Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Rhizoctonia solani* AG-2-2 IIIB Causing Foliar Blight on *Bletilla striata* in China

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Bletilla striata (Thunb.) Rchb. f. (Orchidaceae), a perennial plant, is a traditional Chinese herb (known as baiji) used to treat hemorrhage, scalding injuries, gastric ulcers, pulmonary diseases, and inflammation (Zu et al. 2019). In May 2019, foliar blight symptoms were observed on approximately 25% of *B. striata* (cv. Guiji No.1) plants in three plantations (~4.5 ha in total) in Ziyuan County, Guangxi Province, China. Initial symptoms were light brown, irregular, water-soaked spots on the plant leaves. Several spots often merged, forming large, irregular lesions that extended onto the stem after a week and led to leaf abscission and even to plant death. To determine the causal agent, 5-mm squares cut respectively from the margin of six infected leaves were surface disinfected in 1% sodium hypochlorite solution for 2 min, rinsed three times with sterile distilled water, plated on potato dextrose agar (PDA), and incubated at 28°C (12-h light/dark cycle) for 3 days. The emerging hyphal tip of a single mycelium was transferred to PDA to obtain pure cultures of the isolates. Twenty isolates were obtained, of which 10 isolates (50%) were initially white before turning light brown (~4 days). Septate hyphae were 4.29 to 10.75 µm (average 6.42 µm) in diameter and branched at right angles with a constriction at the origin of the branch point. Staining with 1% safranin O and 3% KOH solution (Bandoni 1979) revealed multinucleated cells (three to nine nuclei per cell, $n = 142$). This morphology was typical of *Rhizoctonia solani* Kühn (Meyer et al. 1990). For species confirmation by molecular identification, three isolates (BJ101.6, BJ101.11, and BJ102.2) were cultured on PDA for 4 days, and then DNA was extracted from the mycelium using the CTAB method (Guo et al. 2000), and the ribosomal ITS1-5.8S-ITS2 region

was amplified by PCR using the universal fungal primers ITS1 and ITS4 (White et al. 1990). Internal transcribed spacer (ITS) sequences of strains BJ101.6, BJ101.11, and BJ102.2 (deposited in GenBank under accession nos. MT406271, MT892815, and MT892814, respectively) had over 99% similarity with those of *R. solani* anastomosis group (AG) 2-2 IIIB in GenBank (accession nos. JX913810 and AB054858) (Carling et al. 2002; Hong et al. 2012). Phylogenetic analysis using ITS sequences showed that the isolates clustered monophyletically with strains of *R. solani* AG-2-2 IIIB. The AG of the isolates was confirmed by their ability to grow well on PDA at 35°C, which separates AG-2-2 IIIB from AG-2-2 IV (Inokuti et al. 2019). Based on morphological characteristics and nucleotide sequence analysis, the isolates were identified as *R. solani* AG-2-2 IIIB. Pathogenicity was tested using 1.5-year-old *B. striata* (cv. Guiji No.1) plants grown in a perlite and peat moss mixture (1:3) in 7-cm pots. Healthy leaves on plants were inoculated with an aqueous suspension (approximately 1×10^5 hyphal fragments/ml, 100 µl) prepared from cultures of strains BJ101.6, BJ101.11, and BJ102.2, and each isolate was inoculated onto three plants; three other plants with sterile water served as controls. All plants were enclosed in transparent plastic bags and incubated in a greenhouse at 28°C for 14 days (12-h photoperiod). Three days postinoculation, leaves exposed to the mycelial fragments had symptoms similar to those originally observed in the field. No symptoms were detected on control plants. Experiments were replicated three times with similar results. To fulfill Koch's postulates, *R. solani* AG-2-2 IIIB was reisolated on PDA from symptomatic leaves and confirmed by sequencing, whereas no fungus was isolated from the control plants. To our knowledge, this is the first report of *R. solani* AG-2-2 IIIB causing foliar blight on *B. striata* in China, and these findings will be useful for further control strategies and research.

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