Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose on *Bletilla striata* Caused by *Colletotrichum fructicola* in China

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Bletilla striata (Thunb.) Rehb. f. (Orchidaceae) is a traditional Chinese medicinal plant. In April 2018 and 2019, a leaf spot disease was observed on ~20% of *B. striata* plants in two fields (~1.4 ha) in Guilin, Guangxi Province, China. Small, circular, brown spots were initially observed on the leaf surfaces, which progressively expanded into large, sunken, dark brown, necrotic areas. As the disease progressed, lesions merged into large, irregular spots, ultimately resulting in abscission. To determine the causal agent, small pieces (5 mm × 5 mm) were collected from the infected leaf tissues ($n = 18$), surface sterilized in 1% NaOCl for 2 min, and rinsed three times with sterile water. Then, the tissues were placed on potato dextrose agar (PDA) with chloramphenicol (0.1 g/liter) and incubated under a 12-h photoperiod at 26°C for 3 days. Seventeen isolates were obtained, of which 12 isolates with similar morphological characteristics were obtained from the germinated spores on PDA. Seven-day-old colonies on PDA appeared cottony, pale white to pale gray from above, and grayish-green from below. Conidia of strain BJ-101.3 were hyaline, aseptate, straight, and cylindrical, with rounded ends, measuring 11.3 to 15.9 μm × 4.0 to 6.4 μm ($n = 50$). Appressoria were brown to dark brown, with different shapes and a smooth edge, measuring 6.3 to 10.0 μm × 4.1 to 8.0 μm ($n = 50$). Morphological features were similar to *C. gloeosporioides* species complex

(Fuentes-Aragón et al. 2018; Weir et al. 2012). For molecular identification, DNA was extracted from two isolates BJ-101.3 and BJ-101.13, following the CTAB method (Guo et al. 2000). The internal transcribed spacer (ITS) region, partial actin (*ACT*), calmodulin (*CAL*), chitin synthase (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), manganese superoxide dismutase (*SOD2*), beta-tubulin (*TUB2*), glutamine synthetase (*GS*), and *Apn2-Mat1-2* intergenic spacer and partial mating-type (*ApMat*) genes were amplified by PCR and sequenced (Silva et al. 2012; Vieira et al. 2017; Weir et al. 2012). The obtained sequences were deposited in GenBank (MW386818, MW386819, MW403508 to MW403519, and MW888410 to MW888413). BLASTn analysis of the obtained sequences showed 99% identity with those of *C. fructicola* (JX010165, JX010033, FJ917508, FJ907426, JX009866, JX010095, JX010327, JX010405, JQ807838) (Liu et al. 2015; Weir et al. 2012). A phylogenetic tree based on the concatenated sequences confirmed the isolates as *C. fructicola*. Furthermore, pathogenicity tests were conducted on six 1.5-year-old *B. striata* plants. Healthy leaves on the plants were inoculated with the conidial suspensions (10⁶ conidia/ml; 10 μl) of the strains BJ-101.3 and BJ-101.13. The conidial suspension of each isolate was inoculated onto at least three leaves. Another three plants inoculated with sterile water served as the control. All plants were covered with transparent plastic bags and incubated in a greenhouse at 26°C for 14 days with a 12-h photoperiod. Nine days postinoculation, the inoculated leaves showed leaf spot symptoms, while the control plants remained symptomless. The experiments repeated three times showed similar results. Finally, *C. fructicola* was consistently reisolated from the infected leaves and confirmed by morphology and sequencing, fulfilling Koch's postulates. The outcome of this study will help in developing effective management measures against anthracnose of *B. striata*.

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