Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* Causing Postharvest Fruit Rot on Guava (*Psidium guajava*) in Malaysia

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Guava (*Psidium guajava* L.) is an economically important tropical fruit crop and is cultivated extensively in Malaysia. In September and October 2019, postharvest fruit rot symptoms were observed on 30 to 40% of guava fruit cultivar Kampuchea in fruit markets of Puchong and Ipoh cities in the states of Selangor and Perak, Malaysia. Initial symptoms appeared as brown, irregular, water-soaked lesions on the upper portion of the fruit where it was attached to the peduncle. Subsequently, lesions then progressed to cover the whole fruit. Lesions were covered with an abundance of black pycnidia and grayish mycelium. Ten symptomatic guava fruits were randomly collected from two local markets for our investigation. For fungal isolation, small fragments (5 × 5 mm) were excised from the lesion margin, surface sterilized with 0.5% NaOCl for 2 min, rinsed three times with sterile distilled water, placed on potato dextrose agar (PDA), and incubated at 25°C with 12-h photoperiod for 2 to 3 days. Eight single-spore isolates with similar morphological characteristics were obtained, and two representative isolates (P8 and S9) were characterized in depth. Colonies on PDA were initially composed of grayish-white aerial mycelium but turned dark gray after 7 days. Abundant black pycnidia were observed after incubation for 4 weeks. Immature conidia were hyaline, aseptate, ellipsoid, thick-walled, and mature conidia becoming dark brown and one-septate with longitudinal striations, 25.0 to 27.0 ± 2.5 × 13.0 to 14.0 ± 1.0 µm ($n = 30$). On the basis of morphology, both representative isolates were identified as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Alves et al. 2008). For molecular identification, genomic DNA of the two isolates was extracted using the DNeasy plant mini kit (Qiagen, U.S.A.). The internal transcribed spacer (ITS) region of rDNA and translation elongation factor 1- α (EF1- α) genes were amplified using ITS5/ITS4 and EF1-728F/EF1-986R primer sets, respectively (Carbone and Kohn 1999; White et al. 1990). BLASTn analysis of the resulting ITS and EF1- α

sequences indicated 100% identity to *L. theobromae* ex-type strain CBS 164.96 (GenBank AY640255 and AY640258, respectively) (Phillips et al. 2013). The ITS (MW380428, MW380429) and EF1- α (MW387153, MW387154) sequences were deposited in GenBank. Phylogenetic analysis using maximum likelihood based on the combined ITS and TEF sequences indicated that the isolates formed a strongly supported clade (100% bootstrap value) to the related *L. theobromae* (Kumar et al. 2016). A pathogenicity test of two isolates was conducted on six healthy detached guava fruits per isolate. The fruits were surface sterilized using 70% ethanol and rinsed twice with sterile water prior inoculation. The fruits were wound inoculated using a sterile needle according to the method of Oliveira et al. (2014), and 5-mm-diameter mycelial agar plugs from 7-day-old PDA culture of the isolates were placed onto the wounds. Six additional fruits were wound inoculated using sterile 5-mm-diameter PDA agar plugs to serve as controls. Inoculated fruits were placed in a sterilized plastic container and incubated in a growth chamber at 25 ± 1°C, 90% relative humidity, with a photoperiod of 12 h. The experiment was conducted twice. Five days after inoculation, symptoms as described above developed on the inoculated sites and caused a fruit rot; the control treatment remained asymptomatic. *L. theobromae* was reisolated from all symptomatic tissues and confirmed by morphological characteristics and by PCR using ITS region. *L. theobromae* has recently been reported to cause fruit rot on rockmelon in Thailand (Suwannarach et al. 2020). To our knowledge, this is the first report of *L. theobromae* causing postharvest fruit rot on guava in Malaysia. The occurrence of this disease needs to be monitored, because this disease can reduce the marketable yield of guava. Preventive strategies need to be developed in the field to reduce postharvest losses.

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