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Occurrence of stem and shoot cankers caused by *Phomopsis fukushii* on mango

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Abstract In July 2014, an outbreak of stem and shoot cankers was observed in mango experimental plots in Jeju, Korea. Initial disease symptoms were dark or reddish brown stem and shoot cankers on branches and leaves. Dieback symptoms followed in the areas above the lesions, progressing to development of numerous small white-brown pycnidia. The causative agent was initially identified as *Phomopsis fukushii* based on morphological characteristics, colony appearance, and shape of alpha and beta conidia of isolates recovered from cankers. Further confirmation was obtained by assessment of DNA sequences of internal transcribed spacer regions and translation elongation factor and actin genes.

Keywords Dieback · *Mangifera indica* · Mango · *Phomopsis fukushii*

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Mango (*Mangifera indica*, Sapindaceae) is cultivated mostly for edible fruit and is one of the 30 major fruits crops in the world. The mango is native to South Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics (Banerjee 2012). More than two hundred fungal isolates have been listed as associated with various diseases of mango worldwide (Farr and Rossman 2017). Several fungi cause stem and shoot diseases of mango, for an example *Botryosphaeria* (dieback), *Diplodia* (twig blight), *Pestalotiopsis* (stem end rot), *Phomopsis* (stem end rot), and *Verticillium* (*Verticillium* wilt). Stem and shoot cankers and stem end rot have reportedly been caused by *Phomopsis amraii* (India and Venezuela), *P. mangiferae* (Australia, Brazil, Asia including China, Japan, India, Malaysia, Pakistan, and Taiwan), and several different uncharacterized strains of *Phomopsis* sp. (Australia, India, Trinidad and Tobago, and the United States) (Farr and Rossman 2017). However, anthracnose caused by *Colletotrichum fructicola* (Joa et al. 2016) is identified as the only fungal disease of mangoes in Korea to date.

Fungi of the genus *Phomopsis* (teleomorph *Diaporthe*) are one of the major pathogens, which cause stem end rot of mango (during post-harvest storage of fruit), and can cause shoot cankers and dieback (Abreu et al. 2012) as well. Disease initiates at the nursery stage or in the orchard with mostly older trees in the orchard displaying susceptibility to the disease. Fungal hyphae initially colonize the floral parts, then move to the fruit pedicel where they remain quiescent until the fruit matures. *Phomopsis* is known to develop endophytically in healthy tissue of all parts of the plant (Johnson et al. 1992; Davidzon et al. 2010).

In July 2014, a widespread stem and shoot canker disorder with approximately 5% incidence was observed in mango trees in Jeju, Korea, which was able to kill entire branches and resulted in high losses of fruit production (Fig. 1a). The

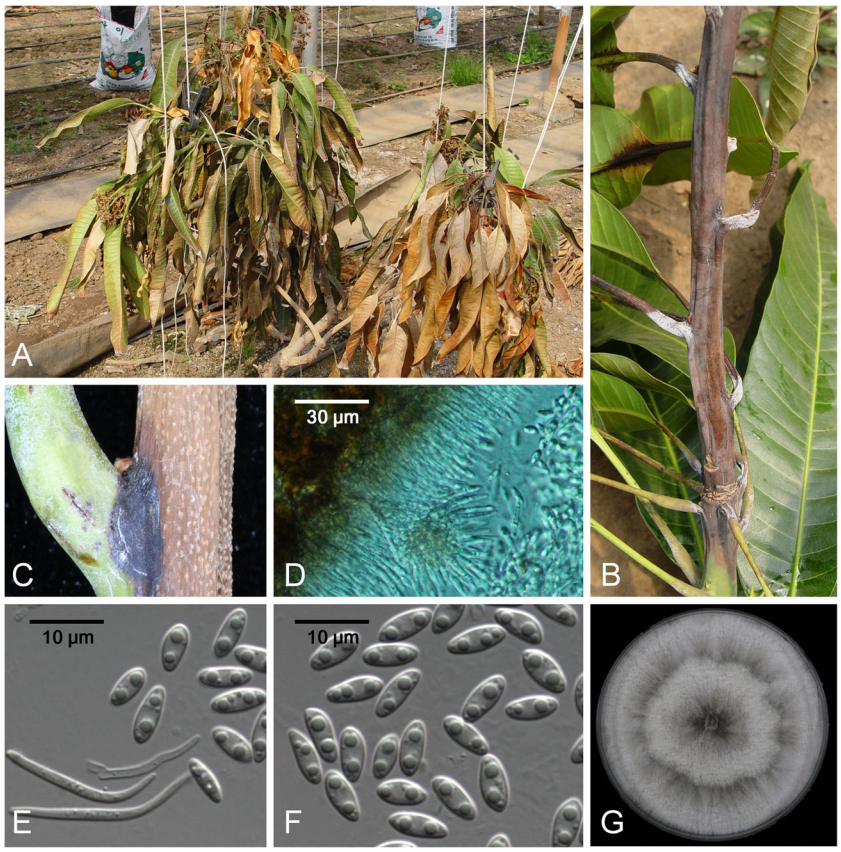

Fig. 1 *Phomopsis* stem and shoot cankers caused by *Phomopsis fukushii* on *Mangifera indica*. **a** Mango plants affected by *Phomopsis* stem and shoot cankers. **b** Magnified view of stem spots. **c** Pycnidia on the diseased bark. **d** Numerous conidiophores formed on the lesions (scale bar: 30 µm). **e, f** Alpha and beta conidia (scale bar: 10 µm). **g** Week-old colony of *P. fukushii* on potato dextrose agar

disease commenced with initial symptoms of shoot and stem cankers. A *Phomopsis* species was consistently isolated from the margins of dark or reddish brown cankered lesions. As a result of disease development, the affected branches started to wilt, leaves were desiccated and general dieback in the areas above the canker lesions became visible (Fig. 1b). In late summer, numerous small white-brown subglobose pycnidia formed on the diseased bark (Fig. 1c). A representative specimen was deposited in the Korea University Herbarium (accession No. KUS-F29461).

To identify the stem and shoot cankers, morphological characters were determined with a Zeiss AX10 microscope equipped with an AxioCam MRc5 digital camera (Carl Zeiss, Göttingen, Germany). To obtain a pure isolate, infected stem tissue with abundant pycnidia was directly placed in a drop of sterile water on a glass slide. Using a disposable bacterial loop, the resulting conidial suspension was streaked onto the surface of 2% water agar plates supplemented with 100 mg/L streptomycin sulfate. After 3 days of incubation at 25 °C, a single conidial colony was transferred to potato dextrose agar (PDA) with a sterile needle under a dissecting microscope. The isolates from infected mango show similar colony morphology. One of two representative monoconidial isolates (JBARES31 and JBARES 32) were deposited in the Korean Agricultural Culture Collection, Rural Development Administration, Wanju, Korea (accession No. KACC47836). After 7 days of incubation at 25 °C with 12 h photoperiod, characterization of colony morphology for the isolates revealed that the fungus formed white-black colonies with dense mycelia and containing at least two wide concentric rings of aerial hyphae (Fig. 1g). Conidiophores were filiform, hyaline, septate at the base, rarely branched, and up to 25 µm long (Fig. 1d). Alpha-conidia were broadly ellipsoidal or fusiform, unicellular, aseptate, guttulate and fusoid with obtuse ends, and 6–10 × 2–4 µm. Beta-conidia were hyaline, aseptate, filiform and curved with rounded ends, and 22–30 × 1.0–1.5 µm (Fig. 1e, f). The morphological characteristics of the organism were identical to the original descriptions of *Phomopsis fukushii* (Gomes et al. 2013).

To confirm the initial identification, a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) was used to extract genomic DNA from mycelia harvested from PDA cultures (KACC47836). Three targeted regions were chosen for multigene analysis. The complete internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified using primers ITS1/ITS4 (White et al. 1990), the primers EF1-728F/EF1-968R were used to amplify the EF-1α region, and ACT-512F/ACT-738R were used to amplify the ACT region (Carbone and Kohn 1999). The possible identity of the isolates was established by comparing their ITS, EF-1α, and ACT sequences with those in the GenBank database (National Center for Biotechnology Information [NCBI] US National Institute of

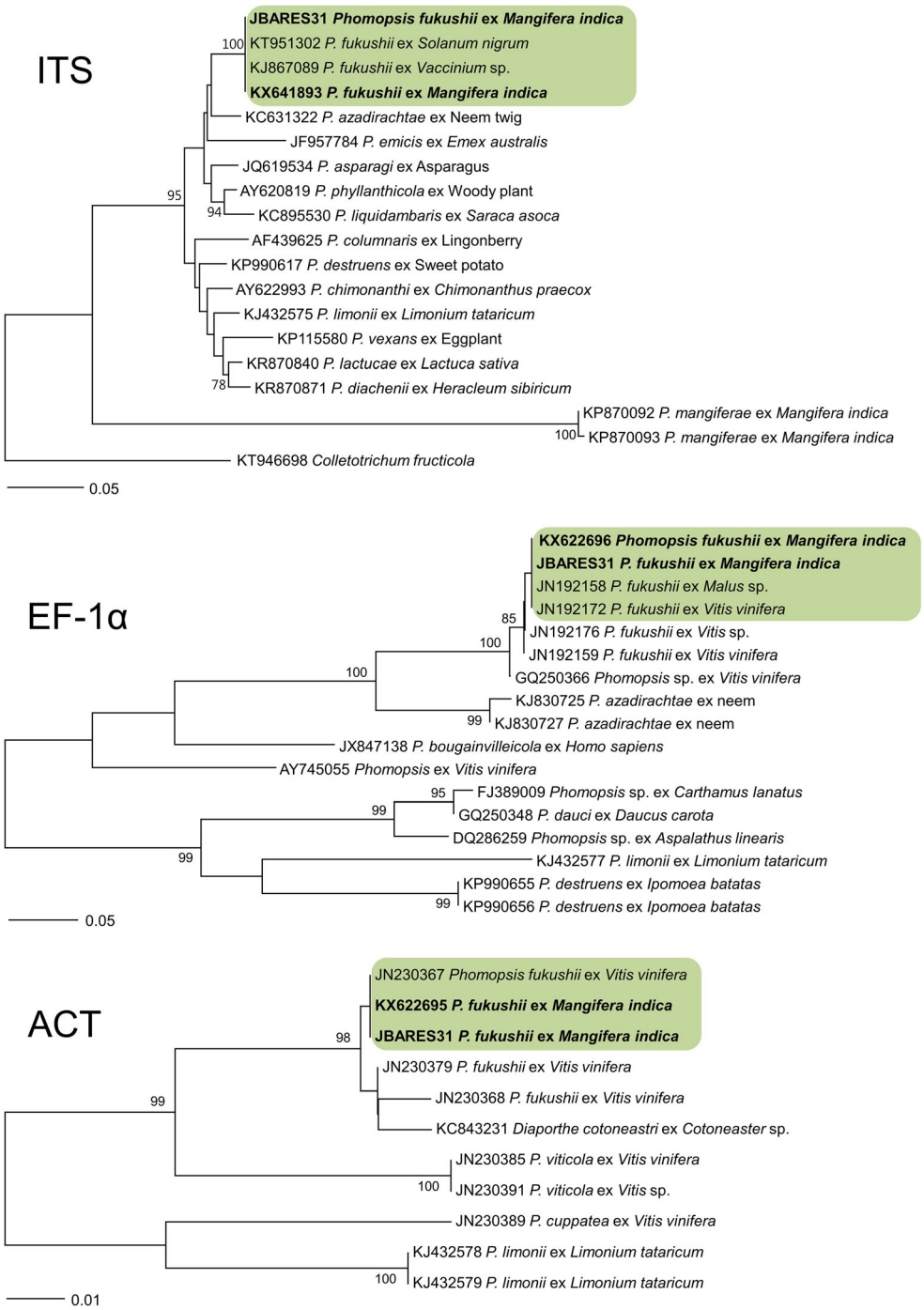

Fig. 2 Neighbor-joining trees based on the internal transcribed spacer [ITS], translation elongation factor 1-alpha [EF-1α], and actin [ACT] sequences of *Phomopsis fukushii* from *Mangifera indica*, and *Phomopsis* spp. retrieved from GenBank. The numbers above the nodes are the bootstrap values obtained from 1000 replicates. The isolates obtained in this study are shown in boldface

Health, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/BLAST). The ITS, EF-1α, and ACT sequences obtained from the two isolates were determined to be identical.

The resulting 544-bp ITS, 370-bp EF-1α, and 284-bp ACT sequences obtained from KACC47836 were deposited in GenBank (accession Nos. KX641893, KX622696, and KX622695, respectively). A GenBank BLAST search of the Korean sequences showed 100% identity with sequences of the three loci from *P. fukushii* isolated from mango fruit (i.e., KT951302 and KJ867089, JN192158 and JN192172, and JN230367).

Phylogenetic analysis of selected *Phomopsis* sequences from GenBank, including ITS, EF-1α, and ACT, was conducted using SeqMan software (Lasergene; DNASTAR, Madison, WI, USA) for editing and assembly. The phylogenetic relationship inferred from the ITS, EF-1α, and ACT sequences showed that the two isolates from our study formed a well-supported sister clade to *P. fukushii*, and revealed a separate clade distinct from other

species in the Diaporthaceae (Fig. 2). However, the EF-1α and ACT sequences varied among *P. fukushii* isolates with identical ITS sequences.

Pathogenicity tests were carried out on five 2-year old 'Irwin' mango plants, using a cork borer 5 mm diameter bark disc was removed, replaced it with a 5-mm fungal plug of which was obtained from 7-day-old *P. fukushii* culture grown on PDA, and covered it with sterile wet cotton wool and sealed immediately using Parafilm™ to prevent contamination and desiccation. Sterile un-inoculated PDA plugs applied to a similar number of plants using the same method served as controls. Plants were kept in a growth chamber for 24 h at 25 °C with 12-h photoperiod and then placed in a greenhouse (25 ± 2 °C, 70% relative humidity). Typical stem rot symptoms were observed in the inoculated plants 30 days after inoculation, and were identical to those observed in the field. No symptoms were observed in control plants. Fungi were re-isolated from the lesions of inoculated plants and confirmed to be *P. fukushii*, fulfilling Koch's postulates. Pathogenicity tests were conducted twice and obtained similar results.

Phomopsis has previously been recorded on several fruit trees belonging to the family Rosaceae, for example *Pyrus pyrifolia*, *P. bretschneideri*, *P. communis*, *Malus pumila*, and *Vaccinium vitis-idaea* and also on some non-rosaceous species (Castlebury et al. 2002). In particular, the association of *Phomopsis* species with mango has been reported in India and Venezuela (*P. amraii*); Australia, Brazil, China, Japan, India, Malaysia, Pakistan, and Taiwan (*P. mangiferae*); and Australia, America, and India (*Phomopsis* sp.) (Farr and Rossman 2017). This is the first report on *P. fukushii* infection of *Mangifera indica* globally and the first report of *Phomopsis* in Korea.

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