Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium commune* Causing Leaf Spot Disease on *Bletilla striata* in China

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Bletilla striata (Thunb.) Rehb. f. (family Orchidaceae, known as Baiji in Chinese) is a perennial herb and has been traditionally used for hemostasis and detumescence in China. In April 2020, a leaf spot disease on *B. striata* was observed in plant nurseries (~0.2 ha) in Guilin, Guangxi Province, China. Approximately 20% of plants were symptomatic, of which 150 were randomly selected for investigation. Initial symptoms included small, circular or irregular, light brown spots, randomly scattered on the edges and surfaces of the leaves, which progressively expand into large, suborbicular or irregular dark brown, necrotic areas. At the severe stage, the lesions coalesced into large necrotic areas and ultimately resulted in leaf abscission. To isolate the pathogen, three representative plants were collected from the nurseries. Leaf tissues (5 × 5 mm) were cut from the margin of necrotic lesions ($n = 18$), surface-disinfected in 1% sodium hypochlorite (NaOCl) solution for 2 min, then rinsed three times in sterile water before isolation. The tissues were plated on potato dextrose agar (PDA) and incubated at 28°C (12-h photoperiod) for 3 days. Hyphal tips from recently germinated spores were transferred to PDA to obtain pure cultures. Nine fungal isolates with similar morphological characteristics were obtained. Three single-spore isolates, BJ23.1, BJ55.1, and BJ91.3, were subjected to further morphological and molecular characterization. Colonies on PDA were villous, had a dense growth of aerial mycelia and appeared white (1A1) to yellowish white (3A2). Macroconidia were smooth, hyaline, straight to slightly curved, usually contained three or five septa, and 23.3 to 42.1 × 3.0 to 6.2 μm (mean ± SD: 31.2 ± 5.1 × 4.2 ± 0.6 μm, $n = 50$). Microconidia were generally cylindrical, straight to slightly curved, aseptate, and 7.2 to 18.8 × 2.5 to 4.3 μm (mean ± SD: 12.1 ± 2.8 × 3.3 ± 0.5 μm, $n = 62$). Morphological characteristics are similar to those of *F. commune* (Skovgaard et al. 2003). For molecular identification, the genomic DNA of isolates BJ23.1, BJ55.1, and BJ91.3 were extracted using the CTAB method (Guo et al. 2000). The internal transcribed

spacer (ITS) region of rDNA, partial translation elongation factor-1 alpha (TEF-1α), RNA polymerase second largest subunit (RPB2), and the mitochondrial small subunit rDNA (mtSSU) genes were amplified using primer pairs ITS1/ITS4 (White et al. 1990), EF-1/EF-2 (O'Donnell et al. 1998), 5f2/11ar (Liu et al. 1999, Reeb et al. 2004), and MS1/MS2 (Li et al. 1994), respectively. The obtained sequences were deposited in GenBank under accession nos. MZ424697–99 (ITS), MZ513467–69 (TEF-1α), MZ513473–75 (RPB2), and MZ513470–72 (mtSSU). BLAST analysis of the deposited sequences showed 99 to 100% identity with those of *F. commune* present in GenBank (DQ016205, MH582348, MH582181, AF077383). In addition, a phylogenetic analysis using concatenated sequences of ITS, TEF-1α, and mtSSU genes showed that BJ23.1, BJ55.1, and BJ91.3 are located on the same clade with strains of *F. commune*. Therefore, based on morphological and molecular characteristics, the isolates were identified as *F. commune* (Skovgaard et al. 2003; Stewart et al. 2006). Pathogenicity was tested using 1.5-year-old *B. striata* plants. Healthy leaves of three plants per isolate were inoculated with 5 × 5 mm mycelial discs of strains BJ23.1, BJ55.1, and BJ91.3 from 3-day-old PDA cultures; three control plants were inoculated with sterile PDA discs. All plants were enclosed in plastic bags and incubated in a greenhouse at 28°C for 14 days (12-h photoperiod). Three days postinoculation, leaf spot symptoms appeared on the inoculated leaves. No symptoms were detected on controls. Experiments were replicated three times with similar results. To fulfill Koch's postulates, *F. commune* was consistently reisolated from symptomatic tissue and confirmed by morphology and sequencing, whereas no fungus was isolated from the control plants. *F. commune* has been reported to cause diseases on some plants, including sugarcane (Wang et al. 2018), maize (Xi et al. 2019), and wax gourd (Zeng et al. 2020). To our knowledge, this is the first report of *F. commune* causing leaf spot disease on *B. striata* in China. Identification of this pathogen provides the information for further studies to develop management strategies to control the disease.

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The author(s) declare no conflict of interest.

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