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Submission date: 11-Apr-2023 12:01PM (UTC+0700)
Submission ID: 2061296867
File name: Jackfruit_Artocarpus.pdf (1.58M)
Word count: 2842
Character count: 15497

Australasian Plant Disease Notes (2021) 16:24
https://doi.org/10.1007/s13314-021-00435-x

Jackfruit (*Artocarpus heterophyllus*), a new host plant of *Ceratocystis* wilt in South Sumatra, Indonesia

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Received: 22 February 2021 / Accepted: 4 August 2021

Abstract
In 2019, wilt and sudden death were observed on *Artocarpus heterophyllus* (jackfruit). Identification was performed by sequence analysis of the concatenated β-tubulin and ITS gene regions. Sequencing of the PCR product confirmed this pathogen was *Ceratocystis fimbriata* sensu stricto. This is the first report of *C. fimbriata* causing sudden death disease in *A. heterophyllus* in Indonesia and worldwide.

Keywords Sudden death disease · Moraceae · *Ceratocystis fimbriata* sensu stricto

Jackfruit (*Artocarpus heterophyllus*, Moraceae) is known in Indonesian as "Nangka", and is cultivated widely in many countries with tropical and subtropical climates. Jackfruit is among the most exported fruits worldwide and has considerable nutrition and health benefits (Ranasinghe et al. 2019).

In July 2019, wilt and die-back symptoms were observed for the first time on *A. heterophyllus* in the agricultural field of Sriwijaya University (Indralaya), Plaju (Palembang) and Gelumbang (Prabumulih), Indonesia. Wood of wilted trees showed a brown to black streaking in the woody xylem. Symptoms on the dying Jackfruit wood produced grey to brown lesions and included a streaking pattern of discoloration in the sapwood (Fig. 1a) and in some cases the lesions extended to heartwood (Fig. 1b). The lesion could be found partially or totally affecting the sapwood from the basal stem to the branches. Leaves of dying trees had yellowing symptoms, followed by wilting of the leaves on several lateral branches, drying of twigs and the wilt of the whole tree (Fig. 1c). This type of wilting was termed as sudden death or wilt (Pratama et al. 2021).

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Wood samples were taken from lesions of wilted trees using a knife sterilised in 70% ethanol. Each sample was wrapped in tissue paper and placed in a cool box. The same day, the wood samples (1–20 mm length, 1–2 mm thick) were sandwiched between two slices of fresh carrot and placed on sterile dry paper in plastic boxes at 25 °C following the method of Moller and DeVay (1968) (Fig. 1d). After 5–10 days, hat-shaped spores of putative *Ceratocystis* pathogens were placed on 2% (w/v) malt extract agar (MEA) (Merck, Germany), and incubated at 25 °C in a laboratory. The isolated fungi were initially identified based on morphological characteristics of a 14 day old culture. Mycelium on MEA grey, reverse side of colony olivaceous grey; submerged mycelium darkening as the ascomata develop forming fine, radiating fibrils.

Morphological traits of fruiting bodies and spores were observed under an optical Olympus CX33 microscope. Ascomatal bases dark brown to black, base subglobose to globose and measured (n = 100), 131.5–250.7 × 101.6–236.5 µm (Fig. 2a). Ascomata necks erect, occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, 324.7–579.1 µm long including ostiolar hyphae (Fig. 2b). Phialides pale brown to hyaline (Fig. 2c). Ascospores hat-shaped, 3.4–6.8 × 2.1–6.2 µm (Fig. 2d). Bacilliform conidia 11.1–36.1 × 2.1–7.4 µm (Fig. 2e). Barrel conidia 4.4–16.1 × 2.7–6.9 µm (Fig. 2f). Chlamydospores oval, thick walled, smooth, 6.7–16.5 × 5.9–12.9 µm (Fig. 2g). Based on these morphological characters, the fungus was identified as *Ceratocystis fimbriata*. Two representative isolates were deposited at the ICBB Culture Collection

Published online: 11 September 2021

Fig. 1 Symptoms of *Ceratocystis fimbriata* wilt disease in *Artocarpus heterophyllus:* **a** vascular discoloration of infected tree; **b** the discolored wood extended to the heartwood of the basal stem; **c** three-year-old tree with wilted, yellowing leaves and rotten fruit; **d** isolation of the fungus from discoloured xylem showing dark mycelium and sporulation on the carrot slices after 7 days

for Microorganisms and Cell Culture, Indonesian Center for Biodiversity and Biotechnology, (Bogor, Indonesia) as accessions ICBB9852 and ICBB9853.

To confirm the species identification, isolates were cultured on potato dextrose broth (PDB) at room temperature for one week. Mycelium was filtered through Whatman filter paper and genomic DNA was extracted from the fungal mycelial mat using YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). PCR conditions and reactions for two gene regions were used to identify the *Ceratocystis* isolates; the Internal Transcribed Spacer (ITS) with primers ITS 1 and ITS4 (White et al. 1990) and part of the β-tubulin (βt) gene with primers βt1a and βt1b (Glass and Donaldson 1995). Amplifications were carried out in 50 µl reactions containing 20 µl DreamTaq Green PCR Master Mix (Eppendorf, Germany) (DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM $MgCl_2$), 1.5 µl of each forward and reverse primer, 4 µl of DNA template and 23 µl sterilised water. The PCRs were performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycling parameters were as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. Amplification was completed at 72 °C for 10 min and the PCR product was stored at 10 °C. The PCR amplicons were sequenced at 1st BASE (Malaysia).

For the ITS and β-tubulin, amplification resulted in fragments of ~ 550 base pairs (bp) in size. The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers isolate ICBB9852 (MT355410; MT412106), isolate ICBB9853 (MT355412; MT412108), and isolate CAAW30817 (MT355413, MT412109) for the ITS and β-tubulin. β-tubulin datasets were generated using ex-type and ex-paratype sequences representing species in the Latin American (LAC) and Asian clade (AC) (Table 1) of the *C. fimbriata* species complex (Fourie et al. 2015; Oliveira et al. 2015; Barnes et al. 2018). To determine relatedness of isolates from jackfruit with known *C. fimbriata* populations, the ITS sequence was manually aligned with known ITS haplotypes as designated by Harrington et al. (2014), Li et al. (2016) and phylogenetic analyses were performed. Maximum Parsimony

Table 1 *Ceratocystis* isolates included in the phylogenetic analyses

Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	β-Tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	–
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	–
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	–
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	–
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	–
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	–
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	–
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	–
	ITS5	**ICBB9852**	***Artocarpus heterophyllus***	**Indonesia**	**MT355410**	**MT412106**
	ITS5	**ICBB9853**	***A. heterophyllus***	**Indonesia**	**MT355412**	**MT412108**
	ITS5	**CAAW30817**	***A. heterophyllus***	**Indonesia**	**MT355413**	**MT412109**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	–
	ITS5	A59662	*Camellia sinensis*	China	KF650948	–
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	–
	ITS5	P20053	*Punica granatum*	China	AM292204	–
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	–
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	–
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	–
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	–
	ITS8c	CMW17808	*Eucalyptus* sp.	Colombia	EF127990	–
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	–
	ITS9	C1558	*M. indica*	Brazil	AY157965	–
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	–
	ITS10	C994	*M. indica*	Brazil	AY157964	–
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	–
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	–
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	–
	ITS14	C1688	*M. indica*	Brazil	AY526291	–
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	–
	ITS16	C924	*G. arborea*	Brazil	HQ157539	–
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	–	DQ371652
	AC	CMW6579	*E. nitens*	Australia	–	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	–	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	–	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	–	EF070430
	AC	CMW19385	*E. grandis*	Australia	–	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	–	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	–	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	–	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	–	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	–	KJ631108

Table 1 (continued)

Species	Haplotype	Isolates no	Host	Origin	GenBank accession no	
					ITS	β-Tubulin
	LAC	CMW15051	*T. cacao*	Costa Rica	–	KJ601510
C. papillata	LAC	CMW8850	*Citrus* × *Tangelo hybrid*	Colombia	–	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	–	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	–	EF433307
	LAC	CMW28907	*M. indica*	Brazil	–	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	–	EF070443
	LAC	C1421	*I. batatas*	USA	–	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	–	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	–	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	–	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	–	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	–	KF302700
	LAC	CBS14653	*C. arabica*	Suriname	–	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	–	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	–	KJ601513

Isolates from jackfruit in Indonesia are marked in bold

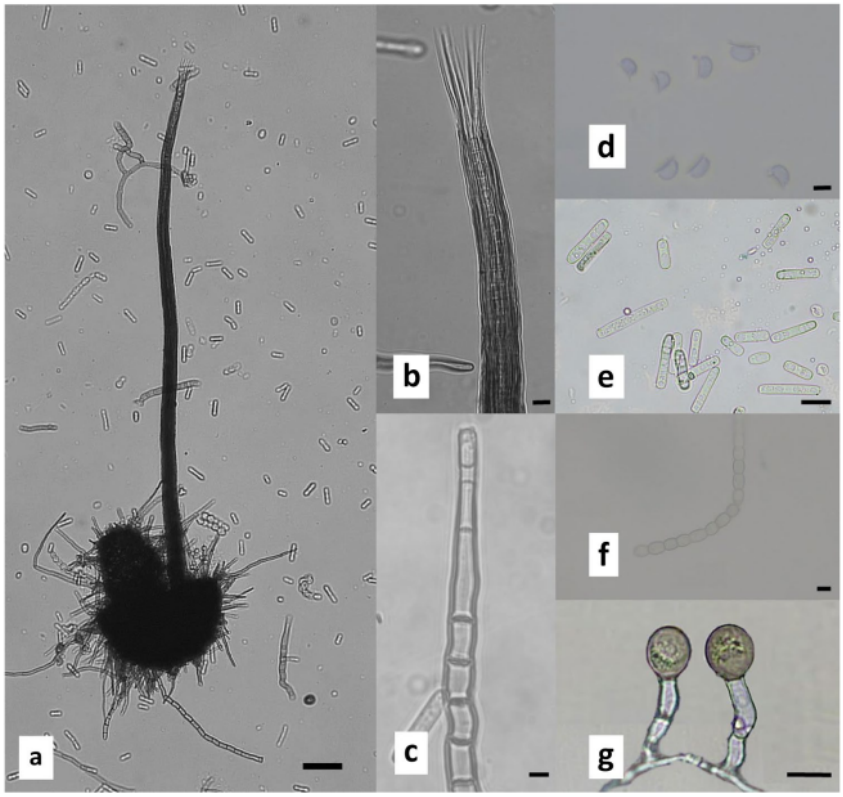

Fig. 2 Morphological characteristics of *Ceratocystis fimbriata* isolated from *Artocarpus heterophyllus* stem lesion: **a** ascomata with pirilliform base, **b** divergent ostiolar hyphae; **c** conidiophore/phialide; **d** hat-shaped ascospores; **e** cylindrical conidia; **f** Chain of barrel-shaped conidia; **g** chlamydospores of various shapes. Scale bars: a = 100 µm; b–c, e–g = 10 µm; d = 5 µm

(MP) analyses were performed in MEGA v. 10 (Kumar et al. 2016; Paul et al. 2018) with 1000 replications. The analysis involved 38 (β-tubulin) and 37 (ITS) nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 408 (β-tubulin) and 518 (ITS) positions in the final dataset. *Ceratocystis variospora* was

used as the out-group. β-tubulin sequence of our isolates confirmed the assignment to LAC of *C. fimbriata* sensu lato (Fig. 3a). Manual alignment of the ITS sequences with previously described ITS genotypes (Harrington et al. 2014; Li et al. 2016) grouped the isolates into ITS5 haplotype of *C. fimbriata* sensu stricto (Fig. 3b). Consistency (CI), retention (RI), and composite indexes (CoI) for β-tubulin were 0.566667, 0.845238, 0.668011, respectively and ITS was 0.933333, 0.976563, 0.932836, respectively.

The pathogenic potential of isolates was evaluated by the under bark inoculation method described by O'Gara et al. (1997) using five-month-old *A. heterophyllus* seedlings with stem diameters of 6–8 mm and heights < 1.5 m were prepared for pathogenicity test. Seedlings were grown in 10 cm diameter plastic pots containing a soil mix (topsoil + peat + chicken manure) under a 50% shading net. Plants were watered daily to maintain humidity, and any mortality occurring before the end of the experiment was recorded. Wounds were made on the stems of the seedlings using a cork borer (4 mm diam.), and mycelial discs (4 mm diam.) taken from an actively growing colony of *C. fimbriata* on 2% MEA (14 days) (Pratama et al. 2021) were placed in the wounds with the mycelium facing inwards. These were covered with Parafilm (Pechiney, Menasha, Wisconsin) to reduce contamination and desiccation. Ten plants of each tree species were inoculated with sterile MEA plugs to serve as controls (Fig. 4a). Fungal isolates were re-isolated and re-identified using morphological characteristics for confirmation of Koch's postulates. In pathogenicity tests, initial symptoms appeared two weeks post-inoculation as brown lesions at the inoculation site on the wood (Fig. 4b). Forty-five days after inoculation, plants exhibited wilt symptoms, lesions of wood discoloration extended to heartwood (Fig. 4c) and length of discolouration (downward + upward) was 17.88 until 34.74 cm. When re-isolated, the fungus was phenotypically identical to the prior isolate of *C. fimbriata* (ICBB9852, ICBB9853, CAAW30817).

This is the first report of *C. fimbriata* causing wilt and dieback in Jackfruit in Indonesia and worldwide. The symptoms

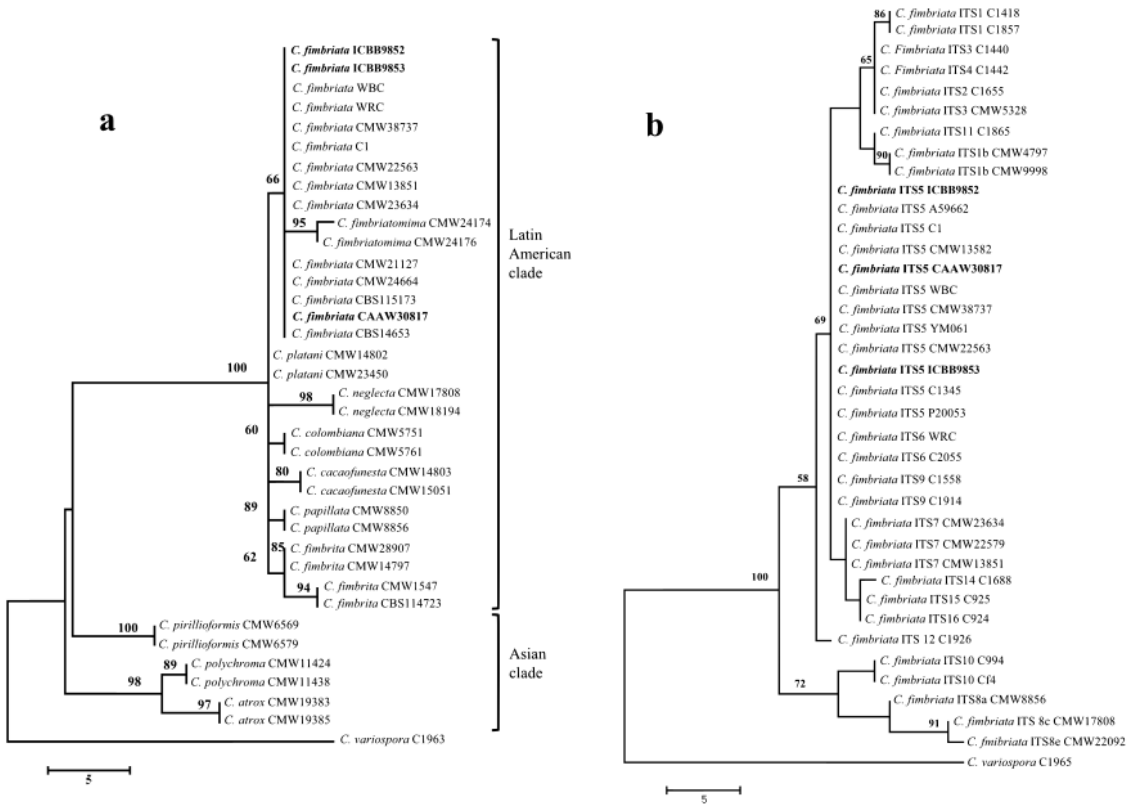

Fig. 3 Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by **a** β-tubulin sequences from Jackfruit tree in Indonesia (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. **b** ITS sequences from Jackfruit tree in Indonesia (marked in bold) and genotypes (sequences) of the *C. fimbriata* sensu stricto

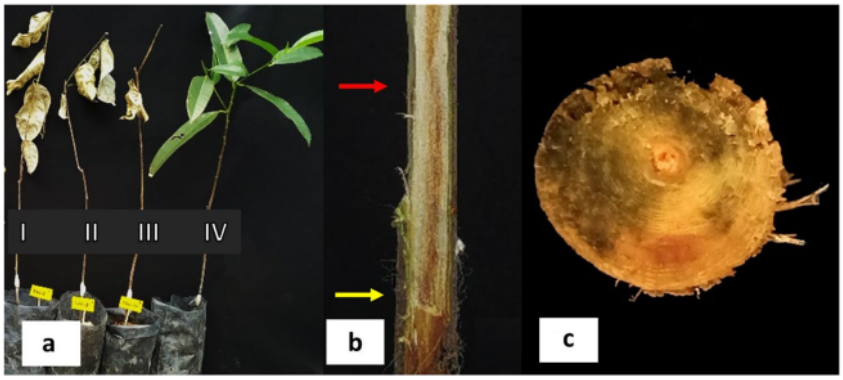

Fig. 4 Response of *Artocarpus heterophyllous* seedlings 45 days after under-bark inoculation with mycelium of *Ceratocystis*. **a** total wilting of plant inoculated with ICBB9852 (I), CAAW30817 (II), ICBB9853 (III) and the healthy control seedling (IV); **b** yellow arrow indicates the point of inoculation and red arrow the lesion boundary; **c** The discoloured wood extended to the heartwood of the basal stem of the seedling

of *C. fimbriata* wilt disease in Jackfruit include cankers on stems, with the stems becoming chapped as though torn apart, fruit rot and progressive loss of the canopy resulting in tree death. *Ceratocystis fimbriata* is a serious wilt pathogen of jackfruit, as well as of *A. mangium* and *A. crassicarpa* in Indonesia (Tarigan et al. 2011), *Lansium domesticum* in Indonesia (Suwandi et al. 2021) and Pomegranate in China (Li et al. 2016). *Ceratocystis fimbriata* infections of native trees in these countries could potentially lead to devastation of important components of the natural biodiversity in Indonesia.

Acknowledgements This research was funded by PMDSU scholarship with budget year of 2019-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service (DRPM), Directorate General for Research and Development, Ministry of Research, Technology, and Higher Education, Number: 068/SP2H/AMD/LT/DRPM/2020.

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