Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Passion Fruit Leaf Blight Caused by *Nigrospora sphaerica* in China

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Passion fruit (*Passiflora edulis* Sims) is a widely cultivated dicotyledonous perennial plant with woody vines (Asande et al. 2020). In November 2020, leaf blight was observed on leaves of *P. edulis* (cv. Panama Red) newly planted in Wangyou, Huishui county, Guizhou province, China (25.8257° N, 106.5049° E). The leaf blight occurred on both young and old leaves, starting from the margins, and then extended to the entire leaves. The color of the affected tissue was brown with a yellow halo in the early period, and then gradually turned to gray. The disease incidence was 60 to 70% on a 0.08-ha field. Following isolation of the potential pathogen from 12 diseased leaves, nine isolates were obtained. The colonies were white with a regular round shape at the early stage and became black with fluffy hyphae after 8 days on potato dextrose agar (PDA) medium, incubated at 25°C in the dark for 10 days. The single cell conidia were solitary, spherical or slightly ellipsoidal, black, shiny, smooth, aseptate, spherical, and 8.1 to

13.5 µm ($n = 50$) in diameter. Conidiophores (5.2 to 9.9×4.4 to 7.2 µm) were mostly reduced to conidiogenous cells and aggregated in clusters on hyphae. Conidiogenous cells were hyaline to pale brown or black, globose to ampulliform or clavate. Morphological characteristics of the isolates matched the description of the genus *Nigrospora* Mei Wang & L. Cai (Wang et al. 2017). For molecular identification, DNA was extracted, and PCRs were performed with primers ITS1/ITS4 for the ITS region (White et al. 1990), primers Bt2a/Bt2b for the β -tubulin gene (*TUB*) (Glass and Donaldson 1995), and primers EF1-728F/EF1-986R for the translation elongation factor 1-alpha gene (*EF1-α*) (Carbone and Kohn 1999). Representative sequences of the ITS region, *EF1-α*, and *TUB* sequences (from isolate WYR007) were deposited in GenBank (accession nos. MW561355, MZ053463, and MZ032030) and are included in the supplementary materials. BLAST analysis against sequences from previously published studies showed 99.58% (ITS region), 99.54% (*EF1-α*), and 99.45% (*TUB*) identity to *Nigrospora sphaerica* sequences (accession nos. MN215808.1, MN864137.1, and KY019606.1). In addition, homology was confirmed with a phylogenetic tree using concatenated sequences from ITS, *EF1-α*, and *TUB* constructed with MEGA 7 for which the maximum likelihood method was used with 1,000 bootstrapping iterations. To complete Koch's postulates, conidia suspensions of isolate WYR007 (prepared from 1-month-old colonies in 0.05% Tween 20 buffer and adjusted to a concentration of 1×10^3 conidia/ml) were sprayed on 15 leaves (200 µl per leaf) of five 1-year-old healthy *P. edulis* plants (cv. Panama Red). The same number of leaves from control group plants was only treated with 0.05% Tween buffer. All plants were incubated at $26^\circ\text{C} \pm 2^\circ\text{C}$ under a 16-h/8-h photoperiod and 70 to 75% relative humidity (RH) after inoculation. After 14 days, symptomatic blight appeared on all inoculated leaves. In contrast, no symptoms appeared on leaves in the control group. The disease assays were repeated three times. Pure cultures were reisolated from diseased leaves and confirmed to be *N. sphaerica* based on the morphological and molecular methods mentioned above (ITS region, *TUB*, and *EF1-α* sequences). To our knowledge, this study is the first report of *N. sphaerica* as a pathogen on *P. edulis* causing leaf blight. The identification of the pathogen could provide relevant background for its future management.

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