Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Nigrospora sphaerica* Causing Fruit Dried-Shrink Disease in *Akebia trifoliata* from China

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Akebia trifoliata, a recently domesticated horticultural crop, produces delicious fruits containing multiple nutritional metabolites and has been widely used as a medicinal herb in China. In June 2020, symptoms of dried-shrink disease were first observed on fruits of *A. trifoliata* grown in Zhangjiatie, China (110.2°E, 29.4°N) with an incidence about 10%. The infected fruits were shrunken, colored dark brown, and withered to death. Symptomatic fruit tissues (6 × 6 mm) were excised from three individual plants, surface disinfested in 1% NaOCl for 30 s and 70% ethanol solution for 45 s, washed, dried, plated and incubated on potato dextrose agar (PDA) containing 50 mg/liter of streptomycin sulfate in the dark, and incubated at 25°C for 3 days. Subsequently, hyphal tips were transferred to PDA to obtain pure cultures. After 7 days, five pure cultures were obtained, including two identical to previously reported *Colletotrichum gloeosporioides* causing leaf anthracnose in *A. trifoliata* (Pan et al. 2021) and three unknown isolates (ZJJ-C1-1, ZJJ-C1-2, and ZJJ-C1-3). The mycelia of ZJJ-C1-1, ZJJ-C1-2, and ZJJ-C1-3 were white and formed colonies of approximately 70 mm (diameter) in size at 25°C after 7 days on potato sucrose agar plates. After 25 days, conidia were formed, solitary, globose, black, shiny, smooth, and 16 to 21 µm in size (average diameter = 18.22 ± 1.00 µm, *n* = 20). These morphological characteristics were similar to those of *Nigrospora sphaerica* previously reported (Li et al. 2018). To identify species of ZJJ-C1-1, ZJJ-C1-2, and ZJJ-C1-3, the internal

transcribed spacer (ITS) region, β-tubulin (*TUB2*), and translation elongation factor 1-α (*TEF1-α*) were amplified using primer pairs including ITS1/ITS4 (Vilgalys and Hester 1990), Bt-2a/Bt-2b (Glass and Donaldson 1995), and EF1-728F/EF-2 (Zhou et al. 2015), respectively. Multiple sequence analyses showed no nucleotide difference was detected among genes tested except ITS, which placed three isolates into two groups. BLAST analyses determined that ZJJ-C1-1, ZJJ-C1-2, and ZJJ-C1-3 had 99.73% identity to *N. sphaerica* strain LC2705 (KY019479), 100% to LC7294 (KY019397), and 99.79 to 100% to LC7294 (KX985932) or LC7294 (KX985932) based on sequences of *TUB2* (MW252168, MW269660, MW269661), *TEF1-α* (MW252169, MW269662, MW269663), and ITS (MW250235, MW250236, MW192897), respectively. These indicated three isolates belong to the same species of *N. sphaerica*. Based on a combined dataset of ITS, *TUB2*, and *TEF1-α* sequences, a phylogenetic tree was constructed using the maximum likelihood method through IQ-TREE (Minh et al. 2020) and confirmed that three isolates were *N. sphaerica*. Further, pathogenicity tests were performed. Briefly, healthy unwounded fruits were surface disinfested in 0.1% NaOCl for 30 s, washed, dried, and needle wounded. Then, three fruits were inoculated with 10 µl of conidial suspension (1 × 10⁶ conidia/ml) derived from three individual isolates, with another three fruits sprayed with 10 µl of sterilized water as a control. The treated fruits were incubated at 25°C in 90% humidity. After 15 days, all three fruits inoculated with conidia displayed typical dried-shrink symptoms like those observed in the farm field. The decayed tissues with mycelium and spores could be observed on the skin or vertical split of the infected fruits after 15 days' inoculation. Comparably, in the three control fruits, there were no dried-shrink-related symptoms displayed. The experiment was repeated twice. The reisolated pathogens were identical to *N. sphaerica* determined by sequencing the ITS, *TUB2*, and *TEF1-α*. Previous reports showed *N. sphaerica* could cause postharvest rot disease in kiwifruits (Li et al. 2018). To our knowledge, this is the first report of *N. sphaerica* causing fruit dried-shrink disease in *A. trifoliata* in China.

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