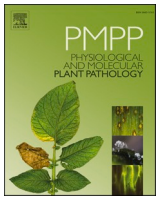
Physiological and Molecular Plant Pathology 134 (2024) 102416

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp



Multi-gene phylogeny and phenotypic analyses revealed an association of different *Colletotrichum* species with inflorescence dieback and leaf spot of arecanut in India

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ARTICLE INFO

Keywords:
Arecanut
Colletotrichum
Inflorescence
Dieback
Leaf spot
Blight

ABSTRACT

Arecanut is one of the economically important plantation crops. Among the various diseases, inflorescence dieback and leaf spot/blight diseases caused by *Colletotrichum* spp. have re-emerged as major factors impeding arecanut production in India in the last few years. To understand the *Colletotrichum* species complex in the arecanut system, we have characterized *Colletotrichum* spp. associated with inflorescence dieback and leaf spot/blight of arecanut based on a combination of phenotypic, multi-locus (*ITS*, *ACT*, *CHS-1*, *GAPDH*, *HIS3* and *TUB2*) phylogeny and pathogenicity studies. A total of 26 *Colletotrichum* isolates were obtained from arecanut inflorescence dieback (13 isolates) and leaf spot/blight (13 isolates) infected tissues collected from disease-endemic regions of Karnataka, Kerala and Tripura states of India. The combined multi-locus sequence analyses and a critical examination of phenotypic characters revealed the association of *Colletotrichum aoteaora* as a dominant species with arecanut inflorescence dieback disease. On the other hand, two *Colletotrichum* species, *viz*., *C. siamense* and *C. fructicola,* were distinctly associated with leaf spot/blight disease. All the *Colletotrichum* isolates studied were pathogenic to arecanut. Significant variations in lesion size were observed among the different species with cross-infection ability on inflorescence and leaves. This study represents the first comprehensive study of *Colletotrichum* spp. associated with arecanut inflorescence dieback and leaf spot/blight diseases. In addition, the study is the first record of *C. aoteaora, C. siamense,* and *C. fructicola* associated with the arecanut crop in this country. It provides valuable information for implementing effective IDM strategies against these diseases in India.

1. Introduction

Arecanut (*Areca catechu* L.) is one of the economically important plantation crops in Southern and Southeast Asia, including India, Bangladesh, China, Indonesia, Malaysia, Sri Lanka, the Philippines, and New Guinea. The crop supports the livelihood of millions of small and marginal farmers or traders (Vision 2050). Although arecanut production is limited to certain regions, commercial products are mostly shared worldwide (Guo et al., 2020). The economic produce of *A. catechu* fruit is called 'betel nut' and is used mainly for masticatory purposes (Peng et al., 2015). Many studies have revealed that arecanut possesses many pharmacological properties, *viz*., anti-oxidant, anti-fungal, anti-inflammatory, anti-bacterial, and anti-allergic effects (Peng et al., 2015). In addition, arecanut has been proven to act as an appetizer and energy booster and possesses cosmetic and anti-diabetic properties [1, 2].

The areca industry forms the monetary backbone of nearly six million people in India, and for most of those people, the crop is a main source of livelihood [3]. India accounts for about 57 per cent of world arecanut production, followed by China, Bangladesh, and Myanmar

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https://doi.org/10.1016/j.pmpp.2024.102416
Received 25 July 2024; Received in revised form 13 September 2024; Accepted 14 September 2024
Available online 16 September 2024

(FAO, www.fao.org/statistics). In India, arecanut is cultivated in an area of 512,266 ha with a total production of 827,639 t (Hort. Division, Department of Agriculture and Cooperation, 2021). It is largely grown in plains, foothills of the Western Ghats and North Eastern regions of India. It is cultivated naturally in valley conditions in a multi-storied cropping system along with black pepper, cardamom, nutmeg, banana, colocasia, ginger, and turmeric [4]. Although the production and productivity of arecanut in India have grown considerably in the past few decades, the prevalence of many diseases in the majority of the arecanut areas has adversely affected the arecanut industry [5].

Arecanut productivity is affected by several diseases and disorders. Among them, inflorescence dieback caused by *Colletotrichum gloeosporioides* is one of the economically important diseases since it causes partial or total crop loss in individual palms (Anonymous, 1970). According to previous studies, inflorescence dieback disease causes crop damage of up to 60 % [6]. The disease is seen throughout the year, but becomes severe from February to May and is accelerated by high temperatures prevailing during summer (Chandrarnohanan and Kaveriappa, 1985). Earlier, the cause of the shedding of female flowers and dieback of inflorescence were attributed to nutritional imbalance and physiological reasons or to lack of pollination and subsequent fertilization failure [7]. The presence of the fungus *Gloeosporium* in the shed nuts and inflorescence was reported by Anonymous [8]. Menon (1961) considered that the shedding of buttons, dieback, and discoloration was due to the effect of toxins produced by the fungus. Saraswathy et al. [6] confirmed the constant association of the fungus *Colletotrichum gloeosporioides* Penz with more than 70 per cent of the shed female flowers and infected inflorescences. *Colletotrichum* spp. can survive in the debris of the previous year's infected bunches for up to eight months [9].

Leaf spot is another important disease in arecanut and is normally observed in arecanut seedlings (one to two and half years old) and young arecanut palms (less than 10 years) [10]. This disease was reported in a severe form in major arecanut-growing districts in Karnataka and parts of the Kasaragod district of Kerala States of India, from 2015 onwards. The infection is restricted to three to four leaves of the outer whorl. The fungus isolated from infected portions was identified as *Colletotrichum* sp [11,12]. *Colletotrichum gloeosporioides* was the most frequently isolated species, and its pathogenicity has been established [13,14].

Earlier studies have shown that characters such as conidial size and shape, the shape of appressoria, the growth rate in culture, color of cultures, presence or absence of setae, and presence of teleomorph were useful tools for distinguishing groups within *C. gloeosporioides* [15–17]. In addition, conidial shape and size have also been utilized to distinguish the *C. gloeosporioides* group (Von Arx, 1970; [18]). However, issues occur because many morphological characteristics can alter with respect to various media, growing environments and repeated sub-culturing. Different isolates with genetically distinct characteristics can cause the same disease, with the shared pathogenicity likely evolving independently. For instance, the apple bitter rot disease is caused by both *C. acutatum* and *C. gloeosporioides* species complexes [19]. There is a lack of systematic studies on identifying isolates belonging to this C. *gloeosporioides* species complex [18]. Based on ITS sequences, ex-epitype isolate is distinct from other species previously confounded with *C. gloeosporioides, C. acutatum,* and *C. boninense* [20,21]. Biological and genetic relationships within the broad *C. gloeosporioides* clade remain obscure, and ITS sequences alone are insufficient to resolve them [22].

There was an increased incidence of inflorescence dieback and leaf spot/blight from 2015 onwards in major arecanut-growing states of India. In addition, no systematic study has been undertaken to understand the *Colletotrichum* species complex in the arecanut system. Thus, the present study aimed to determine the species complex of *Colletotrichum* associated with *Areca catechu* (arecanut) in India based on morphological and multi-locus gene analysis.

2. Materials and methods

2.1. Collection of affected arecanut samples and isolation of the pathogen

Inflorescence dieback and leaf spot-affected arecanut samples were collected from major arecanut-growing southern states (Karnataka, Kerala) and North East region (Tripura) of India during the disease epidemic period from 2016 to 2021. The pathogen was isolated by following the standard tissue isolation technique. In brief, dieback and leaf spot-affected inflorescence and leaf tissues were first washed in sterile distilled water and dried on sterilized tissue paper. Tissues were selected from the disease advancing edge and cut into small pieces (5 mm), then surface disinfected with 1 % sodium hypochlorite for 1 min, followed by washing three times in sterile distilled water and placed on antibiotic streptomycin (100 μg ml^{-1}) amended potato dextrose agar (PDA). Inoculated plates were incubated at 26 ± 1 °C with a 12–h photoperiod for three days in a BOD incubator (Remi, India). The emerging fungal colonies from the tissues were then aseptically transferred to PDA. Fungal isolates were purified using a single spore isolation technique [23]. Cultures were maintained on PDA slants at room temperature for further characterization.

2.2. Phenotypic analysis

For phenotypic characterization, mycelial discs were selected from the actively growing five-day-old cultures, inoculated in the center of 90-mm Petri plates containing PDA, and incubated at 26 ± 1 °C under continuous fluorescent light for seven days. The colony characters (color and texture) were recorded by following the mycological color chart described by Rayner [24]. Also, colony growth rate (mm day^{-1}) was recorded till seven days of incubation. To study conidial morphology, conidia were extracted from each isolate, and the shape and size of 100 randomly chosen conidia were recorded under the Nikon Eclipse 80i microscope. The data was analyzed in R software (Version, Ri3864.0.5, R core team, USA). Appressoria production was induced by following the slide culture technique [25]. Observations on the shape and size of the appressoria were taken on the fifth day after incubation. Three replicates were maintained for each isolate, and all the experiments were repeated thrice.

2.3. Molecular analysis

2.3.1. DNA extraction

Single spore-isolated fungal isolates were cultured in potato dextrose broth (PDB) for seven days to extract genomic DNA. Mycelia were harvested from PDB using sterilized filter paper (Whatman No.3), dried, and ground into a fine powder using liquid nitrogen. Subsequently, DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, USA). The quantity and quality of genomic DNA were measured using a spectrophotometer (Nanodrop ND-100, Thermo Fisher Scientific Inc., USA) and agarose gel (0.8 %), respectively. DNA was stored at −20 °C for further studies.

2.3.2. PCR amplification and sequencing

Six loci, including internal transcribed spacer region (*ITS*), actin (*ACT*), chitin synthase 1 (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), histone (*CYLH3*), and β-tubulin (*TUB2*) were amplified and sequenced. The details of the primer sequences and PCR conditions are given in Table 1. PCR reactions were carried out in a thermal cycler (T100TM, Bio-Rad) for 20 μl reaction volume consisting of 80 ng genomic DNA, primers, and Red Dye PCR Master mix (Genei, Bangalore, India).

The amplified products were separated by electrophoresis in a 1.5 % agarose gel in Tris-Borate-EDTA (TBE) buffer containing 0.5 μg ml^{-1} ethidium bromide, and amplification was detected using Gel Doc System (Alpha Imager, Alpha Innotech). Amplified PCR products of *ITS, ACT,*

Table 1
List of various genes, primer sequences and polymerase chain reaction (PCR) conditions.

Gene	Primer Sequence (5′-3′)	PCR Cycle	Reference
Actin (*ACT*)	ACT-512F 5′-ATGTGCAAGGCCGGTTTCGC-3′ ACT-783R 5′-TACGAGTCCTTCTGGCCCAT-3′	95 °C for 3 min, 35 cycles at 95 °C for 30 s, 54 °C for 45 s, 72 °C for 1 min, and a final step at 72 °C for 15 min	Carbone, I., & Kohn, L. M [26].
Beta tubulin (*β Tub*)	Bt2a 5′-GGTAACCAAATCGGTGCTGCTTTC-3′ Bt2b 5′-ACCCTCAGTGTAGTGACCCTTGGC-3′	95 °C for 4 min, 35 cycles at 95 °C for 30 s, 54 °C for 45 s, 72 °C for 60 s, and a final step at 72 °C for 15 min.	Glass and Donaldson [27]
Chitin synthase (*CHS-1*)	CHS-79 F 5′TGGGGCAAGGATGCTTGGAAGAAG-3′ CHS-354R 5′- TGGAAGAACCATCTGTGAGAGTTG-3′	95 °C 4 min, 35 cycles at 95 °C for 30 s,58 °C for 45 s, 72 °C for 45 s, and a final cycle of 15 min at 72 °C.	Carbone, I., & Kohn, L. M [26].
Glyceraldehyde-3- phosphate dehydrogenase (GAPDH)	GDF1 5′- GCC GTC AAC GAC CCC TTC ATT GA -3′ GDR1 5′- GGG TGG AGT CGT ACT TGA GCA TGT -3′	94 °C for 4 min, 35 cycles at 94 °C for 45 s, 60 °C for 45 s, 72 °C for 1 min, and a final cycle at 72 °C for 15 min.	Templeton et al. [28]
Histone (CYLH3)	CYLH3F 5′- AGG TCC ACT GGT GGC AAG -3′ CYLH3R 5′- AGC TGG ATG TCC TTG GAC TG -3′	95 °C 4 min, 35 cycles at 95 °C for 30 s,58 °C for 45 s, 72 °C for 45 s, and a final cycle of 15 min at 72 °C.	Crous et al. [29]
Internal transcribed spacer region (ITS)	ITS1 5′TCCGTAGGTGAACCTTGCGG3′ ITS4 5′TCCTCCGCTTATTGATATGC3′	94 °C for 2 min, 35 cycles at 94 °C for 45 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min.	White et al., 1990

CHS-1, CYLH3, GAPDH, and *TUB2* were purified using a Nucleospin® PCR clean-up and purification kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. Purified PCR products were sequenced (Agri Genom Labs Pvt Ltd, Kochi, Kerala, India). Sequencing was done in both forward and backward directions for each PCR product. Sequencing reactions used individual primers of all genes used in the PCR, and the sequences were analyzed by BLAST and deposited in Genbank (Supplemental Tables 3 and 4).

2.3.3. Sequence alignment and phylogenetic analyses

Multiple sequence alignment of *ITS, ACT, CHS-1, CYLH3, GAPDH,* and *TUB2* genes of *Colletotrichum* isolates was carried out separately using Clustal Omega (Hall 1999). All the individual sequence alignments were concatenated to produce a single multiple sequence alignment, and a phylogenetic tree was performed using MEGA (Version 11), including reference sequences downloaded from the Fungal Biodiversity Center (CBS-KNAW) (http://www.cbs.knaw.nl/Colletotrichum/) (Supplemental Table 3).

The ideal nucleotide substitution model was determined using the Jukes-Cantor model. The initial tree was created using the maximum parsimony method. Nearest-neighbour-interchange was used for maximum likelihood heuristics. The test for phylogeny was carried out using the bootstrap method with 50 iterations, *Monilochaetes infuscans* was used as an outgroup. The phylogenetic tree was constructed using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei, 1993) in MEGA-X software (Version 10.1.7). The robustness of the tree topologies was evaluated by bootstrap analysis using 1000 replicates (Tamura et al., 2011).

2.3.4. Pathogenicity test

The pathogenicity assays of *Colletotrichum* isolates were carried out on (a) inflorescence and (b) leaves of Mangala cv. by spot application of 20 µl of a conidial suspension (10^6 ml^{-1}) prepared from 10-day-old PDA cultures as described by Than et al. [21] with slight modifications. Control inflorescences and leaves were inoculated with 20 µl of sterilized distilled water and incubated at 26 ± 1 °C for seven days in plastic trays containing a wet cotton pad to maintain humidity. Three replicates were maintained for each isolate, and the experiment was repeated three times. Observations on the development of infection lesions on the inflorescences and leaves were measured, and data was analyzed in R software (Version, Ri3864.0.5, R core team, USA).

3. Results

3.1. Symptomatology and collection of isolates

The characteristic symptoms of inflorescence dieback disease are yellowing of the rachillae of male flowers from tip to end. As the infection spreads downwards, the rachillae turn dark brown and dry off subsequently. The subsequent spread of yellowing and discoloration induces the shedding of female flowers and shriveling of the developing embryos. There is a formation of conidial ooze in the form of concentric rings on infected rachillae (Fig. 1). This disease is one of the reasons for low fruit set and complete yield loss in case of severe infection in arecanut palms. The leaf spot symptoms are characterized as small brown to dark brown spots with varied sizes surrounded by a yellow halo on the leaf lamina. These spots later coalesce to form blighted patches with a typical yellow halo around the spots. Severe infection causes drying, drooping, and shredding of leaves, resulting in total yield loss (Fig. 2).

A total of 26 *Colletotrichum* isolates were purified from arecanut inflorescences (13 isolates) and leaves (13 isolates), showing typical symptoms of dieback and leaf spot diseases, respectively. The samples were collected from Karnataka (Shivamogga, Davanagere, Tumkur, Dakshina Kannada, Udupi, and Uttara Kannada), Kerala (Kasaragod and Kannur) and Tripura (Northeast) states of India, (Fig. 3).

3.2. Phenotypic analysis

Colletotrichum isolates could be divided into three morphological groups after thorough phenotypic analysis based on a combination of cultural and conidial characteristics (Table 2). Considerable variations were observed in the phenotypic characteristics of the *Colletotrichum* isolates, as detailed below.

Morpho group I (*Colletotrichum aoteaora*): This group isolates manifested a whitish-grey and slightly cottony-type colony with conidial ooze in the center. Conidia were hyaline cylindric, straight with a broadly rounded apex, often slightly tapering towards a sub-truncate base (Fig. 4). These were fast-growing, and the mean growth rate of 89 mm was significantly different from the other two groups. The mean conidial length of 19.2 µm is significantly different from other species,

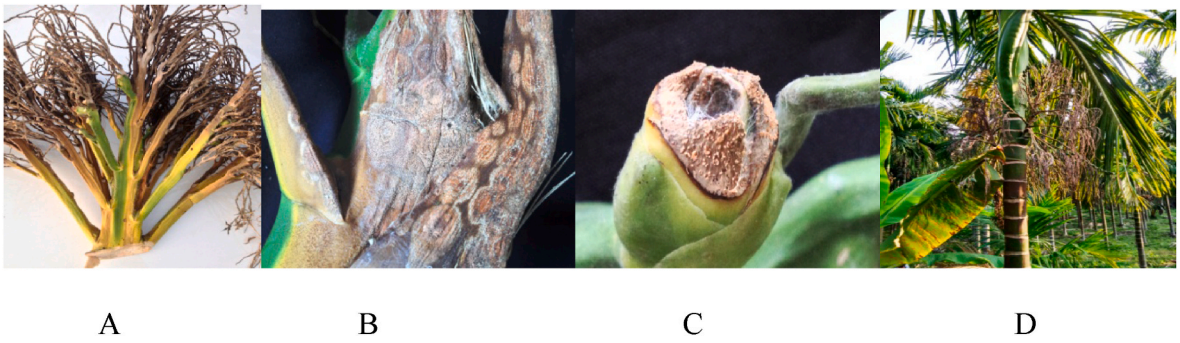

Fig. 1. Inflorescence dieback; yellowing of the rachillae and drying (A), orange color conidial ooze on infected rachillae (B) and nuts (C) and completely infected inflorescence (D).

Fig. 2. Leaf spot/blight; small brown to dark brown spots with yellow halo (A) spots coalesce and form blighted patches (B) severely leaf blight-affected garden.

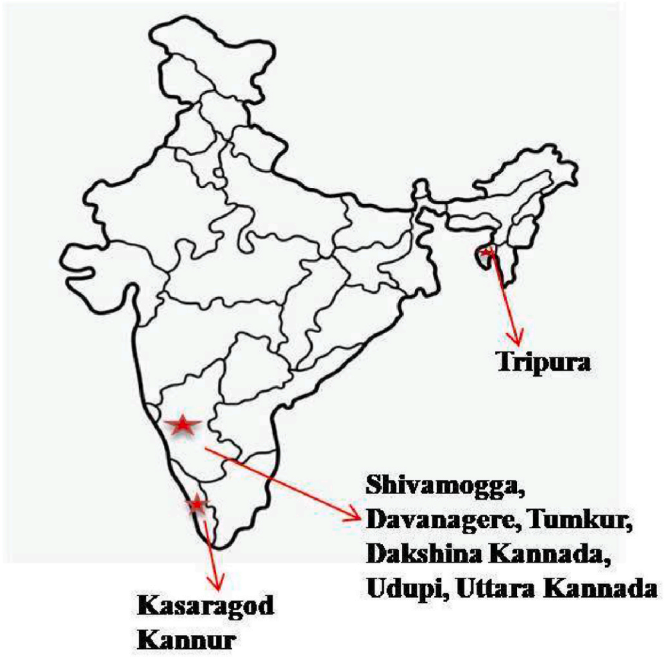

Fig. 3. Location map showing geo-distant *Colletotrichum* isolates sampled from major arecanut growing regions of India.

while there is no significant difference regarding width (5.0 μm). The values mentioned in the 'X' axis represents the number of isolates in replication belonging to each *Colletotrichum* species (Fig. 5). Appressoria were simple to broadly lobed and measures 11 ± 0.4 x 5.0 ± 0.5.

Morpho group II (*Colletotrichum siamense*): Isolates produced white to off-white colonies with dense cottony mycelia and were medium growing type with a mean colony growth rate of 72.5 mm (Fig. 4). Conidia were straight cylindric, fusiform to obtuse end with guttulate, and the mean conidia length and width was 18.1 with 5.0 μm, respectively (Fig. 5). Appressoria were globose to clavate and lobed with 11.5 ± 0.6 x 5.0 ± 0.2 μm in size.

Morpho group III (*Colletotrichum fructicola*): Isolates in this group formed a white-color colony with dense cottony mycelia, and the average growth rate was 60 mm (Fig. 4). Conidia were cylindrical with obtuse to slightly rounded ends and two round guttules with a mean length of 17.0 and width of 5.0 μm in size (Fig. 5). Appressoria were ovoid with 8.0 ± 1.0 x 4.0 ± 0.5 μm in size.

3.3. Pathogenicity test

The pathogenicity test of *Colletotrichum* isolates (*C. aoteaora, C. fructicola,* and *C siamense*) on rachilla of inflorescences of arecanut (Mangala cv.) revealed typical symptoms of dieback disease after four to six days of incubation. An initial characteristic yellowing symptom was noticed on the fourth day, followed by drying on the sixth day after incubation. However, significant variation in lesion size was observed among the different *Colletotrichum* isolates on both inflorescence and leaves. Among them, *C. aoteaora* isolates were more pathogenic, with a mean lesion size of 31.5 mm, followed by *C. siamense* (27 mm). While *C. fructicola,* on the other hand, had a 17 mm lesion size, which was less pathogenic on inflorescences. In the case of leaf spot/blight, the same trend could be observed in the development of infection lesions from four to six days. However, the highest mean lesion size (28 mm) was recorded in *C. siamense* inoculated leaves, followed by *C. aoteaora* (25 mm) and *C. fructicola* (19 mm) (Fig. 6). All the isolates of *Colletotrichum* were re-isolated from the infected inflorescence tissues and depicted the same phenotypic characters as previously observed.

Table 2
Grouping of *Colletotrichum* isolates infecting arecanut based on phenotypic analysis.

Morphology group	*Colletotrichum* species	Colony morphology		Conidial morphology	Appressoria morphology	
		Colony Color	Growth rate (mm/day)	Shape	Shape	Size (μm)
I	*C. aoteora*	Whitish grey colony and slightly cottony with conidial ooze in the center	11.0 ± 0.3	Cylindric, straight, apex broadly rounded, often slightly tapering towards sub-truncate base	Simple to broadly lobed	11 ± 0.4–5.0 ± 0.5
II	*C. siamense*	White to off-white with dense cottony mycelia	8.5 ± 0.5	Straight cylindric, fusiform to obtuse end, and guttulate	Globose to clavate and lobed	11.5 ± 0.6 5.0 ± 0.2
III	*C. fructicola*	White color colony with dense cottony mycelia	7.0 ± 0.2	Cylindric with obtuse to slightly rounded ends and two round guttules	Ovoid	8.0 ± 1.0–4.0 ± 0.5

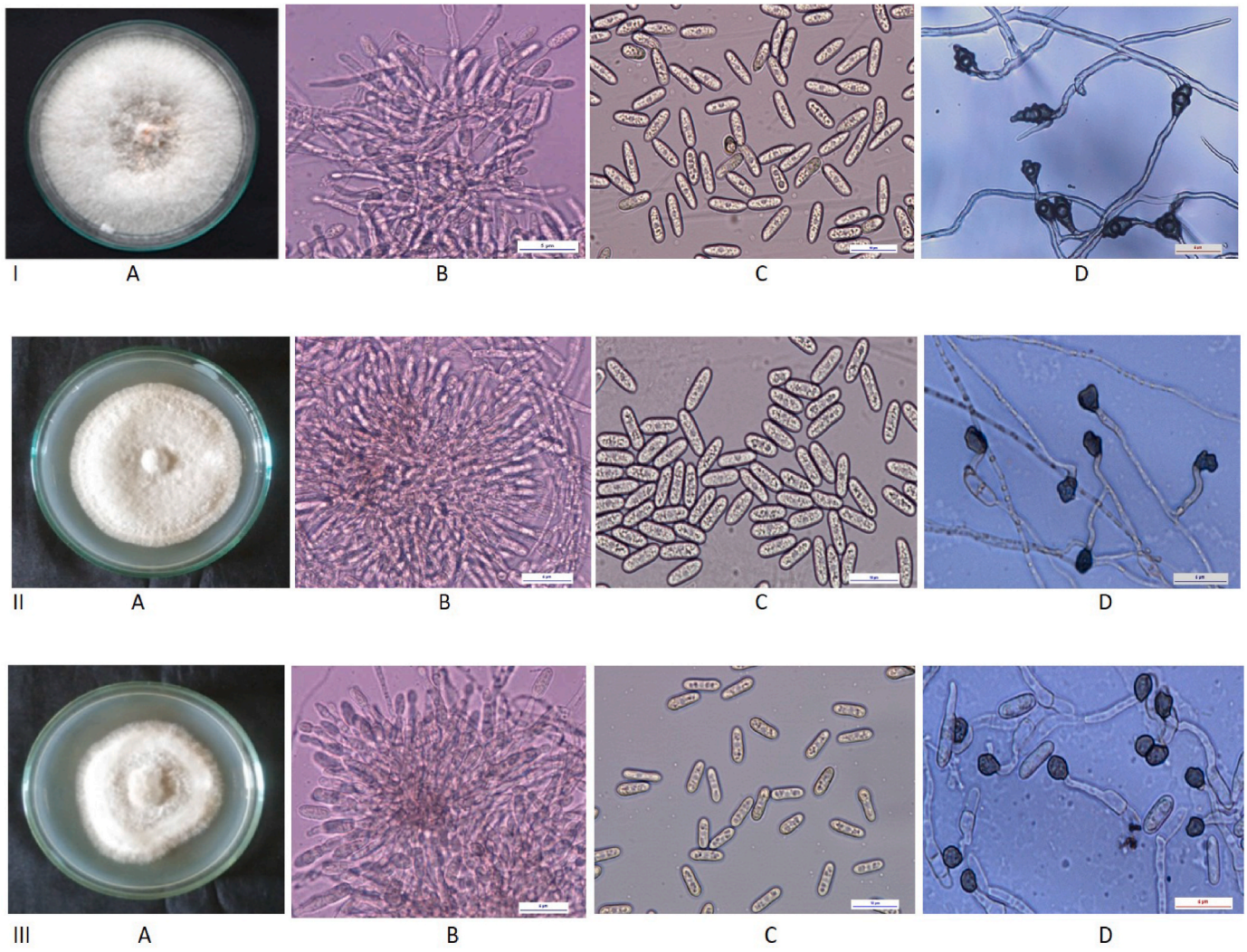

Fig. 4. Morphology of *Colletotrichum aoteaora* [I], *C. siamense*[II], and *C. fructicola*[III] associated with arecanut: Colony on PDA (A), Conidiogenous cells (B), Conidia (C) and Appressoria (D).

3.4. Phylogenetic analyses

The PCR amplification of *ITS, ACT, CHS-1, CYLH3, GAPDH,* and *TUB2*yielded590-600, 275–280, 270–300, 385–400, 280 and 550–600 bp long products, respectively. The dataset of combined multi-locus sequences used for phylogenetic analyses included 4454 characters, including the alignment gaps. The consensus tree constructed from Bayesian analyses confirmed the tree topology by bootstrapping estimations of RA x ML (Fig. 7). *Colletotrichum* isolates associated with arecanut were clustered in three well-defined groups representing three distinct species. The first cluster included isolates of *C. siamense* causing leaf spot/blight along with one isolate from inflorescence dieback. The second cluster consisted of *C. fructicola* isolates from leaf spots, and the third cluster included *C. aoteaora* isolates responsible for inflorescence dieback disease and highly virulent in nature. However, clustering is not congruent with the geographical location of isolates.

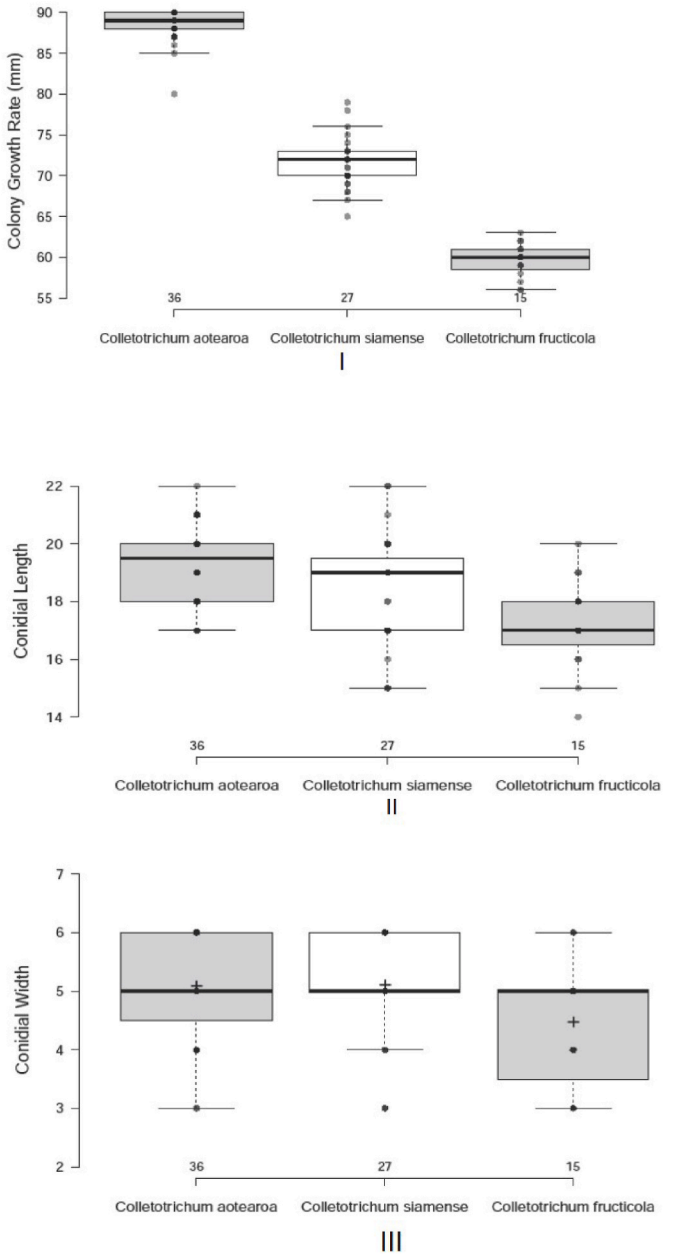

Fig. 5. Box and whiskers diagram showing colony growth rate [I], conidial length [II], and conidial width [III] of *Colletotrichum aoteaora*, *C. siamense,* and *C. fructicola* associated with arecanut.

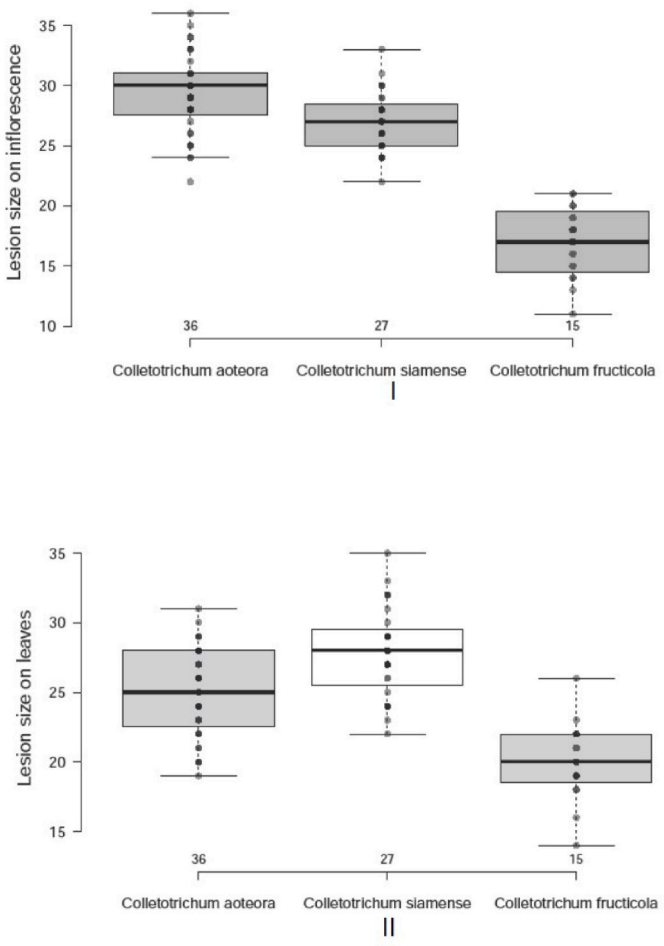

Fig. 6. Box and whiskers plot representing differences in lesion size (mm) manifested by *Colletotrichum aoteaora*, *C. siamense,* and *C.fructicola*on inflorescences [I] and leaves [II] of arecanut.

4. Discussion

Effective plant disease management depends on thoroughly understanding the host, pathogen, and environment. Unfortunately, for *Colletotrichum* spp., each aspect is complex and multi-dimensional. Accurate pathogen identification is important for designing appropriate, effective disease management strategies. The current study is the first attempt to use a polyphasic technique to describe *Colletotrichum* spp. Associated with inflorescence dieback and leaf spot/blight of arecanut. Based on morphological characteristics, *Colletotrichum gloeosporioides* has previously been identified as the causative organism of arecanut leaf spot/blight and inflorescence dieback [6,13,14].

The polyphasic approach, based on phenotypic characters and multilocus phylogeny, has successfully been used earlier too for resolving the taxonomic complexities in *Colletotrichum* [null], [22,30]. Thorough phenotypic and molecular analysis of 26 isolates revealed an association of three species of *Colletotrichum* as the causal agents of leaf spot/blight and one species of inflorescence dieback diseases of arecanut viz., *C. siamense, C. fructicola,* and *C. aotearoa* belongs to *C. gloeosporioides* species complex. *Colletotrichum gloeosporioides* was reported as incitant of leaf spot/blight and inflorescence dieback of arecanut [14]. However, in the present study, none of the isolates were identified as *C. gloeosporioides*. The lack of reproductive phases, limited morphological differences between species, and variations in morphology and pathogenicity have been identified as barriers to genus systematization [31,32].

The pathogen was previously identified and studied in this crop using only morphological tools without molecular methods. In addition, shifts in climatic factors were also attributed to the formation of new pathogenic strains. Out of 26 isolates purified from infected arecanut samples, 12 were identified as *C. aotearoa* based on phenotypic and multigene analysis, which is the only species found to be associated with inflorescence dieback of arecanut in India. *C. aotearoa* is fast growing with a cottony, grey to dark grey colony and cylindric, straight, broadly rounded apex, and often tapering conidia. *C. aotearoa* has been placed in the *C. gloeosporioides* species complex under the 'Kahawae' clade. It causes dieback, leaf and fruit spots in many fruit crops like banana, Berberis, mulberry, and many tree species [22,33,34].

C. siamense has previously been reported as a species complex on several hosts [22,35–38]. *C. siamense* was encountered only in severe leaf blight-affected arecanut grown in Tripura of Northeast India. *C. fructicola* has been identified as another species responsible for leaf spot/blight of arecanut crops in the southern region of India. It is

Fig. 7. Phylogenetic tree of *Colletotrichum* isolates associated with arecanut in India, constructed with concatenated multi-locus (*ITS*, *ACT*, *CHS-1*, *GAPDH*, *HIS3* and*TUB2*) sequences. The present study isolates are marked with red bracket.

important to note that the Musae clade members, *C. siamense* and *C. fructicola,* have similar geographic preferences and host-organ specificities.

C. fructicola is the most important species infecting fruit crops ([22]; Grammen et al., 2019; [32]). Characterizing the species and their aggressiveness on specific tissues is important for laboratory research and field application. Interestingly, *C. aotearoa* consistently produced larger lesions on the inflorescence of arecanut than other *Colletotrichum*species belonging to the same and different clades. In addition, the aggressiveness of *Colletotrichum* species within each complex frequently varies [39]. As witnessed, within the *C. gloeosporioides* complex, *C. siamense* infections on the leaf surface were significantly higher than *C. aotearoa* and *C. fructicola.*

5. Conclusions

This is the first systematic study on *Colletotrichum* species associated with *Areca catechu* in India. The study revealed that *C. aoteaora* is the dominant species associated with inflorescence dieback disease. The association of two *Colletotrichum* species, including *C. fructicola* and *C siamense,* with leaf spot/blight disease of arecanut. In addition, the study is the first record of *C. aoteaora, C. siamense,* and *C. fructicola* associated with the arecanut crop in this country. This, in turn, assists in understanding the *Colletotrichum* pathosystem in the arecanut crop. It also provides information for implementing plant quarantine and warrants the development of effective management strategies.

Funding

This research received funding from the Indian Council of Agricultural Research (ICAR-CPCRI Institute Project Code no. 1000765039).

CRediT authorship contribution statement

V.H. Prathibha: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization. **M.K. Rajesh:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Akshay Dinesh:** Writing – review & editing, Investigation, Formal analysis. **Balanagouda Patil:** Writing – review & editing, Investigation. **N.R. Nagaraja:** Formal analysis, Data curation. **A.A. Sabana:** Writing – review & editing, Methodology, Formal analysis. **K.P. Gangaraj:** Formal analysis, Data curation. **K.P. Thejasri:** Data curation, Writing – original draft. **Sunil S. Gangurde:** Writing – original draft, Formal analysis. **Vinayaka Hegde:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to the Indian Council of Agricultural Research (ICAR-CPCRI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2024.102416.

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