Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum truncatum* Causing Anthracnose on *Piper betle* in China

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Betel vine (*Piper betle* L.) has been an important medicinal agent in the various traditional and folk systems of medicine in southern China. The leaves are the most valued plant part and in the past were routinely used as a chewing agent to prevent halitosis. The leaves are also supposed to harden the gum, conserve the teeth, and prevent indigestion, bronchitis, constipation, congestion, coughs, and asthma. In March 2019, leaf spot disease was detected on *P. betle* plants in Chengmai County, Hainan Province. Symptoms of the disease were characterized by oval-shaped, initially pale to yellow lesions that become necrotic (brown) with yellow borders, which were seen as concentric rings typical of anthracnose disease. A survey of *P. betle* plants revealed that the disease caused serious damage during the spring in Hainan Province, with 20 to 30% incidence in plants. To isolate the pathogen, seven isolates were obtained from 10 leaves after 5 to 6 days of incubation at 25°C. Three single-spore isolates (CHPB-1, CHPB-2, and CHPB-3) were obtained, and they were identical in culture and molecular analysis. Colonies on potato dextrose agar appear cottony, dense, grayish white mycelium, pale gray colony, with numerous black microsclerotia and setae. Conidia were single-celled, hyaline, cylindrical to slightly curved, with a rounded apex and truncated base that measured 18.3 to 21.7 (length) × 2.4 to 3.6 (width) µm ($n = 30$). The appressoria were elliptic to claviform or slightly lobed. The

morphological characteristics and measurements of this fungal pathogen matched the previous descriptions of *Colletotrichum truncatum* (Sawant et al. 2012). Three isolates were identified by molecular analysis; sequences of the internal transcribed spacer (ITS), actin (ACT), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) regions were obtained. These loci were amplified from isolates using the primer pairs ITS1/ITS4 (Mills et al. 1992), ACT-512F/ACT-783R (Weir et al. 2012), and GDF/GDR (Templeton et al. 1992), respectively. The sequences of the ITS-rDNA region (GenBank nos. MT071589, MT071588, and MN216313), the ACT sequences (GenBank nos. MT090050, MT090051, and MT090049), and the GAPDH sequences (GenBank nos. MT090052, MT090053, and MT090054) of the three single-spore isolates (CHPB-1, CHPB-2, and CHPB-3) were 100% identical to each other and 99% identical to *C. truncatum* isolate LJ731 (accession nos. KP748222, KP823769, and KP823769). Maximum likelihood trees based on concatenated sequences of the three genes were constructed using MEGA6.0. The results showed the strains isolated from *P. betle* were closely related to *C. truncatum*, as supported by high bootstrap values. Pathogenicity test was performed with three isolates (CHPB-1, CHPB-2, and CHPB-3) by depositing 10-µl droplets of a suspension (1×10^6 conidia/ml) on the surfaces of five artificially wounded leaves (a small hole made with a pushpin) of 10 healthy 1-year-old *P. betle* plants. An equal number of control leaves were inoculated with sterile water to serve as a negative control. The test was conducted three times. Plants were kept at 25°C with 80% humidity and observed for symptoms every day. One week after inoculation, all the inoculated plants showed symptoms of yellow sunken spots similar to those observed in the field. No symptoms were observed on the controls. The fungus reisolated from the infected tissues showed the same cultural and morphological characteristics of the strain inoculated, fulfilling Koch's postulates. *C. truncatum* was previously reported as the causal agent of anthracnose on begonia (Zhai et al. 2018), strawberry (Bi et al. 2017), and berry stalk on grape (Zhang et al. 2018) in China. To our knowledge, this is the first report of anthracnose on *P. betle* caused by *C. truncatum* in China.

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