Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose on *Kadsura coccinea* Caused by *Colletotrichum siamense* in China

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Kadsura coccinea (Lem.) A. C. Smith, an evergreen liana, is widely cultivated in China for its economic importance in traditional medicine. Many phytochemical studies on the stems and roots of *K. coccinea* have shown numerous biological activities, such as antitumor, anti-HIV, and antioxidant (Yang et al. 2020). In June 2019, an anthracnose on *K. coccinea* was observed in a plantation in Longan (23°03'N, 107°54'E), Guangxi Province. Disease incidence was up to 30% in a plantation. Its symptoms began as small brown spots that expanded into nearly circular spots. To isolate pathogen, diseased leaves were collected. The leaves were sterilized with 75% ethanol for 15 s followed by 2% sodium hypochlorite for 90 s, then rinsed three times in sterilized distilled water, cut into 5 × 5 mm pieces, and placed into potato dextrose agar (PDA) plates. The plates were incubated in an incubator at 25°C in dark for 2 to 3 days. Fungal colonies with similar morphology of 27 isolates were isolated from the 30 infected tissues. Six representative isolates (YB1 to YB6) were selected to further study their characterization. Fungal colonies were grayish-white; orange-yellow conidial masses could be observed in colonies. The mature conidia were colorless and transparent, elliptical, and single-celled, 13.0 to 21.0 × 4.0 to 8.0 µm (average 16.92 × 5.92 µm; $n = 100$). The DNA sequences of ribosomal internal transcribed spacer region (ITS), glyceraldehyde-3-phosphate (GAPDH), calmodulin (CAL), actin (ACT), chitin synthase (CHS-1), and β -tubulin (TUB2) were amplified by PCR using the primer pairs ITS1/ITS4, GDF/GDR,

CL1C/CL2C, ACT-512F/ACT-783R, CHS-79F/CHS-354R, and T1/Bt2b (Wang et al. 2020), respectively. Sequences were submitted to GenBank (accession nos. MZ040489 to MZ040494 for ITS, MZ069043 to MZ069048 for GAPDH, MZ069049 to MZ069054 for CAL, MZ069055 to MZ069060 for ACT, MZ069061 to MZ069066 for CHS-1, and MZ069067 to MZ069072 for TUB2). These sequences were 98 to 100% identical to that of reference isolates JX010278, JX010019, JX009709, GQ856775, GQ856730, and JX010410 of *Colletotrichum siamense* CBS 125378 ex-type recorded in GenBank. Phylogenetic analysis of combined ITS, GAPDH, CAL, ACT, CHS-1, and TUB2 genes with 16 sequences obtained from GenBank using the maximum likelihood method showed that the six isolates clustered with two reference isolates of *C. siamense* as a distinct clade. Based on morphological characteristics and phylogenetic analysis, six isolates were identified as *C. siamense*. Pathogenicity tests were performed on young, fully expanded leaves of 1-year-old seedlings. Every leaf was punctured at six points on the right half and six points on the left half using a sterile needle. A 10 µl conidial suspension (1×10^6 conidia/ml) was inoculated on each wound on the left-half leaf and a 10 µl sterile water was inoculated on each wound on the right-half leaf (control). Each treatment was repeated three times. Inoculated leaves were wrapped in plastic bags for 2 days and after removing the bags, plants were maintained in a growth chamber at 28°C, 80% relative humidity, and a 12-h photoperiod. Anthracnose spots formed 2 to 3 days after inoculation, whereas the control leaves remained symptomless. Morphological characters matched the descriptions of *C. siamense*. The pathogen was previously reported to cause anthracnose on *Aloe vera* (Azad et al. 2020), postharvest anthracnose in mango (Liu et al. 2017), and pod rot in cacao (Serrato-Diaz et al. 2020). To our knowledge, this is the first report of anthracnose on *K. coccinea* caused by *C. siamense* in China.

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