Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Paramyrothecium foliicola* Causing Stem Canker of Cucumber (*Cucumis sativus*) Seedlings in China

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In April 2017, stem canker symptoms were observed on cucumber (*Cucumis sativus* L.) seedlings grown in a greenhouse (0.1 ha) in Wuqing District, Tianjin (39°34' N; 117°07' E), China. Initially, the observed symptoms included small necrotic lesions of a light brown color on the stem base. These lesions subsequently spread and turned a darker brown. The leaves of the affected plants turned yellow and wilted. As the disease progressed the plants eventually died. Years of growing cucumbers and sufficient soil moisture in the greenhouse might have led to a disease incidence of approximately 7%. Symptomatic tissue pieces were surface disinfested in 2% sodium hypochlorite for 60 s, rinsed three times in sterile water, and subsequently plated on potato dextrose agar (PDA) and incubated at 25°C. At 3 days of incubation, mycelia appeared, turned into white and floccose isolated colonies around the excised tissue, and developed olivaceous green concentric rings of sporodochia in the following days. A total of 20 isolates with similar morphology were examined. Five single-spore isolates of isolates designated TJWQPF1 to TJWQPF5 were obtained and maintained on PDA at 25°C. Hyaline, cylindrical conidiogenous cells measuring 9.53 to 16.51 × 1.51 to 2.49 μm ($n = 50$) developed in whorls of three to six on terminal branches. Conidia were single-celled, hyaline, and rod-shaped with rounded ends. Conidia size averaged 5.07 to 7.15 × 1.13 to 2.32 μm ($n = 50$). These characteristics are similar to the morphology of *Paramyrothecium foliicola* (Lombard et al. 2016).

To further identify the isolate TJWQPF1, genomic DNA was extracted, and the internal transcribed spacer (ITS, White et al. 1990), β -tubulin (tub2, Glass and Donaldson 1995), RNA polymerase II largest subunit (rpb2, O'Donnell et al. 2007), and calmodulin (cmdA, Carbone and Kohn 1999; Groenewald et al. 2013) gene regions were amplified using the primer pairs ITS4/ITS5, Bt2a/Bt2b, RPB2-5F2/RPB2-7cR, and CAL-228F/CAL2Rd, respectively. All sequences were obtained and deposited in GenBank. BLAST searches of the NCBI database revealed that the ITS (MW092223), tub2 (MW110635), rpb2 (MW110637), and cmdA (MW110636) sequences of the isolate TJWQPF1 were 100% identical to *P. foliicola* (GenBank accession nos. MT415351 and MT415352 for ITS sequences; MT415353 for tub2 sequences; MN398028 to MN398043 for rpb2 sequences; MN593698 to MN593713 for cmdA sequences). We also sequenced the other four single isolates and identified them as *P. foliicola*. Pathogenicity tests were conducted and repeated three times. Briefly, 10 healthy 45-day-old cucumber seedlings (cultivar: Jinlv No.3) were inoculated with 100 μl of conidial suspension of *P. foliicola* (5×10^5 conidia/ml). Inoculum was applied to the stem with a syringe. Three healthy cucumber seedlings had 100 μl of sterile water injected into the stem to serve as controls. All treated plants were incubated in a climate-controlled growth chamber at 25°C (90% humidity, 12-h/12-h light/dark). Symptoms appeared on all inoculated plants after 7 days. In contrast, control seedlings exhibited no symptoms. The fungus was reisolated from symptomatic tissues and reidentified to be *P. foliicola*, thereby fulfilling Koch's postulates. To our knowledge, this is the first known instance of *P. foliicola* inducing stem canker on cucumber plants in China. Stem canker caused by *P. foliicola* could pose a threat to cucumber production in China. Our results also provide a basis to monitor and manage this potential disease.

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