Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Stem Rot Caused by *Fusarium kyushuense* on *Dendrobium officinale* in China

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Dendrobium officinale Kimura et Migo is a Chinese medicinal plant in the Orchidaceae. In September 2021, *D. officinale* seedlings in a greenhouse farm in the region of Wenzhou City (28.11°N, 120.98°E), Zhejiang Province, China, were affected by stem rot. Symptoms initially occurred at the stem base with dark gray to black lesions, vascular discoloration, wilting, and death. Approximately 30% of the surveyed plants were affected. To determine the causal agent, 10 symptomatic plant samples were collected. The necrotic tissue pieces (3 × 3 mm) from symptomatic stems were disinfected with 10% sodium hypochlorite for 1 min, rinsed three times with sterile water, and dried on sterile paper. Samples were then placed on potato dextrose agar (PDA) and incubated at 25°C in the dark. After 3 days, reddish-white to yellow aerial mycelium with deep red pigments developed on PDA. A total of 15 single-spore isolates from all 10 infected tissues were identified as *Fusarium kyushuense* based on morphological features (Aoki and O'Donnell 1998). DDO-S1 was chosen as a representative isolate for further study. Microconidia were ellipsoidal to clavate, 0 to 1 septate, and 5.2 to 17.6 × 2.3 to 4.7 μm ($n = 100$; length × width). Hyaline and straight or slightly curved macroconidia were observed with three to five septa, 32.4 to 51.7 × 3.9 to 5.3 μm ($n = 100$; length × width). In order to identify the isolate, a portion of RNA polymerase second largest subunit (*RPB2*) (Liu et al. 1999) and translation elongation factor-1α (*TEF-1α*) (Geiser et al. 2004) were amplified by PCR. The gene sequences from isolate DDO-S1

were deposited in the NCBI GenBank nucleotide database with accession numbers OL548912 and OL548913. BLASTn analysis showed highest similarity with *F. kyushuense* strain MRC 2534 99.9% (*RPB2*) and 99.2% (*TEF-1α*) nucleotide sequence identity, which indicated that the pathogen was *F. kyushuense*. A maximum-likelihood phylogenetic analysis based on multilocus sequence analysis using MEGA X showed similar results (Kumar et al. 2018). Pathogenicity of this isolate was confirmed by following Koch's postulates. To verify pathogenicity, 30 healthy *D. officinale* seedlings (1 year old) were used for inoculation tests. Fifteen surface sterilized stems were stab inoculated with 5 × 5 mm colonized agar discs of strain *F. kyushuense* DDO-S1 pierced at three points using a 1-mm sterile needle, and another 15 samples were mock inoculated with sterile PDA disks as negative controls (Xiao et al. 2022). Each experimental seedling was inoculated with three agar disks from a colony of DDO-S1. Seedlings were placed in a humidified chamber (>95% relative humidity) at 25°C for 48 h after inoculation and kept in a growth chamber at 25°C and a daily 12 h photoperiod for 8 days. All inoculated stems showed symptoms identical to those observed in the field. No disease was observed on the controls. The pathogen was reisolated from all 15 diseased stems, and species identification was confirmed by the morphological and molecular methods described above. *Fusarium kyushuense* has been reported to cause diseases on a broad range of plants in China, such as tobacco (Wang et al. 2013), maize (Wang et al. 2014), and rice (Zhao and Lu 2007). To the best of our knowledge, this is the first report of *F. kyushuense* on *D. officinale* in China. *D. officinale* is native to Wenzhou, and spread of the disease may seriously affect the local economy and food security. This report will help diagnose stem rot of *D. officinale* and provide a foundation for development of management tools.

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