Diseases Caused by Fungi and Fungus-Like Organisms

Identification of *Fusarium ipomoeae* as the Causative Agent of Leaf Spot Disease in *Bletilla striata* in China

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Bletilla striata (Thunb.) Rchb. f. (Orchidaceae) is traditionally used for hemostasis and detumescence in China. In April 2019, a leaf spot disease on *B. striata* was observed in plant nurseries in Guilin, Guangxi Province, China, with an estimated incidence of ~30%. Initial symptoms include the appearance of circular or irregular brown spots on leaf surfaces, which progressively expand into large, dark brown, necrotic areas. As lesions coalesce, large areas of the leaf die, ultimately resulting in abscission. To isolate the pathogen, representative samples exhibiting symptoms were collected; leaf tissues (5 × 5 mm) were cut from the junction of diseased and healthy tissue, surface disinfected in 1% sodium hypochlorite solution for 2 min, rinsed three times in sterile water, plated on potato dextrose agar (PDA) medium, and incubated at 28°C (12-h light-dark cycle) 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. Colonies on PDA were villous, had a dense growth of aerial mycelia, and appeared pinkish white from above and greyish orange at the center and pinkish-white at the margin on the underside. Macroconidia were smooth and hyaline, with a dorsiventral curvature, hooked to tapering apical cells, and three- to five-septate. Three-septate macroconidia were 21.2 to 32.1 × 2.4 to 3.9 μm (mean ± SD: 26.9 ± 2.5 × 3.2 ± 0.4 μm, *n* = 30); four-septate macroconidia were 29.5 to 38.9 × 3.0 to 4.3 μm (mean ± SD: 33.5 ± 2.6 × 3.6 ± 0.3 μm, *n* = 40); and five-septate macroconidia were 39.3 to 55.6 × 4.0 to 5.4 μm (mean ± SD: 48.0 ± 3.9 × 4.5 ± 0.3 μm, *n* = 50). These morphological characteristics were consistent with

Fusarium ipomoeae, a member of the *Fusarium incarnatum-equiseti* species complex (FIESC) (Wang et al. 2019). To confirm the fungal isolate's identification, the genomic DNA of the single-spore isolate BJ-22.3 was extracted using the CTAB method (Guo et al. 2000). The internal transcribed space (ITS) region of rDNA, translation elongation factor-1 alpha (TEF-1α), and partial RNA polymerase second largest subunit (RPB2) were amplified using primer pairs ITS1/ITS4 (White et al. 1990), EF-1/EF-2 (O'Donnell et al. 1998), and 5f2/11ar (Liu et al. 1999; Reeb et al. 2004), respectively. The ITS (MT939248), TEF-1α (MT946880), and RPB2 (MT946881) sequences of the BJ-22.3 isolate were deposited in GenBank. BLASTN analysis of these sequences showed over 99% nucleotide sequence identity with members of the FIESC: the ITS sequence showed 99.6% identity (544/546 bp) to *F. lacertarum* strain NRRL 20423 (GQ505682); the TEF-1α sequence showed 99.4% similarity (673/677 bp) to *F. ipomoeae* strain NRRL 43637 (GQ505664); and the RPB2 sequence showed 99.6% identity (1,883/1,901 bp) to *F. equiseti* strain GZUA.1657 (MG839492). Phylogenetic analysis using concatenated sequences of ITS, TEF-1α, and RPB2 showed that BJ-22.3 clustered monophyletically with strains of *F. ipomoeae*. Therefore, based on morphological and molecular characteristics, the isolate BJ-22.3 was identified as *F. ipomoeae*. To verify the *F. ipomoeae* isolate's pathogenicity, nine 1.5-year-old *B. striata* plants were inoculated with three 5 × 5-mm mycelial discs of strain BJ-22.3 from 4-day-old PDA cultures. Additionally, three control plants were inoculated with sterile PDA discs. The experiments were replicated three times. All plants were enclosed in transparent plastic bags and incubated in a greenhouse at 26°C for 14 days. Four days postinoculation, leaf spot symptoms appeared on the inoculated leaves, whereas no symptoms were observed in control plants. Finally, *F. ipomoeae* was consistently reisolated from leaf lesions from the infected plants. To our knowledge, this is the first report of *F. ipomoeae* causing leaf spot disease on *B. striata* in China. The spread of this disease might pose a serious threat to the production of *B. striata*. Growers should implement disease management to minimize the risks posed by this pathogen.

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