Diseases Caused by Fungi and Fungus-Like Organisms

Nigrospora oryzae Causing Black Leaf Spot Disease of *Hibiscus mutabilis* in China

Shan Han, Shutian Yu, Tianhui Zhu,[†] Shujiang Li, Tianmin Qiao, Yinggao Liu, TianTian Lin, and Chunlin Yang

College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan Province, 611130, P.R. China

Funding: Funding was provided by General Program of Science and Technology Department of Sichuan (2019YFN0156). Plant Dis. 105:2255, 2021; published online as <https://doi.org/10.1094/PDIS-08-20-1796-PDN>. Accepted for publication 23 January 2021.

Cotton rose (*Hibiscus mutabilis* L.) is a deciduous shrub native to China. It has been widely cultivated in many provinces in China for its ornamental and ecological value (Shang et al. 2020). In May 2017, an unknown leaf spot symptom was first observed on *H. mutabilis* at the Chengdu Campus of Sichuan Agricultural University (30°42'31" N, 103°51'28" E). The disease occurred from May to September with approximately 81% incidence by field sample survey of 300 plants in Chengdu Greenway. The symptoms at first appeared as irregular black spots on the leaves. Then the lesions grew and coalesced into large, black necrotic areas, which later produced leaf chlorosis and abscission. This disease seriously reduced the ornamental value of *H. mutabilis*. Forty diseased lesions (4 × 5 mm) were surface sterilized with 75% alcohol for 60 s and 3% NaClO for 45 s, rinsed three times in sterile water, placed onto potato dextrose agar (PDA), and then incubated in a dark at 25°C. From the seven obtained isolates, four isolates exhibited the morphology described as *Nigrospora oryzae* (Hao et al. 2020). The fungus produced initially circular white colonies, and then the centers turned dark gray or black with age on the PDA. Hyphae were smooth, branched, septate, hyaline, or pale brown. Conidia ($n = 100$ spores) were abundant and were solitary, dark-brown to black, smooth, aseptate, and measured 11 to 15 µm in diameter. DNA was extracted from the fungal colonies using a DNeasy Plant Mini Kit (Qiagen). The internal transcribed spacer (ITS), β-tubulin gene (TUB), and translation elongation factor 1-α (TEF1) were amplified with primers ITS1/ITS4 (White et al. 1990), BT2A/BT2B (Glass and Donaldson

1995), and EF1-728F/EF1-986R (Carbone and Kohn 1999; O'Donnell et al. 1998), respectively. BLAST results indicated that the ITS, TUB, and TEF1 sequences (GenBank accession nos. MN515070, MN733956, and MN635723, respectively) had 99% identity with *N. oryzae* sequences (GenBank accession nos. KX986031, KY019553, and KY019358). The result was confirmed by multilocus phylogenetic analysis. The morphological characteristics and molecular analyses of the isolate matched the description of *N. oryzae*. To confirm pathogenicity, Koch's postulates were fulfilled under controlled conditions. The seedlings of twenty 2-year-old potted *H. mutabilis* plants were inoculated by spraying conidial suspension at the concentration of 1×10^6 conidia/ml on both sides of leaves. Sterilized distilled water (20 seedlings) was used as a negative control. The experiment was performed three times. All plants were incubated at $25 \pm 2^\circ\text{C}$ under a 16-h/8-h photoperiod and 70 to 75% relative humidity after inoculation and were observed daily for disease development. Two weeks later, the inoculated plants showed the same symptoms as the original diseased plants and the controls remained asymptomatic. The pathogen *N. oryzae* was reisolated from all inoculated plants, and the culture and fungus characteristics were the same as those of the original isolate. But *N. oryzae* was not isolated from the control plants. The results indicated that *N. oryzae* is a causal agent of the disease. *N. oryzae* was reported as a leaf pathogen on cotton (Zhang et al. 2012), but this is the first report of *N. oryzae* causing leaf black spot on *H. mutabilis* in the world. The identification could provide relevant information for adopting appropriate management strategies to control the disease.

References:

- Carbone, L., and Kohn, L. M. 1999. Mycologia 91:553.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Hao, Y. Y., et al. 2020. Mycobiology 48:169.
- O'Donnell, K., et al. 1998. Proc. Natl. Acad. Sci. 95:2044.
- Shang, K. K., et al. 2020. Int. J. Phytoremediation 22:10.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Zhang, L. X., et al. 2012. Plant Dis. 96:1379.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Hibiscus mutabilis*, leaf black spot disease, *Nigrospora oryzae*

[†]Indicates the corresponding author.
T. H. Zhu; zhutianhui63@163.com