Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum siamense* Causing Leaf Spot on *Alocasia macrorrhiza* in China

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Alocasia macrorrhiza (L.) Schott (Araceae), known as alocasia, is widely planted in southern China for its ornamental and medicinal value. It has a range of pharmacological effects and potential antitumor activity (Lei et al. 2013). In July 2019, leaf spots were observed on *A. macrorrhiza* in the Xixiangtang Area, Nanning, Guangxi, China. Symptoms began with water-soaked yellow-green spots and progressed to form brown, round or oval lesions with yellow halos. Under severe conditions, spots merged into larger irregular lesions. Over 60% of the plants in a 0.5-ha field showed symptoms. Symptomatic leaves were collected and cut into 3 × 3-mm pieces. Leaf pieces from the margin of the necrotic tissue were surface sterilized in 75% alcohol for 10 s followed by 2% NaOCl for 2 min and then rinsed three times in sterile distilled water. Tissues were plated on PDA and incubated at 28°C for 5 days in the dark. Among >30 isolates, most shared a similar morphology, the isolation rate was 86.7%, and three (GY1-1A, -1B, and -1C) were chosen for single-spore purification and used for morphological characterization and identification. White feathery aerial mycelia with olivaceous gray mycelia below were observed in 7-day cultures. After 14 days, orange conidial masses were observed. Conidia were hyaline, guttulate, smooth, one-celled, and cylindrical, averaged 13.79 × 5.26, 13.89 × 5.33, and 13.92 × 5.42 μm for GY1-1A, -1B, and -1C, respectively. Appressoria were mostly irregular in outline, deeply lobed or lightly lobed, gray-brown to dark brown; conidial appressoria were 7.93 to 8.74 × 5.26 to 5.42 μm; and mycelial appressoria were

7.15 to 10.11 × 5.60 to 7.44 μm. Morphological characteristics were similar to *Colletotrichum siamense* as previously described (Weir et al. 2012). The partial internal transcribed spacer (ITS) regions, actin (ACT), chitin synthase (CHS-1), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), calmodulin (CAL), β-tubulin (TUB2), and intergenic region of apn2 and MAT1-2-1 (ApMAT) were amplified from genomic DNA for the three isolates using primers ITS4/ITS1 (White et al. 1990), ACT-512F/ACT-783R, CHS-79F/CHS-354R, GDF1/GDR1, CL1C/CL2C, T1/Bt2b (Weir et al. 2012), and AM-F/AM-R (Silva et al. 2012) and sequenced. All sequences showed >99% identity with *C. siamense* and were deposited in GenBank (ITS, MW040179 to MW040181; ACT, MW049220 to MW049222; CHS-1, MW049229 to MW049231; GAPDH, MW049232 to MW049234; CAL, MW049226 to MW049228; TUB, MW049235 to MW049237; ApMAT, MW049223 to MW049225). A maximum likelihood phylogenetic tree was constructed with MEGA 5 using the concatenation of multiple sequences (ACT, CHS-1, GAPDH, ITS, TUB2, CAL). According to the phylogenetic tree, all three isolates were found with *C. siamense* with 95% bootstrap support. To confirm pathogenicity, three sets (three plants per set) of healthy leaves were slightly scratched with autoclaved toothpicks at each of eight locations. Each inoculation location was a cross (2 mm length), and inoculation location was at least 3 cm apart. Conidial suspension (10 μl, 10⁶ conidia/ml in 0.1% sterile Tween 20) was applied to the inoculation areas. A control group was mock inoculated with 0.1% sterile Tween 20. Plants were covered with plastic bags to maintain a high-humidity environment and placed in a 28°C growth chamber with constant light for 7 days. Inoculated leaves showed yellowish brown spots (0.4 × 0.65 cm), but no symptoms were observed in the control group. The fungus was reisolated from inoculated leaves, and these isolates matched the molecular and morphological characteristics of the original isolates, confirming Koch's postulates. Reported hosts of this pathogen include *Coffea arabica*, *Carica papaya*, *Melilotus indicus*, *Litchi chinensis* (Ling et al. 2019; Qin et al. 2017; Weir et al. 2012), and so on. This is the first report of *C. siamense* causing leaf spot on *A. macrorrhiza* in China. The identification of this pathogen provides a foundation for the management of leaf spot on this medicinal plant.

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