Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Anthracnose Caused by *Colletotrichum karstii* of *Piper nigrum* in Yunnan Province, China

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Pepper (*Piper nigrum* L.), one of the economically important spice crops of China, is mainly grown in Hainan and Yunnan provinces. In January 2021, classic anthracnose lesions were observed on pepper leaves at a plantation (24°57'50"N, 98°53'00"E) in Baoshan city, Yunnan, China. Most of the diseased spots occurred at the tips and margins of the old pepper leaves. Lesions were grayish brown or pale white with a slight yellow halo; a concentric whorl of black dots or scattered black dots were observed on the leaves sometimes. Five symptomatic leaves from different parts of the field were sampled for pathogen isolation. Lesion tissues removed from the border between symptomatic and healthy tissue were surface sterilized in 75% ethanol, then air-dried, plated on potato dextrose agar (PDA) medium plates, and incubated in a 12-h photoperiod at 28°C. Similar fungal colonies developed from all plated tissues after 5 days. Five isolates from different leaves (one isolate per leaf) were subcultured using the single-spore method. The colonies appeared white, cottony, aerial mycelium dense and slow-growing (mean 1.01 mm day⁻¹) on PDA plates after 6 days. Conidia were short-cylindric, straight, sometimes slightly constricted near the center, ends broadly rounded, measuring 11.05 to 14.43 × 3.78 to 6.08 μm (average = 12.03 × 5.48 μm, *n* = 200). Appressoria were single, subglobose to elliptic, light brown to dark black. Genomic DNA of two

isolates (21HJ0301-1 and 21HJ0301-2) was extracted from mycelium and used as a template for molecular identification. The internal transcribed spacer (ITS) region of ribosomal DNA, and partial sequences of chitin synthase (CHS-1), actin (ACT), and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene regions were amplified with primer pairs ITS1/ITS4, CHS-79F/CHS-354R, ACT-512F/ACT-783R, and GDF/GDR, respectively (Weir et al. 2012). These four gene sequences were deposited in GenBank (accession nos. MZ725047 and MZ725048 for ITS, MZ733415 and MZ733416 for GAPDH, MZ733408 and MZ733409 for ACT, and MZ733422 and MZ733423 for CHS-1). A multilocus phylogenetic analysis performed with the reference sequences revealed that both 21HJ0301-1 and 21HJ0301-2 isolates clustered with *C. karstii*. Based on morphology and molecular results, isolates were confirmed to be *C. karstii*. Pathogenicity tests were carried out on potted seedlings in the greenhouse; six healthy leaves per isolate were inoculated with 6-day-old cultures of *C. karstii* mycelial discs of 5 mm in diameter after being wounded with a needle or nonwounded. Control leaves were inoculated with PDA agar. Inoculated plants were incubated under high relative humidity at room temperature. Anthracnose symptoms appeared within 5 days using nonwounded or wounded inoculation methods. All control leaves remained asymptomatic. The fungus was reisolated from inoculated leaves, fulfilling Koch's postulates, but not from controls. *C. karstii* has a wide range of hosts, such as rubber tree, tea-oil tree, chili, and some plants belonging to the family Orchidaceae in China (Cai et al. 2016; Diao et al. 2017; Jiang and Li 2018; Youlian et al. 2011). To the best of our knowledge, this is the first report of *C. karstii* on *P. nigrum* in China. This report will help us to recognize the anthracnose disease of *P. nigrum* and establish a foundation for future studies on *C. karstii* to address effective management strategies.

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