Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diplodia mutila* Causing Leaf Blight on *Magnolia grandiflora* in China

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Magnolia grandiflora is a widely cultivated ornamental tree in China. In June 2020, a leaf blight disease was observed on *M. grandiflora* at Guizhou University (26.4457°N, 106.6594°E) in Guiyang, China. The initial symptoms on leaves were expanding round necrotic lesions with a gray center and dark brown edge, and twigs were withered when the disease was serious. Out of 100 measured plants, the disease incidence was around 65%. To isolate the potential causal pathogen, diseased leaves were collected from an *M. grandiflora* tree at Guizhou University. Isolations were made from the junction between healthy and symptomatic tissue and disinfested by immersing in 75% ethanol for 30 s and 3% NaOCl for 2 min, and washing three times in sterile distilled water. Symptomatic tissue was then plated on potato dextrose agar (PDA) and incubated at 25°C with 12 h of light for 3 to 5 days. Three isolates (GUCC 21235.1, GUCC 21235.2, and GUCC 21235.3) were obtained. Colonies on PDA after 7 days were dark brown, and pycnidia embedded in the mycelium were dark brown to black, single, and separated. Conidiophores were transparent, measuring 7 to 12.5 × 2.5 to 4.5 µm (mean = 9.5 × 3.6 µm, *n* = 30) in length. Conidia were transparent, becoming brown when mature with a diaphragm, with round ends measuring 21 to 27 × 10 to 15 µm (mean = 23.6 × 12.6 µm, *n* = 30). To confirm the pathogen by molecular characterization, four genes or DNA fragments, ITS, LSU, *tefl*, and *β-tubulin*, were amplified using the following primer pairs: ITS4-F/ITS5-R (White et al. 1990), LR0R/LR5 (Rehner and Samuels 1994), EF1-688F/EF1-986R (Carbone and Kohn 1999), and Bt2a/Bt2b (O'Donnell and Cigelnik 1997), respectively. The sequences of four PCR fragments of GUCC 21235.1 were deposited in GenBank, and the accession numbers were MZ519778 (ITS), MZ520367 (LSU), MZ508428 (*tefl*), and MZ542354 (*β-tubulin*). Bayesian inference was

performed based on a concatenated dataset of ITS, LSU, *tefl*, and *β-tubulin* genes using MrBayes 3.2.10, and GUCC 21235.1 formed a single clade with the reference isolates of *Diplodia mutila* (*D. mutila* strain CBS 112553). BLASTn analysis indicated that the sequences of ITS, LSU, *tefl*, and *β-tubulin* revealed 100 (546/546 nucleotides), 99.82 (568/569 nucleotides), 100 (302/302 nucleotides), and 100% (437/437 nucleotides) similarity with that of *D. mutila* in GenBank (AY259093, AY928049, AY573219, and DQ458850, respectively). For confirmation of the pathogenicity of this fungus, a conidial suspension (1×10^5 conidia ml⁻¹) was prepared from GUCC 21235.1, and healthy leaves of *M. grandiflora* trees were surface-disinfested with 75% ethanol, rinsed with sterilized distilled water, and dried with absorbent paper. Small pieces of filter paper (5 × 5 mm) dipped with 20 µl of conidial suspension (1×10^5 conidia ml⁻¹) or sterilized distilled water (as a control) were placed on the bottom left of the leaves for inoculation. The leaves were then sprayed with sterile distilled water and wrapped with plastic film and tin foil successively to maintain high humidity in the dark. After 36 h, the plastic film and tin foil on the leaves were removed, and the leaves were sprayed with distilled water three times each day in natural conditions (average temperature was about 25°C, 14 h light/10 h dark). After 10 days of inoculation, the same leaf blight began to appear on the leaves inoculated with conidial suspension. No lesions appeared on the control leaves. The fungus was reisolated from the symptomatic tissue. Based on the morphological information and molecular characterization, the isolate GUCC 21235.1 is *D. mutila*. Previous reports indicated that *D. mutila* infects a broad host range and gives rise to a canker disease of olive, apple, and jujube (Feng et al. 2019; Úrbez-Torres et al. 2013, 2016). This is the first report of leaf blight on *M. grandiflora* caused by *D. mutila* in China.

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