Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on *Nelumbo nucifera* Plants in China

Xin Gong,¹ Weigang Kuang,¹ Xinwen Zheng,² Lianhu Zhang,¹ Xiaotang Sun,¹ Jian Ma,¹ and Ruqiang Cui^{1,†}

¹ College of Agronomy/Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, China

² Guangchang White Lotus Research Institute, Guangchang, Jiangxi 344900, China

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White lotus (*Nelumbo nucifera*) is an aquatic plant of the Nymphaeaceae family that primarily serves as an ornamental plant and is an important cash crop in China (Wu et al. 1994). In May 2020, an unknown leaf disease affecting these plants was first detected in White Lotus Science and Technology Expo Park in Guangchang County, Fuzhou City, Jiangxi Province (26.79° N, 116.31° E). The disease caused approximately 30 to 40% of leaves to die, and led to 15 to 20% seed yield losses. This disease was characterized by the formation of irregular yellow-brown to dark-brown spots during the initial phases of infection. As the disease developed, these spots expanded until they were generally round and brown to purple-brown, with a yellow halo surrounding the expanding spots. To characterize the causative pathogen, a small ~5 × 5 mm leaf tissue section from the boundary between normal and diseased tissue was collected and sterilized with the following regimes: 30 s with 75% ethanol, soaked in 0.1% mercuric chloride for 30 s, and washed thrice with sterile water. Tissues were then transferred onto a potato dextrose agar (PDA) plate and placed in an illumination incubator (12 h light/dark) at 28 ± 1°C for 5 days. Seven pure cultures were obtained from 10 diseased leaves. For pathogenicity testing, a hyphal inoculation strategy was employed (Fang 1998), with all studies being conducted at the Plant Pathology Laboratory of Jiangxi Agricultural University. Five-millimeter discs were selected from three separate cultures and one control (PDA). Healthy leaves of lotus seedlings were treated with four treatments per leaf including three separate cultures and one control that were treated

with the test pathogen. The experiments were repeated three times with three biological replicates. Healthy leaves were covered with moisturized sterile cotton balls and fixed to the leaf surface with transparent tape. The inoculated lotus seedlings were kept in a greenhouse incubator at 28 ± 3°C and relative humidity of 70 to 80%. Following a 14-day incubation period, brown spots began to manifest at all sites inoculated with the test pathogen whereas the control sites remained healthy. Diseased spots were then separated. The same pathogen was again successfully isolated and identified microscopically, thus fulfilling Koch's postulates. Six isolates were characterized. Ovoid or elliptical conidia were brown to light-brown with a short beak, one to five transverse septa, and zero to three longitudinal septa. The conidia were large (13.8 to 44.0 × 7.5 to 16.3 µm [average: 24.0 × 11.9 µm]; *n* = 50). These morphological characteristics were consistent with *Alternaria alternata* (Simmons 2007). The ITS4/ITS5, EF1-728F/EF1-986R, AltF/AltR, OPA10-2R/OPA10-2L, EPGF/EPGR, and KOG1058F2/KOG1058R2 primer sets were used to conduct molecular identification by amplifying key transcription elongation factor and internal transcriptional spacer regions (Andrew et al. 2009; Bihon et al. 2015; Peever et al. 2004; Woudenberg et al. 2015), yielding sequences that were 99 to 100% similar to *A. alternata* (GenBank nos.: MK396606, MT178329, MN184998, MN894688, MT849815, and KP125234). Sequences were deposited in GenBank with accession numbers MW898580 (ITS, 620 bp), MW981281 (EF1-α, 284 bp), MZ514094 (Alt a1, 477 bp), MZ514095 (OPA10-2, 716 bp), MZ514096 (endoPG, 465 bp), and MZ514097 (KOG1058, 877 bp). *N. nucifera* is an important aquatic cash crop in China, and this is the first study we are aware of demonstrating the presence of a leaf disease caused by *A. alternata* in *N. nucifera* plants anywhere in the world. These findings may offer a foundation for efforts to prevent diseases caused by *A. alternata*.

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[†]Indicates the corresponding author.
R. Cui; cuiruqiang@jxau.edu.cn