Diseases Caused by Fungi and Fungus-Like Organisms

Leaf Spot Caused by *Epicoccum laticollum* on Tobacco in China

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Tobacco (*Nicotiana tabacum* L.) is a leafy, annual, solanaceous plant grown commercially for its leaves in China. In continuing research on foliar diseases of tobacco in Guizhou province in August 2019, diseased leaves of tobacco that had sandy beige, elliptical or irregular shaped lesions, with a brown edge, and surrounded by yellow halos on 40% of leaves on 5% of plants were obtained (cv. Yunyan 87) in Zhenan (28.55° N, 107.43° E), Guizhou, China. Diseased leaf segments were surface sterilized and plated on potato dextrose agar (PDA). One isolate (T41) was selected for identification. The colonies had white aerial hyphae, with orange-red on the underside when cultured on PDA. The colonies had woolly aerial hyphae, white to gray eventually, and produced pycnidia on oatmeal agar. Pycnidia were dark, spherical or flat spherical, and 69.2 to 178.0 µm in diameter. Conidia were oval mostly, aseptate, usually guttulate, and the size was 5.0 to 6.5 × 3.2 to 5.4 µm. The rDNA internal transcribed spacer region (ITS) with primers ITS1f/ITS4 (Gardes and Bruns 1993; White et al. 1990), 28S ribosomal RNA gene (*LSU*) with primers LROR/LR7 (Rehner and Samuels 1994), beta-tubulin gene (*TUB2*) with primers Btub2Fd/Btub4Rd (Woudenberg et al. 2009), and RNA polymerase II second largest subunit gene (*RPB2*) with primers RPB2-5F2/IRPB2-7cR (Liu et al. 1999) of T41 were

sequenced (GenBank accession nos. MN704804, MN710367, MN718012, and MN718013, respectively). Maximum likelihood (ML) and Bayesian inference (BI) analyses based on these four concatenated sequences were conducted with RAxML version 7.2.6 and MrBayes version 3.2.1, respectively, which showed that T41 was part of a clade with *Epicoccum laticollum* strains (CGMCC 3.18346 and LC 8153) (ML/BI = 100/1). Based on morphological and multigene molecular data, isolate T41 was identified as *E. laticollum*, described as a new taxon by Chen et al. (2017). To verify pathogenicity, tobacco plants at seedling stage (seven to eight leaves) without visible disease were inoculated using conidial suspension (10⁶ spores/ml), following the method of Guo et al. (2020). All inoculated plants were maintained in a greenhouse with relative humidity ranging from 50 to 85% at 28°C under a 12-h/12-h light/dark cycle. Seven days after incubation, typical symptoms were observed on inoculated leaves but not on control leaves. Koch's postulates were fulfilled by reisolation of *E. laticollum* from diseased leaves. *E. laticollum* has been reported to cause black root on yam in China (Han et al. 2019). Meanwhile, leaf spot could be caused on many plants by this genus, such as *Lablab purpureus* (Mahadevakumar et al. 2014) and *Bletilla striata* (Zhou et al. 2018). However, to the best of our knowledge, this is the first report of *E. laticollum* causing leaf spot on tobacco in China. Because considerable loss occurred due to infection from *E. laticollum* on tobacco leaves, this pathogen is worthy of further study, and disease management practices need to be developed to prevent further losses.

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