Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia pseudotheobromae* Causing Stem End Rot of Mango Fruit in Pakistan

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Mango (*Mangifera indica* L.) is considered a desirable fruit in international markets and is grown throughout tropical and subtropical countries around the world (Alemu 2014). Stem end rot is the most damaging and complex postharvest disease of mango, resulting in losses of up to 40% in Pakistan, which is the leading producer and exporter (Alam et al. 2017). A field survey was conducted in June of 2017 and 2018 in Rahim Yar Khan and Multan—major mango producing regions of Punjab Province. After mature but unripe mango fruit (cv. Samar Bahisht Chaunsa) were stored at 12°C for 2 weeks to permit ripening, water-soaked, dark brown to purplish black decay began to appear around the stem end portion. The decay gradually enlarged and covered the whole fruit after 7 days. Disease incidence was estimated at 30%. Small pieces (3 to 4 mm²) from the periphery of 15 diseased fruit were surface disinfected with 1% sodium hypochlorite for 2 min, rinsed three times in sterilized distilled water, air dried, and then placed aseptically onto potato dextrose agar medium and incubated at 25°C under a 12-h light/dark photoperiod for 7 days. Twelve single-spore isolates with similar morphology were isolated from the infected tissues. Initially the fungus produced thick, fluffy, and greyish-white aerial mycelium, which later turned into dark gray colonies. Conidia were unicellular, ellipsoidal, and initially hyaline but with age became dark brown and developed a central septum. Conidia measured 24.5 to 31.5 × 11.4 to 15.7 µm ($n = 60$). Conidiophores were inflated at their base with one diaphragm, which reduced to conidiogenous cells. Conidiogenous cells were hyaline and cylindrical. On the basis of morphological

characteristics, the fungus was tentatively identified as *Lasiodiplodia* sp., a member of the family Botryosphaeriaceae (Alves et al. 2008). For molecular identification, genomic DNA was extracted from mycelium following the CTAB method. The internal transcribed spacer (ITS) region of rDNA and translation elongation factor 1-alpha (TEF1- α) gene were amplified using ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R primer sets (Carbone and Kohn 1999), respectively. BLASTn searches of sequences revealed 99 to 100% identity with the reference sequences of various *Lasiodiplodia pseudotheobromae* isolates (GenBank accession nos. MH057189 for ITS and MN638768 for TEF1- α). The sequences were deposited in GenBank (accession nos. MW439318 and MW433883 for ITS and MW463346 and MW463347 for TEF1- α). To fulfill Koch's postulates, a suspension of 10⁵ conidia/ml from a 7-day-old culture of *L. pseudotheobromae* was used to inoculate fully mature but unripe mango fruit (cv. Samar Bahisht Chaunsa). Fruit were pricked with a sterilized needle to a depth of 4 mm at the stem end portion, injected with 50 µl of the prepared spore suspension (Awa et al. 2012), and stored at 12°C for 3 weeks under 70 to 80% relative humidity. Twenty mango fruit were inoculated, and 10 were inoculated with sterile water only. After 15 days, most fruit showed typical symptoms at the stem end. Reisolations from symptomatic fruit following the procedures described above for isolating and identifying the fungal cultures from infected field samples consistently yielded a fungus identical to *L. pseudotheobromae*. Control fruit remained disease-free. Although *L. pseudotheobromae* was previously reported on several forest and fruit trees (Alves et al. 2008; Awan et al. 2016), this is the first report of the pathogen causing stem end rot disease of mango in Pakistan. This report is important for the new studies aiming at management of stem end rot disease of mango caused by *L. pseudotheobromae* in Pakistan.

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