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RESEARCH NOTE

First report of *Phytophthora palmivora* (E. J. Butler) E. J. Butler, 1919 causing fruit rot in *Areca triandra* Roxb. ex Buch.-Ham. from India

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Abstract

Areca triandra Roxb. ex Buch.-Ham., a wild relative of cultivated arecanut (*Areca catechu* L.) is known as a resistant source for fruit rot caused by *Phytophthora meadii* Mc Rae. However, during monsoon 2019–20, a heavy shedding of immature nuts with whitish mycelial growth was observed from *A. triandra* plantations in Dakshina Kannada district, Karnataka, India. Pure culture of the associated oomycete was isolated from the symptomatic nuts and pathogenicity was confirmed. Based on morphological characteristics and internal transcribed spacer (ITS) sequences the pathogen was identified as *P. palmivora* E. J. Butler. To the best of our knowledge, this is the first report of *Phytophthora palmivora* infecting *Areca triandra* in the world.

Keywords *Areca catechu* · Plant pathogenic · Oomycete · Disease resistance · Nut fall

Areca triandra Roxb. ex Buch.-Ham. (Family-Arecaceae) is a shrubby subtropical palm growing mainly in the forest habitat. It is a wild relative of the commonly cultivated arecanut (*Areca catechu* L.) and mainly used as an ornamental palm, for chewing and timber purpose. Nuts contain tannins and alkaloids that aids in the stimulation of salival flow, accelerate heart and respiration rates, suppress hunger and offer positive protection against intestinal worms (Brown 1995).

During South-West monsoon of 2019–2020, heavy shedding of immature nuts of *A. triandra* was observed from plantations located at Sullia taluk of Dakshina Kannada district, Karnataka, India. Though fruit rot caused by *Phytophthora palmivora* E. J. Butler (Das and Cheeran 1986), *P. meadii* Mc Rae (Sastry and Hegde 1985), *P. heveae* (Chowdappa et al. 2002) and *P. arecae* Peth. (Pethybridge 1913) is prevalent in arecanut (*A. catechu*) growing tracts during monsoon season in India, there has been no previous record of fruit rot disease in *Areca triandra*. In India, *Areca triandra* was identified as a resistance source to arecanut fruit rot pathogen, *P. meadii* (Pratibha et al. 2015; Muralikrishna et al. 2018).

Present investigations recorded the symptoms of fruit rot of *A. triandra* as follows; i) Appearance of dark green water-soaked lesions near perianth during initial infection ii) presence of whitish mycelial growth over the entire fruit surface, iii) shedding off severely infected nuts, iv) discoloration of kernel with reduction in weight, v) infected nuts remains mummified without shedding at the end of the season (Fig. 1a). The aim of this study was to identify the causal organism associated with fruit rot disease of *A. triandra* observed in Karnataka, India (see Fig. 2).

A. triandra nuts showing typical fruit rot symptoms were collected from farmers' garden in Sullia, Dakshina Kannada district, Karnataka, India during South-West monsoon of 2019–2020 (Fig. 1a, b, and c). A total of 10 symptomatic nuts from five different palms were collected, cut into small pieces, washed under running tap water, surface sterilized in 2% NaOCl for 60 s, rinsed in distilled water three times, and air dried. A small bit of infected tissue was placed on carrot agar (CA) plates and incubated at 24 ± 2° C for 4–6 days (Ribeiro 1978).

Cultural and morphological characteristics of the isolated pathogen were recorded. Microscopic characteristics were confirmed based on at least 50 measurements of each structure under Leica DM LB2 compound microscope (Leica Microsystems Wetziar GmbH, Germany). Total genomic

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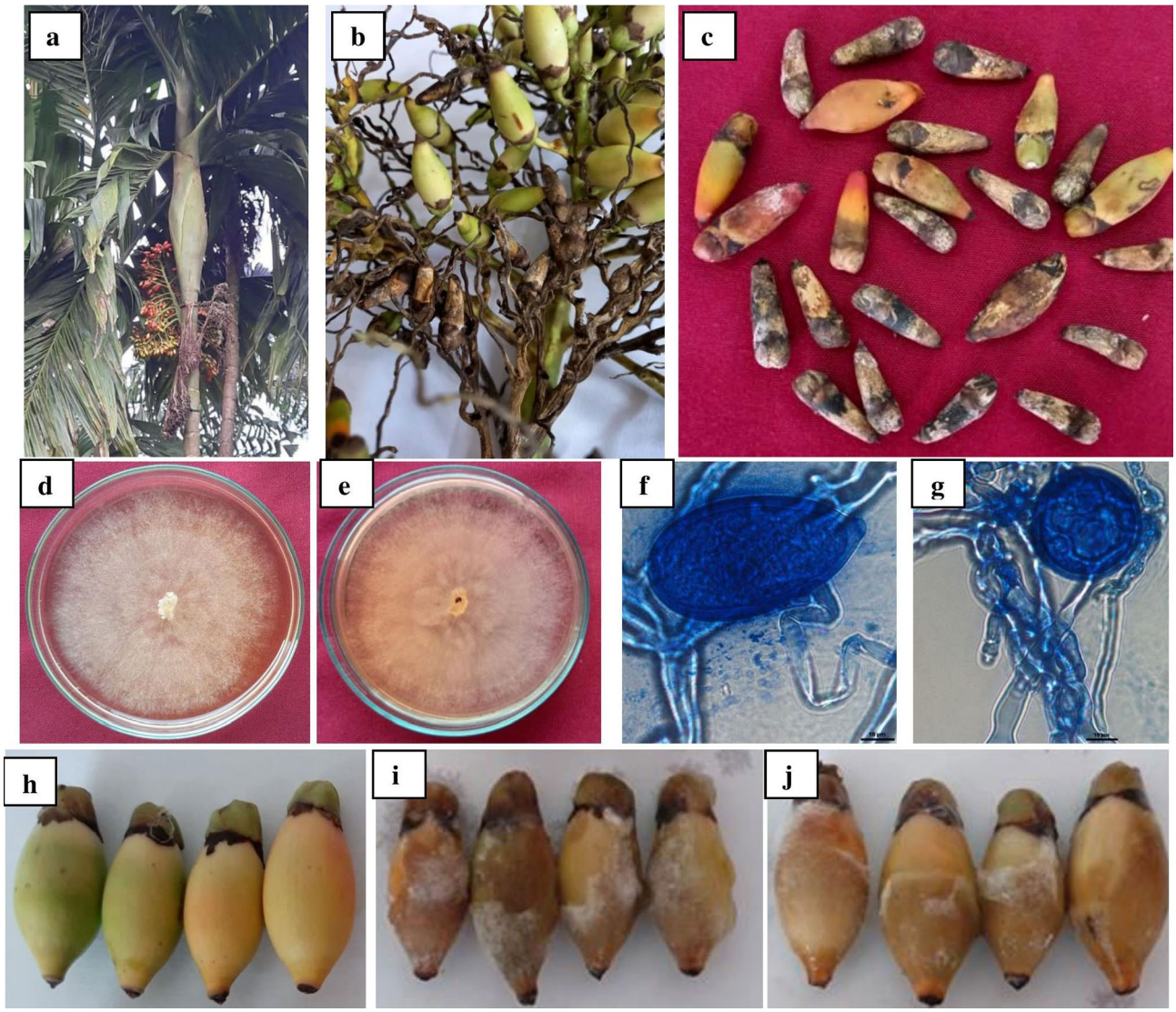

Fig. 1 **a** *Areca triandra* palms showing fruit rot symptoms; **b** Bunch and **c** Nuts showing typical fruit rot symptoms; **d** and **e** Pure culture of *Phytophthora palmivora* showing stellate type of mycelial growth on carrot agar plates; **f** *Phytophthora palmivora* producing typical papillate sporangia; **g** *Phytophthora palmivora* producing chlamydospore; Pathogenicity test of *P. palmivora* on **h** control and **i** inoculated *A. triandra* nuts; **j** Pathogenicity confirmation of re-isolated *P. palmivora* on *A. triandra* nut

DNA was isolated from the pure culture of the pathogen grown on Ribeiro's media (Ribeiro 1978) following CTAB method with minor modifications (Pandian et al. 2018). Molecular amplification of internal transcribed spacer region of ribosomal DNA using ITS1 (5'-TCCGTAGGT GAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGA TATGC-3') primers was carried out to confirm the identity of the pathogen (White et al. 1990). The amplified product was evaluated with electrophoresis (Major Science, USA) using a 1.2% agarose gel (Sambrook and Russell 2001). The PCR amplified products were purified using PCR purification kit (Geneaid, Taiwan) and purified products were sent for Sanger sequencing (Agrigenome labs Pvt. Ltd., Cochin, India). Sequences obtained were aligned using BioEdit (Biological sequence alignment editor – Tom Hall, http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and compared with the available sequences in NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) and BOLD database (http://www.boldsystems.org/). The multiple sequence alignment was performed by using the Clustal-W program with the pathogen sequence along with available sequences and *Colletotrichum gloeosporioides* as an out group (Thompson et al. 1994). Phylogenetic analysis was performed with MEGA6 software using the Maximum livelihood (ML) method with a bootstrap of 5000 replicates (Tamura et al. 2013). End trimmed pathogen sequence was deposited in NCBI.

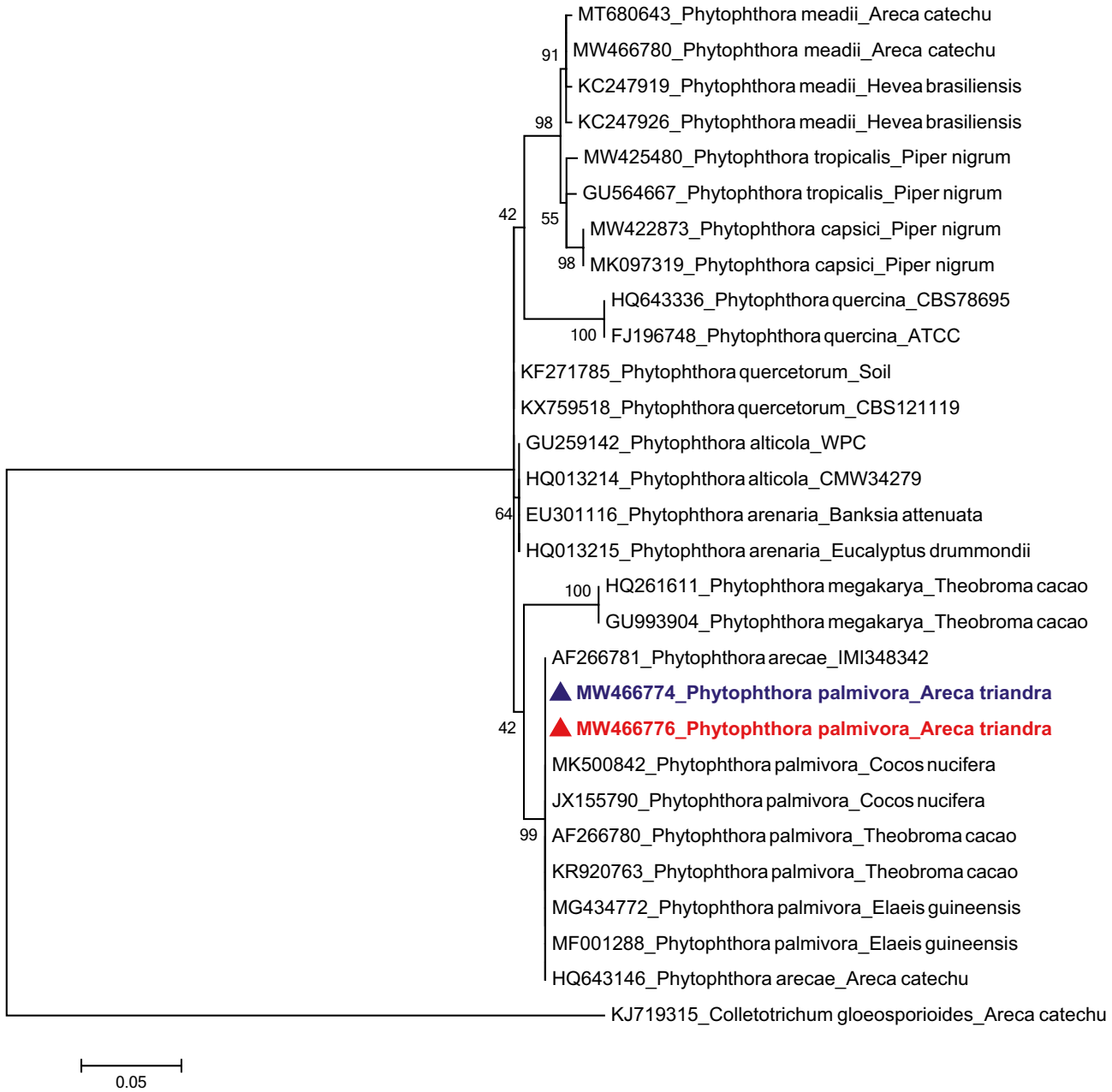

Fig. 2 Phylogenetic tree representing the genetic relatedness of *Phytophthora palmivora* isolated from *Areca triandra*, *Theobroma cacao*, *Cocos nucifera*, and *Elaeis guineensis*; with *Phytophhtora meadii* isolated from *Areca catechu* and *Hevea brasiliensis* and *Colletotrichum gloeosporioides* is used as outgroup retrieved from GenBank, inferred by the Maximum-Likelihood method using the ITS sequences. The robustness was evaluated with 5,000 bootstrap replicates, and numbers above the branches of the phylogenetic tree represent bootstrap values over 50%. Our strain is shown in bold triangle mark with blue (Primary culture) and red colour (re-isolated culture)

Pathogenicity was established by inoculating the spore suspension (1×10^6 zoospores ml^{-1}) on 10 healthy surface disinfected (70% ethanol) detached immature *A. triandra* nuts. Sterile distilled water (SDW) inoculated nuts were kept as control. Inoculated nuts were placed in a plastic box to maintain humidity and kept in the incubation chamber at 22 ± 2 °C with 95% humidity for 5 days. Five replications were maintained in a completely randomized design, and the experiment was repeated thrice. The identity of the pathogen re-isolated from the inoculated nuts showing typical symptoms of fruit rot disease was confirmed based on cultural, morphological and molecular characterization. Pathogenicity test was repeated with the re-isolated culture as explained above.

The culture of the isolated pathogen on CA plates showed stellate pattern with hyaline and aerial mycelium

(Fig. 1d, and e). Microscopic observations recorded aseptate mycelium and sympodial branching of sporangiophores with short pedicels and caducous sporangia. Sporangia are ovoid-ellipsoid to obpyriform in shape, distinctly papillate at the tip and round at the bottom (Fig. 1f). Sporangial size varies, but the average recorded is 28–33 × 42–58 µm. Globose to sub-globose shaped chlamydospores are produced (Fig. 1g). With these key microscopic features the isolated pathogen was identified as *Phytophthora palmivora* (Erwin and Ribeiro 1996). A reference specimen (isolate no-CPCRIVTLTriaS) is maintained in Plant Pathology repository, ICAR-CPCRI, Regional Station, Vittal, Karnataka, India.

The partial ITS region from the rDNA sequence of *P. palmivora* CPCRIVTLTriaS was deposited in GenBank (Accession number MW466774). The BLAST homology sequence analysis revealed 100% nucleotide similarity with *P. palmivora* ITS sequences available in NCBI with Accession No. AF266780. In the phylogram constructed using ITS sequences, *P. palmivora* infecting coconut, oil palm and cocoa formed a monophyletic clade with *P. arecae* infecting arecanut. The fruit rot pathogen from *A. triandra* was also grouped in the *P. palmivora* clade. The *P. meadii* pathogenic to rubber and arecanut formed a separate clade. However, *P. quercetorum, P. arenaria* and *P. alticola* formed a separate monophyletic group (Fig. 2). Cultural, morphological and molecular characterization confirmed the fruit rot pathogen infecting *A. triandra* as *P. palmivora.*

Pathogenicity test showed water soaked lesions on all the inoculated *A. triandra* nuts 2–3 days post inoculation (dpi). Whitish mycelial growth was observed on all the inoculated nuts 7-dpi and nuts became rotten 15-dpi (Fig. 1i). Control nuts inoculated with SDW remain healthy (Fig. 1h). The oomycete re-isolated from the infected nuts was identical to the primary culture originally isolated from the diseased palms with reference to morphology, microscopy and ITS sequences (GenBank Accession No. MW466776), thus fulfilling the Koch's postulates (Fig. 1j).

Earliest description on fruit rot in *Areca catechu* (*Phytophthora omnivora* de Bary) was recorded by Sydow and Butler in 1907. Since then, many reports on fruit rot incidence have been reported from different arecanut growing tracts in India. Regardless of confusion in taxonomy, *P. meadii* (Sastry and Hegde 1985; 1987), *P. palmivora* (Das and Cheeran 1986) and *P. heveae* (Chowdappa et al. 2002) were reported as causal organisms of fruit rot in *A. catechu*. Of these, *P. meadii* is predominant among all the arecanut growing tracts in India. *A. triandra* is gaining importance as breeder's choice due to its resistance against fruit rot caused by *P. meadii* in cultivated arecanut (Pratibha et al. 2015; Muralikrishna et al. 2018). However, its further role in breeding for disease resistance programme needs to be assessed with caution as arecanut is a high value crop in most South Asian countries. Changing weather conditions may support the emergence of *P. palmivora* in the arecanut ecosystem. Being wind borne in nature, this pathogen may spread effortlessly to new host plants. Hence, the occurrence and infestation of *P. palmivora* on the economically important arecanut crop is of very serious concern. Detailed studies on the host range, altitudinal distribution, and management aspects of this pathogen are in progress. Findings of the present study will aid the development of effective integrated disease management package for combating fruit rot disease caused by *P. palmivora* in *A. triandra*.

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Declarations

Ethical Approval This article follows the experimental guidelines of the country.

Conflict of interest The authors declare that they have no conflict of interest.

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