Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum clivicola* Causing Leaf Spot on Tobacco (*Nicotiana tabacum*) in Hunan Province of China

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Tobacco (*Nicotiana tabacum* L.) is an annual leafy herb of the genus *Nicotiana* in the family Solanaceae. It is an important commercial crop in China. In 2020, a leaf spot disease occurred on tobacco leaves in commercial tobacco fields, in the Hunan Province of China. Symptoms appeared as water-soaked, yellow-green spots, which then turned dark brown and coalesced into larger necrotic lesions, often leading to leaf wilt. Approximately 20% of the plants in a 50-ha area were infected, exhibiting symptomatic spots on 60% of these leaves. Symptomatic leaf samples were collected and cut into small pieces, sterilized with 70% ethanol for 10 s followed by 0.1% HgCl₂ for 40 s, rinsed with sterile distilled water three times, plated on potato dextrose agar (PDA), and incubated at 26°C in the dark. Isolates with similar morphology were developed from 10 samples. Fungal isolates produced dense, white to dark green, aerial mycelium. Conidia were straight, hyaline, aseptate, and cylindrical, contained oil globules, and were 15 to 25 × 3.0 to 4.0 μm (*n* = 50). Appressoria were dark brown, irregularly shaped, and 5.5 to 10.0 × 4.5 to 6.5 μm (*n* = 50). These morphological characteristics were typical of *Colletotrichum clivicola* (Yang et al. 2011). For molecular identification, the internal transcribed spacer (ITS) region of rDNA, actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and chitin synthase (CHS-1) genes of representative isolate CS16-2 were amplified and sequenced using the primer pairs as described previously (Weir et al. 2012).

These sequences were deposited in GenBank (accession nos. MW649137 for ITS, MW656181 for ACT, MW656182 for GAPDH, and MW656183 for CHS-1). BLAST analysis showed that they had 99.46 to 100% identity to the corresponding sequences of *C. clivicola* strains. A concatenated phylogenetic tree was generated, using the ACT, GAPDH, and CHS-1 sequences of the isolate CS16-2 and other closely matching *Colletotrichum* species obtained from GenBank. It showed that CS16-2 was grouped with the *C. clivicola* clade with 97% bootstrap support, including the *C. clivicola* strain AH1B6 (Y. C. Wang et al. 2016). Pathogenicity was tested by spraying 2-month-old potted tobacco plants until runoff with a conidial suspension (10⁵ spores/ml). Leaves were mock inoculated with sterilized water. The pathogenicity tests were performed twice, with three replicate plants each. Plants were kept in humid chambers at 26°C with a 12-h photoperiod. Five days postinoculation, the inoculated plants showed symptoms of yellow-brown necrosis resembling that observed in fields, whereas the control plants remained symptomless. *C. clivicola* was specifically reisolated and identified by morphological and molecular methods as described above. Currently, *C. clivicola* has been reported to be the causal agent of anthracnose in some plants, such as soybean (Zhou et al. 2017) and *Zamioculcas zamiifolia* (Barbieri et al. 2017). However, to our knowledge, this is the first report of *C. clivicola* causing leaf spot on tobacco in China and even in the world. Because this disease might damage the yield and quality of tobacco production, attention should be paid by growers. The identification of the pathogen fungus might provide important insights for disease diagnosis on tobacco, because some other *Colletotrichum* species, such as *C. fructicola* (H. C. Wang et al. 2016) and *C. karsti* (Zhao et al. 2020), have also been responsible for anthracnose on tobacco.

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