Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Bipolaris cactivora* Causing Flower Rot of Pitaya (*Hylocereus costaricensis*) in China

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Pitaya (*Hylocereus costaricensis*), belonging to the Cactaceae family, has rich functional substances, such as a variety of amino acids, which are popular with consumers (Wichienchot et al. 2010). In May 2019, flowers showed symptoms of rot, with an incidence of 15% in a plantation (233.3 ha) in Changjiang (19°46'N; 108°93'E), Hainan province, China. The initial disease symptoms of flowers were small, scattered purple-red spots (1 to 2 mm), including circular, long oval, or irregular in shape. The spots gradually expanded and coalesced, forming abundant reddish-brown lesions. Later, this disease resulted in rotting and blackening of the whole flower. Many black mildew layers (conidiophores and conidia) on the surface of the lesions were observed under compound microscopy. Symptomatic flower tissue (4 cm²) from collected samples was disinfected in 75% ethanol for 25 s followed by 1 min in 5% sodium hypochlorite, rinsed three times with sterile water, plated on potato dextrose agar (PDA) for 3 days, and incubated at 28°C. A fungus was consistently isolated from symptomatic flower samples with a 90% isolation rate. The resultant colony of the fungus was circular, dark green, velvety, and hairy after 7 days of incubation at 28°C. Hyphae were septate, 6.2 to 8.9 µm (average 7.6 ± 0.5) in diameter. Conidia were straight, obclavate, pale to mid brown, two to six septate, 23.0 to 42.2 µm (average 31.0 ± 3.2) × 6.5 to 9.8 µm (average 8.0 ± 0.6) (*n* = 100). The conidia normally produced germ tubes from one end or both ends. The width of conidiophore was 5.1 to 6.6 µm (average 5.8 ± 0.4) (*n* = 50). Sequences were generated from the isolate using primers for the internal transcribed spacer region (ITS) (ITS1/ITS4) (White et al. 1990),

ribosomal large subunit (LSU) (LROR/LR5) (Vilgalys et al. 1990), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GPD1/GPD2) (Berbee et al. 1999) loci. The resulting sequences were deposited in GenBank with accession numbers MN960109, MN966852, and MT542865. BLAST analysis demonstrated that these sequences were 99% similar to ITS (HM193535), LSU (MH869295), and GAPDH (HM598681) of *Bipolaris cactivora*. A maximum likelihood phylogenetic analysis based on combined dataset of ITS, LSU, and GAPDH sequences using MEGA7.0 revealed that the isolate was placed in the same clade as *B. cactivora* with 100% bootstrap support. A conidial suspension (1 × 10⁵ conidia/ml) of the fungal isolate was prepared by harvesting conidia from pure culture of the fungus grown on PDA for 25 days. The 10-ml suspension was sprayed onto 10 flowers with no wounding. Ten additional flowers sprayed with sterile distilled water served as controls. All flowers were covered with plastic bags to maintain high humidity and incubated under natural conditions. Typical symptoms of purple-red spots were observed on all the inoculated flowers on the third day. Abundant dark-brown to dark lesions were observed on the surface of flowers and were similar to those observed on the naturally infected flowers after 5 days. The control flowers remained asymptomatic. The fungal isolate of *B. cactivora* was reisolated from lesions of the flowers and reidentified by morphological and molecular characteristics, thus fulfilling Koch's postulates. Pathogenicity tests were repeated thrice with the same results. *B. cactivora* had been reported causing flower and fruit rot of pitaya in south Florida (Tarnowski et al. 2010). This is the first report of *B. cactivora* causing flower rot of pitaya (*H. costaricensis*) in China. The flower rot may provide inoculum for the fruit rot, which will cause reduction of pitaya yield.

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