



Association of *Colletotrichum* species with anthracnose of tropical fruit crops in Southern Karnataka and Andhra Pradesh, India

DURGA VENKATA RAVI TEJA AMULOTHU^{1, 2} and S SRIRAM^{1*}

ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka 560 089, India

Received: 29 June 2025; Accepted: 20 March 2026

ABSTRACT

Severe pre- and post-harvest losses occur in tropical fruit crops by fruit rot pathogens especially *Colletotrichum* spp., causing anthracnose disease. The wide host range of the pathogen is associated with the prevalence of different species in the fruit orchards. Hence, for the study, samples were collected during the winter (*rabi*) season of 2020 and the rainy (*kharif*) season of 2021 from Southern Karnataka and Andhra Pradesh to explore the diversity of *Colletotrichum* spp. for sustainable disease management and plant biosecurity. Characteristic symptoms of anthracnose included dark, sunken lesions with visible acervular aggregates at advanced stages of infection. Preliminary identification of nine isolates (IIHR_COL_C1-9) was based on morpho-cultural characteristics. Eight isolates were assigned to the *C. gloeosporioides* species complex, producing cylindrical conidia and white aerial mycelial colonies, while one isolate (IIHR_COL_C8) was identified as *C. truncatum*, producing falcate conidia and dark grey colonies with concentric zonation. Multigene phylogenetic analysis using partial sequences of *ITS*, *ACT*, *GD*, *SOD2* and *TUB2* in MEGA X further resolved the isolates into three species: *C. siamense* (n=7), *C. musae* (n=1), and *C. truncatum* (n=1). The mean conidial size of *C. siamense* ranged from 10.16–14.18 $\mu\text{m} \times 3.76$ –5.34 μm , *C. musae* measured 12.01 $\mu\text{m} \times 3.83$ μm , and *C. truncatum* is 21.45 $\mu\text{m} \times 2.57$ μm . The mean growth rate of *C. siamense* ranged from 10.56–12.92 mm/day, *C. musae* was 12.61 mm/day, and *C. truncatum* was 7.47 mm/day. *C. siamense* was identified as the dominant species causing anthracnose disease across all seven fruit crops, viz. Banana, Custard Apple, Guava, Grape, Mango, Papaya and Pomegranate, along with *C. musae* identified from Banana and *C. truncatum* from Papaya.

Keywords: Diversity, Fruit crops, Molecular characterization, Multigene phylogeny

Post-harvest damages reduces market value and renders the fruits unfit for consumption. *Colletotrichum* spp. causes huge yield losses in many fruit crops, especially by quiescent infections. *Colletotrichum* spp. are regarded as the eighth most important plant pathogen in the world on account of their scientific and economic importance (Dean *et al.* 2012). This genus consists of cosmopolitan pathogens causing serious epidemics due to their potential to cross infect a wide variety of hosts (Teja *et al.* 2022). *Colletotrichum* species exhibit two lifestyles, namely hemi-biotrophic and endophyte and have implications for plant biosecurity (De Silva *et al.* 2017). The morphology alone does not provide sufficient information for precise identification, especially for those in *Colletotrichum* spp. complexes (Cai *et al.* 2009). *C. gloeosporioides* species complex has been associated with 283 plant species of 212 genera, of which the majority share is of eudicots (80.6%) (Talhinhas and Baroncelli 2021). The knowledge of the diversity of *Colletotrichum* spp. and

host range studies is essential for understanding the key processes in the molecular host-pathogen interaction and can be exploited for more sustainable disease management and plant biosecurity.

Rapid progress in molecular and bioinformatics techniques has helped in efficiently recognising stable and well-resolved clades within the genus *Colletotrichum* (Hyde *et al.* 2009). Molecular identification of species cannot rely merely on the sequence data of rDNA-*ITS* alone as multigene phylogenetic studies give a better understanding of the diversity in *Colletotrichum* spp. infecting various crops. The combined use of traditional morpho-cultural characters (for diagnosis) and advanced techniques like molecular markers along with sequence data (for detection) can be currently viewed as a salient approach for studying the *Colletotrichum* spp. complexes. The present study was taken up with the null hypothesis that the diversity of *Colletotrichum* spp. infecting tropical fruit crops can be accurately detected up to species level using morpho-cultural characteristics, molecular and bioinformatic tools.

MATERIALS AND METHODS

Collection, isolation and identification of pathogen: The

¹ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka; ²College of Horticulture, Dr. YSR Horticultural University, Anantharajupeta, Andhra Pradesh. *Corresponding author email: Subbaraman.Sriram@icar.org.in

samples for the study were collected during the winter (*rabi*) season of 2020 and the rainy (*khariif*) season of 2021 from Southern Karnataka and Andhra Pradesh from symptomatic leaves of grapes (*Vitis vinifera* L.), flowers of pomegranate (*Punica granatum* L.), fruits of banana (*Musa* spp.), custard apple (*Annona squamosa* L.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), and papaya (*Carica papaya* L.). The pathogen was isolated by tissue isolation technique on Potato Dextrose Agar (PDA) medium. The fungus was primarily identified based on the morphological and cultural characteristics as described by Damm *et al.* (2009), and Weir *et al.* (2012). The pathogenicity of all isolates was tested by inoculating the corresponding pathogen culture on detached leaves/ flowers/ fruits (Cai *et al.* 2009). Isolates that fulfilled the Koch's postulates were used in further studies. Pure cultures were maintained on PDA slants with five replications per isolate and stored in a refrigerator at 4±1°C.

Morpho-cultural characterization: A single 5 mm mycelial disc from an actively growing periphery of 5-day-old pure culture was aseptically punched and transferred onto the PDA. Cultures were incubated at 25±1°C with a photoperiod of 24 h for 7 days. Three replicate cultures of each isolate were maintained. All the morpho-cultural characters were recorded from pure cultures of the isolates grown on PDA. Colony colour of the isolates was recorded on the 7th day after inoculation. Observations and measurements of the size and shape of 30 conidia were recorded by mounting conidia in lactophenol cotton blue (LPCB) stain. Length and width of conidia were taken using an automatic microscope calibrated scale in micrometres (µm). For each isolate, observations were recorded by observing under a microscope at 100X magnification (objective lens) using cedarwood oil immersion with a bright field microscope (Zeiss make) using Axio vision software. Acervuli, the asexual fruiting bodies of *Colletotrichum* spp. were picked by needle and placed on a clean glass slide containing a drop of LPCB stain. Later, a clean microscopic cover slip was placed over the sample. The length and width of acervuli (mm) were recorded under a microscope at 10X magnification (objective lens). Mycelial growth of 1 cm² size was taken on a cover slip of 1.8 cm² size and the number of acervuli in the 1 cm² area was counted with the naked eye. The square bits were randomly selected from three areas of a pure culture plate of three replicate cultures of each isolate. The colony diameter of the isolates was recorded in two perpendicular directions from the reverse side of the petri dish till 7th day after inoculation. The mean growth (cm) of three replicates of each isolate was recorded on the 3rd, 5th and 7th day after inoculation. The growth rate was calculated as the 7-day average of daily growth (mm/day) (Cai *et al.* 2009).

Scanning electron microscopy (SEM): The SEM analysis of conidia was performed using TM36030Plus Scanning Electron Microscope (Hitachi, Japan) facilitated at ICAR-Indian Institute of Horticulture Research, Bengaluru as per the manufacturer's instruction. The images were captured in SEM at various magnifications of 1800X, 3000X,

4000X and 6000X. The size of conidia was calculated based on calibrated scale.

Molecular identification of *Colletotrichum* spp.: Molecular identification was carried out to precisely identify different isolates of *Colletotrichum* spp. upto species level. The cetyl trimethyl ammonium bromide (CTAB) method was used for DNA extraction as described by Zhang *et al.* (2010). The extracted DNA was quantified using a NanoDrop UV Spectrophotometer. The *ITS* region and four genes, viz. *ACT*, *GAPDH*, *SOD2* and *TUB2* with their respective primer pairs were used for multigene phylogeny analysis of isolates of *Colletotrichum* spp. The Actin (*ACT*) region was amplified with primers ACT-512F (Forward: ATG TGC AAG GCC GGT TTC GC) and ACT-783R (Reverse: TAC GAG TCC TTC TGG CCC AT) as described by Carbone and Kohn (1999). The Glyceraldehyde-3-phosphate dehydrogenase (*GD*) gene was amplified using GDF (Forward: GCC GTC AAC GAC CCC TTC ATT GA) and GDR (Reverse: GGG TGG AGT CGT ACT TGA GCA TGT) following Templeton *et al.* (1992). The Internal Transcribed Spacer (*ITS*) region was amplified with ITS-1F (Forward: CTT GGT CAT TTA GAG GAA GTA A) and ITS-4 (Reverse: TCC TCC GCT TAT TGA TAT GC) as reported by White *et al.* (1990) and Gardes and Bruns (1993), respectively. The Manganese-superoxide dismutase (*SOD2*) gene was amplified using SODglo2F (Forward: CAG ATC ATG GAG CTG CACCA) and SODglo2R (Reverse: TAG TAC GCG TGC TCG GAC AT) according to Moriwaki and Tsukiboshi (2009). The β-Tubulin 2 (*TUB2*) gene region was amplified with primers T1 (Forward: AAC ATG CGT GAG ATT GTA AGT) and T2 (Reverse: TAG TGA CCC TTG GCC CAGT TG) as described by O'Donnell and Cigelnik (1997). The PCR master mix (25 µl) per reaction consisted of 10X Taq buffer mixed with MgCl₂ of 2.5 µl, Taq DNA polymerase (5-unit/µl) of 0.3 µl, dNTPs (10 mM each) of 0.5 µl, template DNA (10 ng/ µl) of 1 µl, forward and reverse primer (10 picomolar) of 0.5 µl each. Amplification was done by simple PCR technique. The PCR was performed in a Thermocycler (Applied Biosystems). Using gradient PCR technique, a set of temperatures, viz. 50 °C, 52 °C, 54 °C, 56 °C, 58 °C and 60 °C was set for standardization of annealing temperatures for five primer pairs. The PCR amplifications were repeated three times to confirm reproducibility.

Agarose gel electrophoresis: The PCR products were separated in 1.5% agarose gel, stained using ethidium bromide (0.5 µg/ml) in 1 × Tris-borate-EDTA (TBE) buffer. 2 µl of PCR products and 3 µl of DNA loading dye were mixed well and loaded into the wells along with 100 bp DNA ladder. The gel was run for 1 h at constant voltage of 95 V. After the run, gel was observed under the UV light using gel documentation system and recorded the photographs digitally. The size of the amplicons was compared with 100 bp DNA ladder molecular weight standard (Thermo Fisher Scientific).

Purification of PCR product: The PCR products were purified by using PCR product purification kit (HIMEDIA)

as per manufacturer's instructions to remove PCR impurities prior to sequencing.

Sequencing and homology search: The sequencing results were obtained using Sanger sequencing technology by outsourcing in FASTA files for all isolates of *Colletotrichum* spp. The consensus sequences were generated using BIOEDIT v. 7.2.5 (Hall 1999) and further analysed in NCBI BLAST data base with the nucleotide search using 'BLASTn' for sequence homology (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Best model selection for multigene data set and multigene phylogeny: Multiple sequence alignment was done using ClustalW (Larkin *et al.* 2007) in MEGA X v. 10.2.4 (Kumar *et al.* 2018) along with reference sequences. The reference sequences of ITS, ACT, GAPDH, SOD2 and TUB2 genes were retrieved from NCBI data base for construction of multigene phylogenetic analysis (Weir *et al.* 2012). Concatenation of the sequences of ITS region, ACT, GAPDH, SOD2 and TUB2 genes was also performed in MEGA X. The concatenated multigene nucleotide data set was fed to the best model parameter test in MEGA X. Models with the lowest BIC scores (Bayesian Information Criterion) were considered to describe the substitution pattern the best. These sequences were further subjected to multigene phylogenetic analysis along with the reference sequences retrieved from NCBI data base (Supplementary Table 1). The phylogeny reconstruction was performed using maximum likelihood statistical method with a bootstrap method of test of phylogeny with 1000 bootstrap replications and the lowest BIC model for the dataset obtained in MEGA X. The sequences of ITS were deposited to NCBI GenBank and ACT, GAPDH, SOD2 and TUB2 sequences were deposited to NCBI BankIt

Statistical design: Three replicate cultures of each isolate were studied and the data obtained for all the parameters of morpho-cultural characterization were statistically analysed using a completely randomized design (CRD) in WASP-1.0

(Web Agri Stat Package) (Jangam and Thali 2002).

RESULTS AND DISCUSSION

Symptomatology: Anthracnose symptoms in tropical fruit crops were characterized predominantly by brown to black sunken lesions bearing salmon pink acervuli on fruits of banana, guava, mango, papaya and pomegranate. Lesions initially appeared as small black spots that enlarged, coalesced and resulted in extensive surface damage and fruit deformation; in severe cases, entire fruits were rendered unmarketable, particularly in papaya. Guava exhibited large zonate lesions with prominent conidial masses, while mango showed spherical spots that later merged to cover the fruit surface. Custard apple developed pre-harvest dark brown to black discoloration without distinct sunken lesions. In grape, symptoms appeared as black leaf spots progressing to shot-hole formation due to necrosis. In pomegranate, black necrotic lesions occurred on leaves, flowers and fruits, often leading to defoliation (Supplementary Fig. 1). Nine *Colletotrichum* isolates were collected from tropical fruit crops in Karnataka and Andhra Pradesh. IIHR_COL_C1 (Banana) was from Ivarkandapura, Hesaraghatta (Karnataka), and IIHR_COL_C2 (Banana) and IIHR_COL_C7 (Papaya) were from Chinnaramannagaripalle, Kadiri (Andhra Pradesh). IIHR_COL_C3 (Custard apple), IIHR_COL_C4 (Grapes), IIHR_COL_C6 (Mango), IIHR_COL_C8 (*Carica papaya*) and IIHR_COL_C9 (Pomegranate) were collected from ICAR-IIHR, Hesaraghatta (Karnataka). IIHR_COL_C5 (Guava) was obtained from Devanahalli, Karnataka. All locations fall in Karnataka except Kadiri, which is in Andhra Pradesh.

Morphological and cultural characterization: Nine isolates obtained from seven fruit crops were designated as IIHR_COL_C1 to IIHR_COL_C9 (Table 1, Fig. 1). All isolates induced symptoms within 3–6 days of inoculation and were successfully re-isolated from infected tissues, fulfilling Koch's postulates (Supplementary Fig. 2).

Table 1 Morphological characteristics of *Colletotrichum* isolates isolated from tropical fruit crops

Isolate	Mean conidial width (μm)	Mean acervuli length (μm)	Mean conidial length (μm)	Mean acervuli width (μm)	Mean number of acervuli	Mean growth of mycelium (cm) on PDA			Mean growth rate (mm/day)
						3 rd day	5 th day	7 th day	
IIHR_COL_C1	5.34 \pm 0.26	0.57 \pm 0.03	12.19 \pm 0.29	0.52 \pm 0.03	10.11	2.33	4.73	7.41	10.59
IIHR_COL_C2	3.83 \pm 0.54	0.57 \pm 0.02	12.01 \pm 0.35	0.54 \pm 0.02	21.53	3.41	6.06	8.83	12.61
IIHR_COL_C3	3.76 \pm 0.31	0.43 \pm 0.03	13.05 \pm 0.27	0.38 \pm 0.03	31.10	2.45	4.83	7.40	10.56
IIHR_COL_C4	3.80 \pm 0.28	0.63 \pm 0.025	13.16 \pm 0.48	0.56 \pm 0.02	28.77	2.65	5.06	7.65	10.92
IIHR_COL_C5	4.08 \pm 0.63	0.59 \pm 0.027	10.16 \pm 0.32	0.46 \pm 0.02	18.99	2.53	5.00	7.78	11.11
IIHR_COL_C6	4.15 \pm 0.26	0.42 \pm 0.03	12.23 \pm 0.18	0.35 \pm 0.03	11.44	3.60	6.23	8.98	12.92
IIHR_COL_C7	4.03 \pm 0.38	0.53 \pm 0.01	13.20 \pm 0.41	0.48 \pm 0.01	41.55	3.25	5.76	8.41	12.02
IIHR_COL_C8	2.57 \pm 0.34	0.65 \pm 0.032	21.45 \pm 0.92	0.53 \pm 0.03	54.66	1.70	3.20	5.16	7.47
IIHR_COL_C9	4.26 \pm 0.22	0.54 \pm 0.015	14.18 \pm 0.57	0.5 \pm 0.019	25.77	2.65	5.10	7.71	11.01
SEM \pm	0.07	0.05	0.09	0.05	2.01	0.08	0.12	0.14	0.19
CD ($p=0.05$)	0.23	0.16	0.28	0.15	5.97	0.23	0.35	0.42	0.58
CV	3.40	16.94	1.24	18.64	12.84	5.10	4.09	3.22	3.09

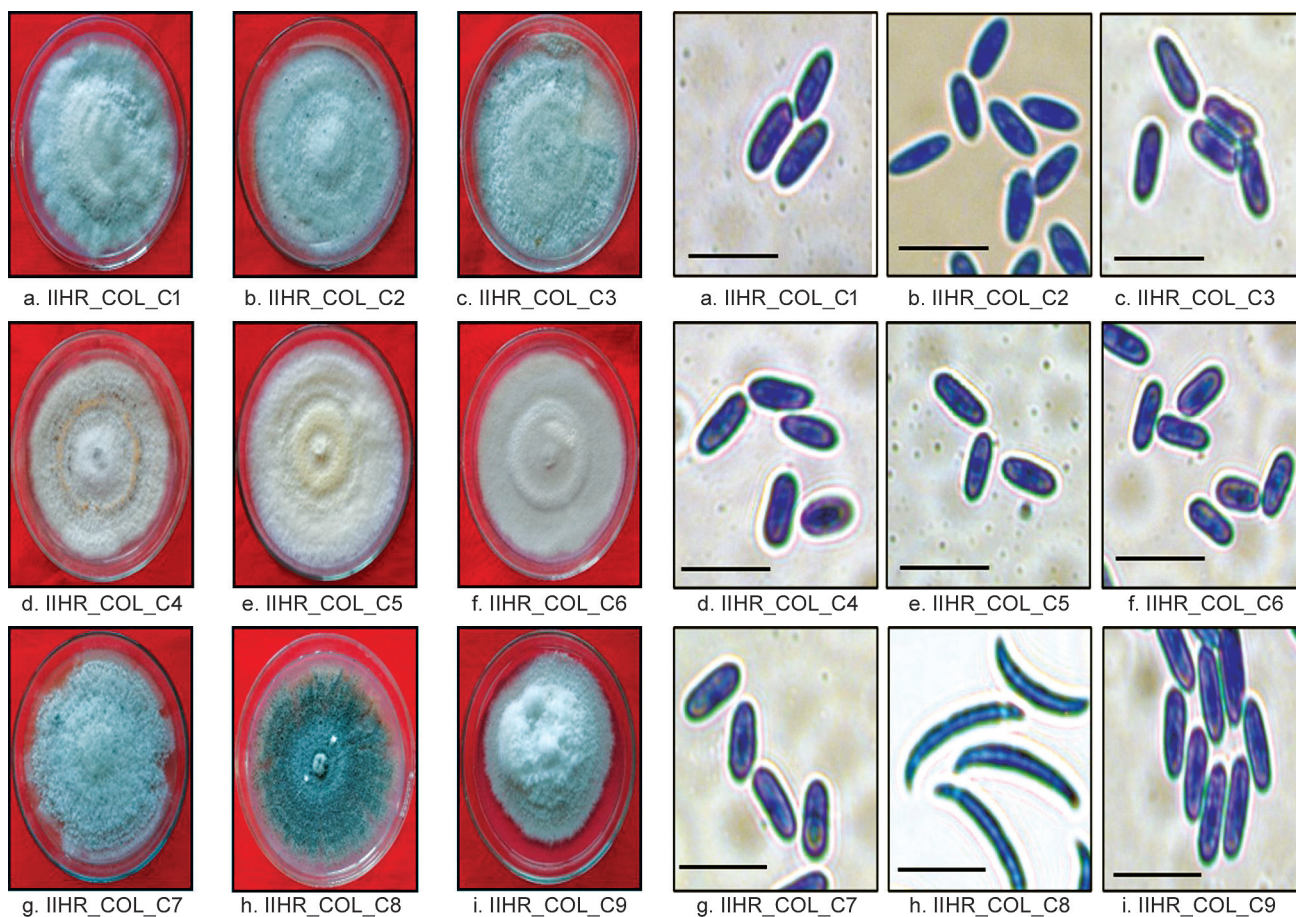


Fig. 1 Colony and conidial morphology of isolates of *Colletotrichum* spp. on PDA.

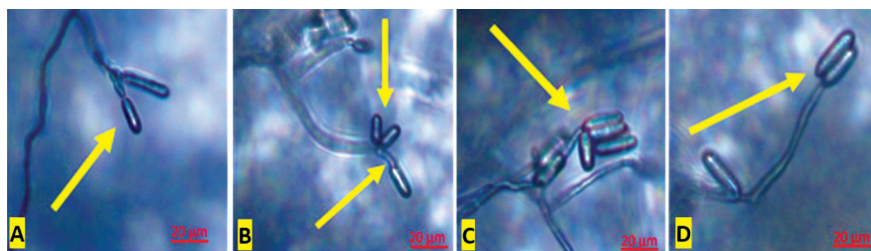
Conidial morphology of *Colletotrichum* isolates (100 × magnification) (Objective lens); Scale bar: 10 µm

Colonies were pure white and sporulation positive, except IIHR_COL_C8, which produced greyish-black mycelium. All isolates developed septate mycelium and conidia within 7–10 days. Conidia were predominantly cylindrical with rounded ends and oil globules; however, IIHR_COL_C8 produced falcate, slender conidia. Mean conidial dimensions ranged from 10.16–21.45 µm × 2.57–5.34 µm with significant variation: maximum length in IIHR_COL_C8 (21.45 µm) and minimum in IIHR_COL_C5 (10.16 µm); maximum width in IIHR_COL_C1 (5.34 µm) and minimum in IIHR_COL_C8 (2.57 µm). Radial growth was highest in IIHR_COL_C6 (3.60, 6.23 and 8.98 cm on 3rd, 5th and 7th day, respectively) with the greatest growth rate (12.92 mm/day), while IIHR_COL_C8 showed the least growth (1.70, 3.20 and 5.16 cm; 7.47 mm/day). Conidial development in IIHR_COL_C7 showed solitary to clustered arrangements of young and mature conidia on conidiophores (Fig. 2). SEM analysis of IIHR_COL_C7 (1800×–6000×) revealed smooth, non-ornamented conidial surfaces with parallel clustering and a distinct central cavity-like structure, likely corresponding to vacuolar inclusions a feature of potential diagnostic value within the *C. gloeosporioides* species complex (Fig. 2). Acervuli measured 0.42–0.65 × 0.35–0.56 mm (100×) with non-significant size variation, but number differed significantly, highest in IIHR_COL_C8

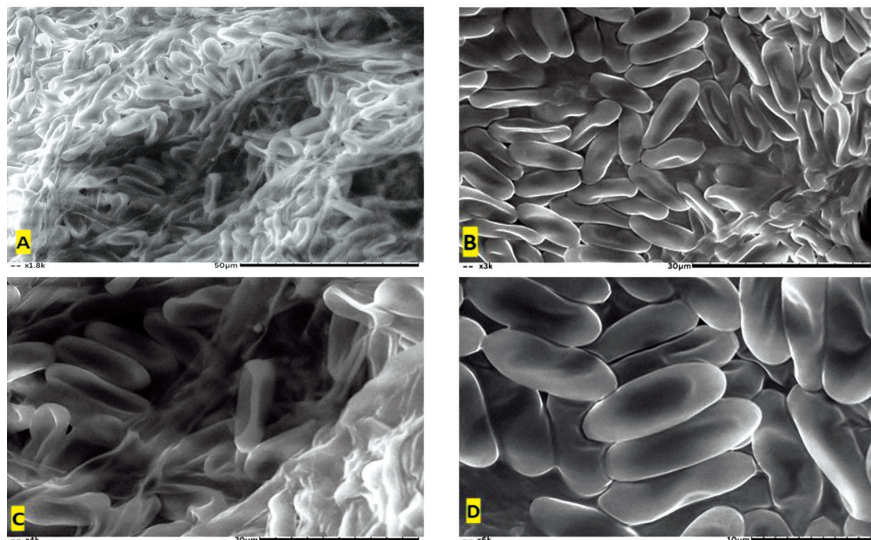
(54.66) and lowest in IIHR_COL_C1 (10.11). Visible setae were observed only in IIHR_COL_C8 (Supplementary Fig. 3 and Supplementary Fig. 4). Cylindrical conidia in most isolates align with reports on *C. gloeosporioides* and *C. siamense*, whereas the falcate conidia, greyish-black colony and setose acervuli of IIHR_COL_C8 correspond to earlier descriptions. The *C. gloeosporioides* and *C. siamense* exhibited a cylindrical shape of conidia with rounded ends (Athinuwat *et al.* 2024). The characters of IIHR_COL_C8 like falcate shaped conidia, greyish-black colour of mycelium, and black colour acervuli with presence of setae were similar to that reported by dos Santos *et al.* (2025).

Molecular characterization: The size of the amplified product of *Colletotrichum* isolates was different for each primer. The fragment of DNA amplified with *ITS-1* and *ITS-4* was ~600 bp, with *ACT*, *GAPDH* at ~300 bp, with *TUB2* at ~700bp and with *SOD2* at ~350 bp (Fig. 3). All isolates were amplified with all primers with one exception of IIHR_COL_C8 failed to amplify with *SOD2* which didn't get amplified with *SOD2* primer.

Best model selection for multigene data set: The Evolutionary analysis involved 52 nucleotide sequences. A total of 2421 positions were included in the final dataset. analyses were conducted in MEGA X. The Tamura-Nei (TN93) model with lowest BIC score (24151.506) was



(i) Light Microscopy depicting the formation of conidia on conidiophore at varying stages on pure culture of *C. siamense* IIHR_COL_C7



(ii) Scanning electron microscopy of *C. siamense* IIHR_COL_C7 conidia at magnifications of 1800X (A), 3000X (B), 4000X (C) and 6000X (D)

Fig. 2 Light and scanning electron microscopy of conidial development in *C. siamense*.

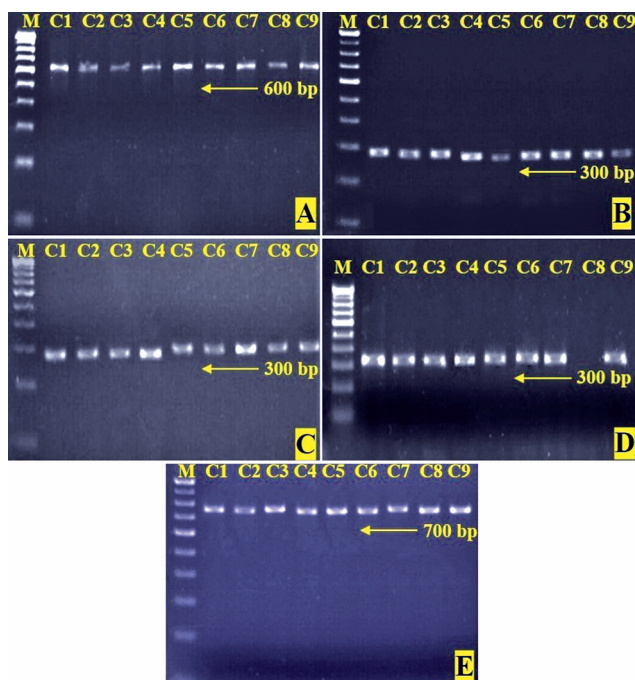


Fig. 3 Amplicons of nine isolates of *Colletotrichum* spp. in 1.5% agarose gel electrophoresis; A- *ITS* region; B- *ACT*; C- *GAPDH*; D- *SOD2*; E- *TUB2*; M- 100 bp DNA ladder.

selected as the best-fit substitution model (Tamura and Nei 1993) with gamma distributed (+G) rates among sites with

5 discrete gamma categories.

Multigene phylogeny analysis: The five gene phylogenetic tree (Fig. 4) resolved the nine representative isolates into the clades corresponding to their closest reference species. IIHR_COL_C1 clustered with *C. siamense* ICMP 12567. IIHR_COL_C2 clustered with *C. musae* ICMP 17817. IIHR_COL_C3 clustered with *C. siamense* ICMP 17795. IIHR_COL_C4, IIHR_COL_C6 and IIHR_COL_C9 clustered with *C. siamense* CBS 130417. IIHR_COL_C5 and IIHR_COL_C7 clustered with *C. siamense* CBS 125378. IIHR_COL_C8 clustered with *C. truncatum* CBS120709.

With the aid of morpho-cultural, molecular and bioinformatic tools, IIHR_COL_C2 and IIHR_COL_C8 were identified as *C. musae* and *C. truncatum* while all other seven isolates were identified as *C. siamense*. All the nine representative isolates identified to species level were deposited in GenBank and the accession numbers are provided in Table 2. Species diversity of *C. musae* and *C. siamense* was identified from Andhra Pradesh isolated from banana (IIHR_COL_C2)

and papaya (IIHR_COL_C7).

A polyphasic approach integrating morphology and multigene phylogeny enables accurate delineation of *Colletotrichum* spp. (Cai *et al.* 2009, Damm *et al.* 2009, Cannon *et al.* 2012, Weir *et al.* 2012, Fuentes-Aragon *et al.* 2021, Talhinhos and Baroncelli 2021). Although many species exhibit broad host ranges, *C. siamense* has been reported on custard apple (Borges *et al.* 2021), mango (Giblin *et al.* 2018, Li *et al.* 2019, Fuentes-Aragon *et al.* 2020), banana (Fuentes-Aragón *et al.* 2021) and pomegranate (Xavier *et al.* 2019). *C. siamense* and *C. musae* belong to the *C. gloeosporioides* spp. complex and cluster within the same clade (Weir *et al.* 2012, Talhinhos and Baroncelli 2021), whereas *C. truncatum* represents a distinct complex (Cannon *et al.* 2012). Both *C. siamense* and *C. gloeosporioides* are cosmopolitan with wide host ranges (Talhinhos and Baroncelli 2021, Athinuwat *et al.* 2024, Kaur *et al.* 2024, Prathibha *et al.* 2024, Praveena *et al.* 2025), while *C. musae* showed strong specialization to *Musa* spp. (Weir *et al.* 2012). *C. truncatum* is also polyphagous, reported on papaya (Aktaruzzaman *et al.* 2018, dos Santos Vieira *et al.* 2020), *Citrus* spp. (Guarnaccia *et al.* 2017) and Vitaceae hosts (Zhang *et al.* 2018).

Gene-wise comparisons showed identical coding regions in partial ACT and GAPDH sequences among *C. siamense*, *C. musae* and *C. truncatum*, resulting in 100% similarity at the protein level; interspecific variation was

Table 2 GenBank accession numbers for the sequences deposited in NCBI

Accession code	Host	Species	GenBank accession number				
			ITS	ACT	TUB2	GD	SOD2
IIHR_COL_C1	Banana	<i>C. siamense</i>	OK356680	OQ079577	OQ079586	OQ079595	OQ079604
IIHR_COL_C2	Banana	<i>C. musae</i>	OK041515	OQ079578	OQ079587	OQ079596	OQ079605
IIHR_COL_C3	Custard apple	<i>C. siamense</i>	OK356681	OQ079579	OQ079588	OQ079597	OQ079606
IIHR_COL_C4	Grapes	<i>C. siamense</i>	OK356682	OQ079580	OQ079589	OQ079598	OQ079607
IIHR_COL_C5	Guava	<i>C. siamense</i>	OK356683	OQ079581	OQ079590	OQ079599	OQ079608
IIHR_COL_C6	Mango	<i>C. siamense</i>	OK356684	OQ079582	OQ079591	OQ079600	OQ079609
IIHR_COL_C7	Papaya	<i>C. siamense</i>	OK356685	OQ079583	OQ079592	OQ079601	OQ079610
IIHR_COL_C8	Papaya	<i>C. truncatum</i>	OK356686	OQ079584	OQ079593	OQ079602	-
IIHR_COL_C9	Pomegranate	<i>C. siamense</i>	OK356687	OQ079585	OQ079594	OQ079603	OQ079611

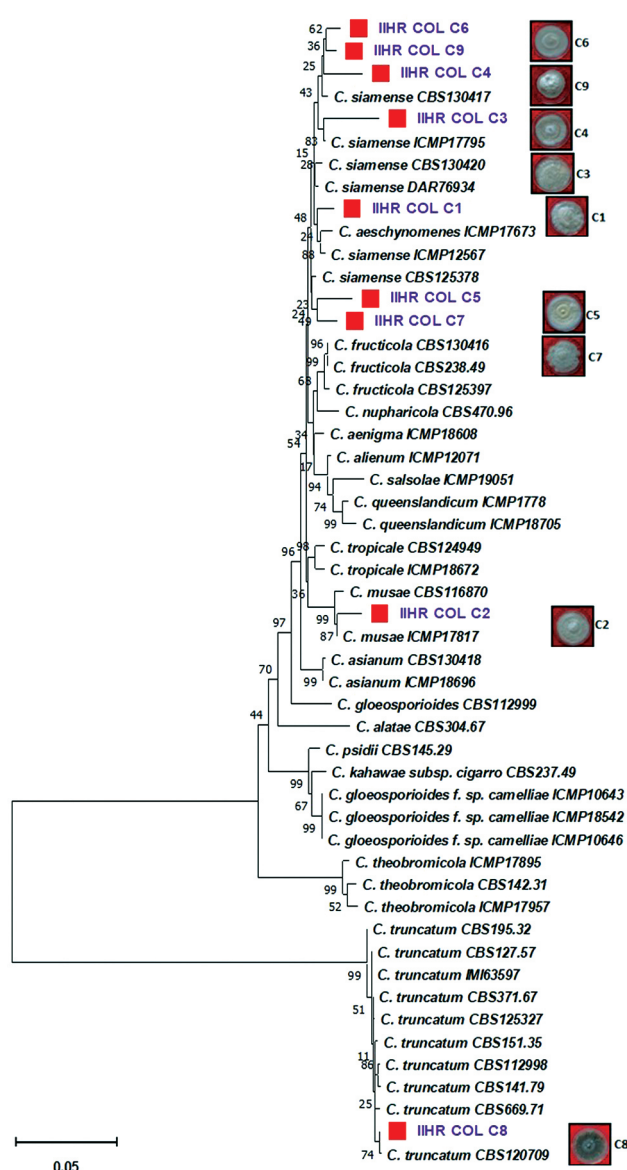


Fig. 4 Multigene phylogenetic tree using concatenated sequences of ITS region, ACT, GAPDH, SOD2 and TUB2 genes of Colletotrichum spp. isolates infecting tropical fruit crops by the maximum likelihood method in MEGA-X.

mainly confined to intron regions, enabling phylogenetic separation. In GAPDH, major intronic divergence distinguished *C. truncatum* from *C. siamense* and *C. musae*, whereas the latter two shared high similarity, consistent with their placement in the same complex. In TUB2, *C. musae* differed from *C. siamense* by a ~14 bp intron deletion and 4 bp insertion, supporting clade separation. Partial SOD2 sequences (intronless) were 100% identical between *C. siamense* and *C. musae*, while *C. truncatum* failed to amplify. Overall, extensive intronic divergence in ACT, GAPDH, TUB2 and SOD2 clearly separated *C. truncatum* as an independent clade, corroborating its distinct species complex status (Cannon *et al.* 2012) and supporting the uniqueness of isolate IIHR_COL_C8 in morphology and cross-infectivity (Teja *et al.* 2022). The high sequence similarity between *C. siamense* and *C. musae* may reflect a close evolutionary relationship, although further genomic studies are required to elucidate mechanisms of underlying host specificity. The results support the hypothesis that integration of morpho-cultural, molecular and bioinformatic tools enables accurate species-level identification of *Colletotrichum* associated with anthracnose in tropical fruit crops.

REFERENCES

Aktaruzzaman M, Afroz T, Lee Y G and Kim B S. 2018. Post-harvest anthracnose of papaya caused by *Colletotrichum truncatum* in Korea. *European Journal of Plant Pathology* **150**: 259–65.

Athinuwat D, Ruangwong O U, Harishchandra D L, Latehnuering F and Sunpapao A. 2024. Morphology and molecular characterization of *Colletotrichum siamense* associated with leaf spot disease of rubber tree (*Hevea brasiliensis*) in southern Thailand. *Physiological and Molecular Plant Pathology* **130**: 102248.

Borges R C, Rossato M, Santos M D M, Macedo M A, Fonseca M E N, Boiteux L S and Reis A. 2021. *Colletotrichum siamense* as a causal agent of leaf anthracnose in seedlings of *Annona muricata* in nurseries from the Federal District, Brazil. *Journal of Plant Diseases and Protection* **128**(2): 583–88.

Cai L, Hyde K D, Taylor P W J, Weir B, Waller J, Abang M M, Zhang J Z, Yang Y L, Phoulivong S, Liu Z Y and Shivas R G. 2009. A polyphasic approach for studying *Colletotrichum*.

- Fungal Diversity* **39**(1): 183–204.
- Cannon P F, Damm U, Johnston P R and Weir B S. 2012. *Colletotrichum*: Current status and future directions. *Studies in Mycology* **73**(1): 181–213.
- Carbone I and Kohn L M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**(3): 553–56.
- Damm U, Woudenberg J H C, Cannon P F and Crous P W. 2009. *Colletotrichum* species with curved conidia from herbaceous hosts. *Fungal Diversity* **39**: 45.
- De Silva D D, Crous P W, Ades P K, Hyde K D and Taylor P W. 2017. Life styles of *Colletotrichum* species and implications for plant biosecurity. *Fungal Biology Reviews* **31**(3): 155–68.
- Dean R, Van Kan J A, Pretorius Z A, Hammond-Kosack K E, Di Pietro A, Spanu P D, Rudd J J, Dickman M, Kahmann R, Ellis J and Foster G D. 2012. The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* **13**(4): 414–30.
- dos Santos Viera W A, dos Santos Nunes A, Veloso J S, Machado A R, Balbino V Q, da Silva A C, Gomes A A M, Doyle V P and Camara M P S. 2020. *Colletotrichum truncatum* causing anthracnose on papaya fruit (*Carica papaya*) in Brazil. *Australasian Plant Disease Notes* **15**: 1–3.
- dos Santos M H, de Oliveira Costa J F, da Silva França K R, dos Santos T F, de Medeiros Ferro M M, de Melo M P, Lima V B C, Cunha J L X, de Andrade Lima G S and Assunção I P. 2025. Characterization and pathogenicity of *Colletotrichum* species causing anthracnose on pitaya (*Hylocereus* spp.) in Brazil. *Physiological and Molecular Plant Pathology* **138**: 102657.
- Fuentes-Aragón D, Rebollar-Alviter A, Osnaya-González M, Enciso-Maldonado G A, González-Reyes H and Silva-Rojas H V. 2021. Multilocus phylogenetic analyses suggest the presence of *Colletotrichum chrysophilum* causing banana anthracnose in Mexico. *Journal of Plant Diseases and Protection* **128**(2): 589–95.
- Fuentes-Aragón D, Guarnaccia V, Rebollar-Alviter A, Juárez-Vázquez S B, Aguirre-Rayó F and Silva-Rojas H V. 2020. Multilocus identification and thiophanate-methyl sensitivity of *Colletotrichum gloeosporioides* species complex associated with fruit with symptoms and symptomless leaves of mango. *Plant Pathology* **69**(6): 1125–38.
- Gardes M and Bruns T D. 1993. ITS primers with enhanced specificity for basidiomycetes: Application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–18.
- Giblin F R, Tan Y P, Mitchell R, Coates L M, Irwin J A G and Shivas R G. 2018. *Colletotrichum* species associated with pre- and post-harvest diseases of avocado and mango in eastern Australia. *Australasian Plant Pathology* **47**: 269–76.
- Guarnaccia V, Groenewald J Z, Polizzi G and Crous P W. 2017. High species diversity in *Colletotrichum* associated with citrus diseases in Europe. *Persoonia-Molecular Phylogeny and Evolution of Fungi* **39**(1): 32–50.
- Hall T A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. (In) *Nucleic Acids Symposium Series* **41**: 95–98.
- Hyde K D, Cai L, McKenzie E H C, Yang Y L, Zhang J Z and Prihastuti H. 2009. *Colletotrichum*: A catalogue of confusion. *Fungal Diversity* **39**(1): 1–17.
- Jangam A K and Thali P. 2002. WASP-Web Agri Stat Package. ICAR Research Complex for Goa, Ela, Old Goa, Goa. 403 402. India. <https://ccari.icar.gov.in/waspnew.html>
- Kaur D, Kaur A, Singh H and Arora A. 2024. First report of *Colletotrichum siamense* causing fruit drop in kinnow mandarin (*Citrus nobilis* × *Citrus deliciosa*) in Punjab, India. *Crop Protection* **175**: 106464.
- Kumar S, Stecher G, Li M, Knyaz C and Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**(6): 1547.
- Larkin M A, Blackshields G, Brown N P, Chenna R, McGettigan P A, McWilliam H, Valentin F, Wallace I M, Wilm A, Lopez R and Higgins D G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**(21): 2947–48.
- Li Q, Bu J, Shu J, Yu Z, Tang L, Huang S, Guo T, Mo J, Luo S, Solangi G S and Hsiang T. 2019. *Colletotrichum* species associated with mango in southern China. *Scientific Reports* **9**(1): 18891.
- O'Donnell K and Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**(1): 103–16.
- Prathibha V H, Rajesh M K, Dinesh A, Patil B, Nagaraja N R, Sabana A A, Gangaraj K P, Thejasri K P, Gangurde S S and Hegde V. 2024. Multi-gene phylogeny and phenotypic analyses revealed an association of different *Colletotrichum* species with inflorescence dieback and leaf spot of arecanut in India. *Physiological and Molecular Plant Pathology* **134**: 102416.
- Praveena S, Sujanalal P and Jose P A. 2025. First global report of pathogens causing anthracnose in threatened seedlings of *Hopea erosa* and *Cynometra beddomei*: A molecular and pathological study from the Southern Western Ghats, India. *Physiological and Molecular Plant Pathology* **140**: 102870.
- Talhinhas P and Baroncelli R. 2021. *Colletotrichum* species and complexes: Geographic distribution, host range and conservation status. *Fungal Diversity* **110**(1): 109–98.
- Teja A D V R, Samuel D K, Ruth C, Sakthivel T and Sriram S. 2022. Cross infectivity of *Colletotrichum* spp. on tropical fruit crops and *Ageratum* spp. (weed host) in Southern Karnataka and Andhra Pradesh. *Pest Management in Horticultural Ecosystems* **28**(1): 168–74.
- Templeton M D, Rikkerink E H, Solon S L and Crowhurst R N. 1992. Cloning and molecular characterization of the glyceraldehyde-3-phosphate dehydrogenase-encoding gene and cDNA from the plant pathogenic fungus *Glomerella cingulata*. *Gene* **122**(1): 225–30.
- Weir B S, Johnston P R and Damm U. 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* **73**: 115–80.
- White T J, Bruns T, Lee S and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. (In) *PCR Protocols: A Guide to Methods and Applications*, pp. 315–22. Innis M A, Gelfand D H, Sninsky J J and White T J (Eds). Academic Press, New York.
- Xavier K V, Kc A N, Peres N A, Deng Z, Castle W, Lovett W and Vallad G E. 2019. Characterization of *Colletotrichum* species causing anthracnose of pomegranate in the Southeastern United States. *Plant Disease* **103**(11): 2771–780.
- Zhang Y J, Zhang S, Liu X Z, Wen H A and Wang M. 2010. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. *Letters in Applied Microbiology* **51**(1): 114–18.
- Zhang Y, Xu H, Jiang S, Wang F, Ou C, Zhao Y, Ma L and Li Y. 2018. First report of *Colletotrichum truncatum* causing anthracnose on the berry stalk and the rachis of kyoho grape (*Vitis labruscana* × *V. vinifera*) clusters in Hebei, China. *Plant Disease* **102**(10): 2040–2040.