Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Blight on *Mentha canadensis* Caused by *Nigrospora sphaerica* in China

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Mentha canadensis L. is a widely cultivated and economically important aromatic plant around the world. It is used as a fresh or dried flavoring, herbal tea, essential oil crop, and ornamental. The leaves are used as an important medicine source for the Chinese medicinal industry. During the summers of 2018 and 2019, approximately 30 to 35% of the plants showed leaf spot symptoms in the affected fields in Chengmai, Hainan Province. Initial lesions on infected leaves were irregularly shaped, light yellow to light brown spots. After several days, the lesions turned grayish to dark brown with yellow borders. Then, the infected leaves turned gray brown, leading to defoliation. Leaf tissues of 10 diseased plant samples collected from the fields were surface sterilized 70% ethanol for 30 s, 0.1% HgCl₂ for 30 s, rinsed thrice with sterile distilled water for 30 s, placed on potato dextrose agar amended with 30 mg/liter of kanamycin sulfate, and incubated at 28°C in the dark for 3 to 5 days. Colonies were initially white and later turned grayish-brown with the onset of sporulation. Conidia were single-celled, black, smooth, spherical to subspherical, and measured 14.2 to 18.7 µm long and 11.2 to 14.8 µm wide ($n = 30$). Each conidium was terminal and borne on a colorless vesicle at the tip of conidiophores.

Morphological characteristics of the isolates were similar to those of *Nigrospora sphaerica* (Wang et al. 2017). To confirm the identity of a representative isolate, the internal transcribed spacer (ITS) region, translation elongation factor 1-alpha (*TEF1-α*), and partial beta-tubulin (TUB2) genes were amplified with primers ITS1/ITS4 (Mills et al. 1992), EF1-728F (Carbone and Kohn 1999)/EF-2 (O'Donnell et al. 1998), and Bt-2a/Bt-2b (Glass et al. 1995), respectively. A BLAST search of GenBank showed that the ITS (525-bp; MN153452), *TEF1-α* (482-bp; MN864137), and TUB2 (411-bp; MT090055) sequences of the isolate were 99, 100, and 100% homologous with *N. sphaerica* (GenBank nos. KU360636, KY019401, and KY019565), respectively. Maximum likelihood trees based on concatenated nucleotide sequences of the three genes were constructed using MEGA7.0, and bootstrap values indicated that our isolate was closely related to *N. sphaerica*. A pathogenicity test was performed by depositing 10 µl of a suspension (1×10^6 conidia/ml) on the surfaces of five artificially wounded leaves (a small hole made with a pushpin) of 10 healthy 1-year-old *M. canadensis* plants. An equal number of leaves that were wounded and inoculated with sterile distilled water served as the control. Treated plants were kept at 28°C with 80% humidity in a greenhouse. Ten days after inoculation, all the inoculated leaves developed blight symptoms similar to those observed in the field. No symptoms were observed on the control leaves. The fungus was reisolated from the infected tissues, fulfilling the Koch's postulates. *N. sphaerica* was previously reported as the causal agent of leaf spot on *Sesamum indicum* L. (Zhao et al. 2014), *Curcuma wenyujin* (Zhang et al. 2011), and *Camellia sinensis* (Liu et al. 2016) in China. To our knowledge, this is the first report of leaf blight of *M. canadensis* caused by *N. sphaerica* in China. Given its wide host range, *N. sphaerica* has great potential to become an economically important plant pathogen.

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