Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Tobacco Fusarium Root Rot Caused by *Fusarium verticillioides* in China

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In July 2019, diseased tobacco plants (*Nicotiana tabacum* L.) exhibiting wilting and chlorosis of lower leaves were observed in various fields in Yunnan Province, which is a main tobacco cultivation area in China, and accounts for more than one third of China's total tobacco output. In the early stage of disease development, the lower leaves showed chlorosis and the roots became necrotic. When the disease was severe, the whole plant became wilted and stunted, had rotted roots, and an average of 5% of the affected plants died. To isolate the causal agent, five symptomatic roots (cv. Yunyan 87) were collected from Lincang (24.45°N, 99.45°E). Representative lesions and healthy tissue were cut, successively disinfested with 2% sodium hypochlorite and 70% ethanol, rinsed three times in sterile distilled water, dried on sterilized filter paper, and then plated on potato dextrose agar (PDA), and incubated at 25°C in the darkness for 3 days. Five isolates were obtained and purified from a single spore and examined using morphological and molecular analyses. Colonies with white to purple mycelia on PDA were observed after 5 days of incubation. Microconidia were slender, mostly one-celled, and measured 4.4 to 8.2 × 1.7 to 3.8 µm (*n* = 50). Macroconidia observed on synthetic low nutrient agar (SNA) medium were slightly curved and measured 24.0 to 41.4 × 3.5 to 4.9 µm (*n* = 50), with three to five septa. Chlamydospores were absent. The morphology of representative isolate GMGH02 was similar to

Fusarium spp. (Leslie and Summerell 2006). For molecular analyses, the internal transcribed spacer (ITS) region rDNA and translation elongation factor 1α (*EF-1α*) were amplified and sequenced using primers ITS1/ITS4 (White et al. 1990) and EF-1H/EF-2T (Geiser et al. 2004), respectively. The 549-bp ITS sequence (MW386816) resulted in 99% identity to *F. verticillioides* isolate JO-B466 (MN335233.1) after basic local alignment search (BLAST) in NCBI GenBank, and the 709-bp *EF-1α* sequence (MW383503) showed 100% identity with GenBank KF993992 belonging to *F. verticillioides*. To confirm its pathogenicity, 1 ml of conidial suspension (1×10^6 spores/ml) was poured over the roots of tobacco seedlings, which were six-leaf-stage seedlings (cv. Yunyan 87). Three seedlings were treated with sterile water as control. All the seedlings were maintained at 25°C, 70% relative humidity, and watered each day during the postinoculation period. After 5 days, the lower leaves showed symptoms of wilting and the roots of all inoculated seedlings became discolored, whereas the control seedlings remained symptomless. The same fungus was reisolated from the symptomatic roots and identified using the *EF-1α* gene sequence. *Fusarium* spp. diseases previously reported in China were caused by *F. kyushuense* (Wang et al. 2013) and *F. solani* (Yang et al. 2020). *F. verticillioides* has been reported to cause root rot or stalk and stem rot on maize plant (Cook 1981). However, to the best of our knowledge, this is the first report of *F. verticillioides* causing root rot on tobacco in China. Identification of *F. verticillioides* as a root rot agent might provide important insight for disease management practices on tobacco caused by *Fusarium* species.

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