Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Macrophomina phaseolina* Causing Root Rot of *Curcuma longa* in China

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Curcuma longa L. is one of the most important crop plants, with great medicinal and economic significance, in the Zingiberaceae family. Its medicinal uses are diverse, including antitumor, anti-inflammatory, antioxidant, antimicrobial, treating Alzheimer's disease, and HIV activities, so this plant is considered to be one of the most promising economic crops in China (Xiao et al. 2011). In 2018, symptoms of wilting were observed on *C. longa* plants in the fields in ChengMai scientific base of Hainan Academy of Agricultural Sciences, and the disease incidence was approximately 15%. Infected plants initially showed leaf chlorosis and wilting, and the plants eventually died with the leaves remaining attached. When affected plants were examined, the basal stem pith was rotted, and a brown discoloration was observed after splitting the roots and stems. Diseased root tissues from six *C. longa* plants were sliced into 0.1-cm-thick

cross sections, surface sterilized by immersion in HgCl₂ (0.1%) for 1 min followed by immersion in ethanol (75%) for 1 min, and rinsed with sterile double distilled water three times, prior to aseptic transfer to potato dextrose agar (PDA) plates. Cultures were grown at ambient temperature (28°C) for 7 days in the dark. Four pure cultures were obtained for subsequent experiments. Colonies of the isolates were black on PDA; the mycelia were initially hyaline and turned from gray to dark with age. Masses of dark oblong microsclerotia that averaged 91 (length) × 68 (width) μm (*n* = 30) formed on the cultures 3 days after incubation. The molecular identification was done by amplifying the sequences of internal transcribed spacers (ITS) using ITS1/ITS4 primers (Mills et al. 1992) and β-tubulin (TUB) using Bt2a/Bt2b primers (Glass et al. 1995). BLAST searches in GenBank database indicated that the ITS and TUB sequences had 99 and 100% similarity to *Macrophomina phaseolina* ex-type strain CPC 21535 (KF951785, KF952227), respectively. Representative sequences were deposited in GenBank (ITS, MN629245; TUB, MN635700). Based upon the morphological and molecular results, the isolates were identified as *M. phaseolina* (Nouri et al. 2019). For the pathogenicity test, six 1-year-old healthy *C. longa* plants were inoculated with a 5-mm fungal disc on PDA of a 7-day-old *M. phaseolina* colony, buried 1.5 cm deep, approximately 2 cm from the base of the plant, and covered with sterile soil mixture. PDA plugs without mycelia were used for control plants. All plants were placed in the greenhouse at 28°C and 80% relative humidity. Symptoms of wilting were observed after 4 weeks on all inoculated plants, and no symptoms were observed in the control plants. *M. phaseolina* was reisolated from inoculated plants, and the experiment was repeated with similar results, fulfilling Koch's postulates. To our knowledge, this is the first report of root rot of *C. longa* caused by *M. phaseolina* in China.

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