273
14. Cheng YJ, Wu YJ, Lee FW, Ou LY, Chen CN, Chu YY, Kuan YC. Impact of storage condition on chemical composition and antifungal activity of pomelo extract against *Colletotrichum gloeosporioides* and anthracnose in post-harvest mango. Plants. 2022;11(15):2064.
15. Prakash O, Srivastava KC. Mango diseases and their management. A world review. Today and Tomorrow's Printers and Publishers: New Delhi; 1987.
16. Prabakar K, Raguchander T, Parthiban VK, Muthulakshmi P, Prakasam V. Post harvest fungal spoilage in mango at different levels marketing. Madras Agric. J. 2005;92(1):42-48.
17. Prema Ranjitham T, Prabakar K, Faisal MP, Kathikeyan G, Raguchander T. Morphological and physiological characterization of Colletotrichum musae the causal organism of banana anthracnose. World Journal of Agricultural Sciences. 2011;7(6):743-754.
18. Knapp JE, Chandlee JM. RNA/DNA mini-prep from a single sample of orchid tissue. Biotechniques. 1996;21:54-56.
19. Dofuor AK, Quartey NK, Osabutey AF, Antwi-Agyakwa AK, Asante K, Boateng BO, Ablormeti FK, Lutuf H, Osei-Owusu J,

Osei JHN, Ekloh W, Loh SK, Honger JO, Aidoo OF, Ninsin KD. Mango anthracnose disease: the current situation and direction for future research. Front Microbiol. 2023 Aug 24;14:1168203. DOI:10.3389/fmicb.2023.1168203. PMID: 37692388; PMCID: PMC10484599.

20. Maymon M, Zveibil A, Pivonia S, Minz D, Freeman S. Identification and characterization of benomyl resistant and sensitive populations of *Colletotrichum gloeosporioides* from statice (Limonium spp.). Phytopathology. 2006;96:542-548.
21. Sutton BC. The Coelomycetes: Fungi imperfecti with Pycnidia, Acervuli, and Stromata, Common-wealth Mycological Institute, Kew, UK. 1980;196.
22. Sutton BC. The genus Glomerella and its anamorph Colletotrichum. In: Colletotrichum: Biology, Pathology and Control. Bailey JA, Jeger MJ. (Eds.) Wallingford, UK: CAB International. 1992; 1-26.
23. Martinez CPV, Barrio E, Suarez-Fernandez MB, Garcia-Lopez MD, Querol A. RAPD analysis of Colletotrichum species isolated from strawberry and the design of specific primers for the identification of C. fragariae. J. Phytopathol. 2002;150:680-686.
24. Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. Meth. Enzymol. 1987;155: 335-350
25. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 1993;2: 113–118.
26. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH., Snisky JJ. and White TJ. (Eds.) PCR protocols: a guide to methods and applications.1990;315-322.
27. Xiao CL, Mackenzie SJ, Legard DE. Genetic and pathogenic analyses of *Colletotrichum gloeosporioides* isolates from strawberry and no cultivated hosts. Phytopathology. 2004;94: 446-453.
28. Tussell RT, Ramayo AQ, Velazquez PL, Saavedra AL, Brito DP. PCR based detection and characterization of the fungal pathogens *Colletotrichum gloeosporioides* and C. capsici causing anthracnose in Papaya (Carica papaya L.) in the Yucatan Peninsula. Mol. Biotechnol. 2008;8: 9093-9095.
29. Xie L, Zhang J, Yao Wan, Dong-wei Hu. Identification of Colletotrichum spp. isolated from strawberry in Zhejiang Province and Shanghai City, China. Biomed. Biotechnol. 2010;11(1):61-70.
30. Sherriff C, Whelan MJ, Arnold GM, Lafay JF, Brygoo Y, Bailey JA. Ribosomal DNA sequence analysis reveals new species groupings in the genus Colletotrichum. Exp. Mycol. 1994;18:121-138.
31. Dunne CP, Glen M, Tommerup IC, Shearer BL, Hardy GE. Sequence variation in the rDNA ITS of Australian Armillaria species and intra-specific variation in A. Aust. Plant Pathol. 2002;31: 241–251.
32. Freeman S. Genetic diversity and host specificity of Colletotrichum species on various fruits. In: Colletotrichum: host specificity, pathology and host pathogen interaction. Prusky, D., Freeman, S. and Dickman, M.B. (Eds.). Minnessota, USA: American Pathological Society Press. 2000;131–144

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