Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* Causing Leaf Blight of *Kadsura longipedunculata* in China

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The medicinally important plant kadsura (*Kadsura longipedunculata*), belonging to the family Schisandraceae, is widely distributed in China and other Asian countries. This plant is an evergreen scandent vine and is primarily used as a traditional Chinese medicine to treat rheumatoid arthritis and gastrointestinal diseases. In October 2019, a disease survey was conducted in an herbal medicine production base with 305 *K. longipedunculata* trees in five fields in Kaili city (26°39'16"N, 107°47'18"E), Guizhou province, southwestern China, and leaf blight was found to be widely occurring on kadsura, with about 10 to 20% of kadsura plants affected. The disease was initiated with chlorotic spots, which became necrotic and black lesions with a chlorotic halo following the disease development, thus causing leaf death in the end. Leaf samples were collected, and surface disinfection was performed by soaking samples in 75% alcohol for 45 s and 0.1% HgCl₂ for 1 min. After triple rinses with sterile distilled water and drying on sterilized paper, symptomatic tissue pieces were placed on potato dextrose agar (PDA) medium for incubation at 25°C in the dark. Fungal colonies with similar morphology of five single-spored isolates were consistently isolated from the infected tissues and showed greenish gray aerial mycelium after 3 days incubation, and dark olive colored colonies were observed at 5 days on PDA plates. Young conidia were subovoid, hyaline, without septum, and mature conidia of

23.2 to 27.5 × 12.8 to 14.1 μm (*n* = 30) were oval-shaped, dark, along with a diaphragm. Based on the cultural and conidial morphology, the isolates were identified as *Lasiodiplodia theobromae* (Rodríguez-Gálvez et al. 2015). To confirm the identification, PCR analysis was conducted using the DNA of the five isolates with the primers of ITS1/ITS4 and EF1-728F/EF1-986R targeting the internal transcribed spacer (ITS) region and translation elongation factor 1-α (*EF1-α*) gene (Alves et al. 2008). Sequencing analysis indicated that these isolates shared the same sequences in both of the regions. Hereby, nucleic acid sequences of ITS and *EF1-α* gene of one isolate, KL-1, were deposited into GenBank with accession numbers MN901218 and MN906449, respectively. According to BLASTn results, sequences showed 100 and 99.6% identities with those of *L. theobromae* from GenBank (accession nos. EF622068 and EF622048, respectively). Furthermore, a phylogenetic tree generated by the maximum-likelihood method using MEGA with the ITS and *EF1-α* gene sequences of 37 reference isolates (Alves et al. 2008) along with the isolate KL-1 in the current study showed that isolate KL-1 clustered in the same clade with *L. theobromae*. A pathogenicity assay was performed by inoculating 20 μl of conidial suspension (2 × 10⁵ spores/ml) onto needle wounds on healthy *K. longipedunculata* leaves (Sun et al. 2018). Mock wounds were inoculated with sterilized water. In total, six plants were used, and three leaves of each plant were employed for inoculation. The experiment was repeated twice. These treated samples were kept in a growth chamber at 25°C and 80% relative humidity. Chlorotic spots were first observed around the inoculation sites 10 days postinoculation, and *L. theobromae* was reisolated and identified by morphology and sequencing, whereas the controls remained asymptomatic, thus confirming Koch's postulates. To the best of our knowledge, this is the first report of *L. theobromae* causing leaf blight on *K. longipedunculata* in China, and it may potentially become a serious problem for some medicinal plant growers.

References:

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