Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium* Root Rot of Tobacco Caused by *Fusarium sinensis* in Henan Province, China

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Tobacco (*Nicotiana tabacum* L.) is an economically important crop in China, with an estimated production of 2.2 million tons every year. In June 2018, tobacco plants within the municipality of Sanmenxia (Henan, China) showed symptoms of wilting with leaf yellowing and stunting. Diseased plants exhibited severe necrosis that advanced through the main root. The symptoms were observed in 19 surveyed tobacco fields, 60 ha in total, and approximately 25% of the plants were symptomatic. The disease resulted in a severe loss in tobacco leaf production. Five symptomatic tobacco plants were sampled. Diseased tissues from roots were surface sterilized in 75% ethanol and placed on potato dextrose agar (PDA) medium. Eighteen of the 25 diseased tissues had cultures growing from them, and all the cultures were white colonies with abundant aerial mycelium and produced scarlet pigmentation on PDA. One pure culture was obtained by single-spore culturing (SL1). A 10-day-old culture grown on carnation leaf agar (CLA) produced macroconidia that were falcate, straight or slightly curved, three-septate, and 25 to 35 × 3.5 to 4.5 µm (average 26.8 × 3.7 µm) (*n* = 50). Two types of microconidia (napiform and fusiform) were formed on CLA that were hyaline, with one to two cells. Napiform conidia were 4.5 to 9.3 × 3.8 to 5.9 µm (average 7.3 × 5.0 µm) (*n* = 50); fusiform conidia were 6.9 to 15.8 × 1.8 to 3.1 µm (average 9.9 × 2.5 µm). Spherical chlamydospores (7 to 12.5 µm) (*n* = 50) were terminal or intercalary and produced in clumps or in chains. Morphological

characteristics of the isolate were similar to the features of *Fusarium sinensis* previously described by Zhao and Lu (2008). Molecular identification was performed using partial sequences of *EF1-α* gene (primers EF1/EF2, O'Donnell et al. 1998). Maximum parsimony and maximum likelihood-based methods were fitted using MEGA 7 (Moreira et al. 2019). The isolate was also sequenced for *β-tubulin* (primers T1/Bt-2b, O'Donnell and Cigelnik 1997), ribosomal RNA gene (LSU, LROR/LRS primers, Vu et al. 2019), and rDNA-ITS (ITS 1/ITS 4 primers, White et al. 1990). Sequences were deposited in GenBank under accession numbers MT947797 (*EF1-α*), MW484999 (*β-tubulin*), MW486649 (LSU), and MT907471 (ITS). The obtained *EF1-α* sequence had 98.10% identity with those of *F. sinensis* (MG670388.1) in the GenBank database, whereas the *β-tubulin*, LSU, and ITS sequences showed 100% identities to the corresponding DNA sequences in *F. sinensis* (GenBank accession nos. KX880370.1, NG_067454.1, and MH863232.1, respectively). Morphological and molecular results confirmed this species as *F. sinensis* (Zhao and Lu 2008). Pathogenicity tests were performed on tobacco seedlings grown on an autoclaved matrix (YC/T310-2009). Healthy six-leaf-stage tobacco seedlings were inoculated by pouring a 20 ml of conidial suspension (1 × 10⁶ conidia/ml) around the stem base of each plant, and 30 plants were inoculated. Thirty control seedlings received sterilized water. All treatments were maintained for 30 days under greenhouse conditions with a 12-h light/dark photoperiod at 25 ± 0.5°C and 70% relative humidity. The assay was conducted three times. Root rot and foliage chlorosis similar to the ones observed on infected plants in the field were observed on the inoculated tobacco seedlings, whereas the control seedlings remained asymptomatic after 30 days. The pathogen isolated from the inoculated plant exhibited morphological characteristics identical to *F. sinensis* and was identified by a partial *EF1-α* gene sequence. This disease has previously been reported as the causal agent of root and crown rot of wheat in China (Xu et al. 2018; Zhao and Lu 2008). To our knowledge, this is the first report of *F. sinensis* causing root rot on tobacco in China.

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