Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Root Rot Caused by *Dactylonectria torresensis* on *Bletilla striata* (Baiji) in Yunnan, China

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Bletilla striata (Thunb.) Rchb.f. (Orchidaceae, “baiji” in Chinese) is an endangered species with medicinal value in China. *B. striata* plants with wilting, leaf yellowing, and rotting on underground parts were found in Shizong (24.82822 N, 103.99084 E), Yunnan Province, China, in July 2016. In the following years, this disease became prevalent when high temperature and humidity prevailed from May to August. Disease incidence was 45 to 75%, with yield losses of 40 to 65% in different fields. To identify the causal agent, symptomatic vascular tissue fragments were soaked in 2% NaOCl for 2 min, rinsed twice with sterile distilled water, and placed on 4% (w/v) PDA plates. Plates were incubated at 26°C in 12-h light/dark for 3 days. Mycelia grown from the edges of the plant fragments were transferred to PDA plates and incubated at 26°C in 12-h light/dark. After 3 days, hyphal tips were isolated from the edge of the colonies to PDA plates. Three hyphal-tip isolates from different plants were further studied. Colonies of these isolates were dark red, with cottony mycelia of moderate density. Hyphae were transparent and branched. Numerous hyphae anastomosed frequently and formed hyphal coils. For further analysis, sporulation was induced as described by Cabral et al. (2012) and Lombard et al. (2014). Macroconidia were abundant, 37.2 to 44.0 × 5.2 to 8.7 µm ($n = 20$ conidia per isolate). Ascospores divided into two cells of equal size, ellipsoid to oblong-ellipsoid, 12.5 to 14.8 × 4.8 to 5.9 µm ($n = 20$ spores per isolate). Conidiophores simple or complex, sporodochial. Simple conidiophores arising laterally or terminally from aerial mycelium,

solitary to loosely aggregated, unbranched or sparsely branched, more or less cylindrical. Morphological characteristics were consistent with *Dactylonectria* spp. (Cabral et al. 2012; Lombard et al. 2014). From one isolate, the ITS region of ribosomal DNA and the β -tubulin (*tub2*) gene were amplified by PCR using primer pairs ITS1/ITS4 (White et al. 1990) and T1/Bt-2b (Cabral et al. 2012), respectively. PCR products were sequenced and deposited in GenBank as MH458779 (ITS) and MH626485 (*tub2*). BLAST search revealed that both sequences showed 99 to 100% homology with the corresponding sequences of previously identified *Dactylonectria torresensis* isolates. Specially, MH458779 shares 100% identity with the entire 463-bp sequence of KP411806, the ITS sequence of a *D. torresensis* isolate identified from olive trees (Nigro et al. 2019); MH626485 shares 99% identity with the entire 320-bp sequence of KP411801, the *tub2* sequence of the same isolate. In addition, the entire 609-bp sequence of MH626485 shares 99% identity with JF735478, the *tub2* sequence of a *D. torresensis* isolate identified from grapevines (Cabral et al. 2012). To test pathogenicity, *B. striata* plants in plastic pots filled with sterilized nursery soil were inoculated with each of the three isolates by placing a fungal-colonized wheat kernel adjacent to each healthy plant. Plants inoculated with noncolonized wheat kernels were used as controls. Plants in three pots (replicates), with one plant per pot, were inoculated by each isolate. The pots were maintained in a greenhouse with a 12-h photoperiod at 25°C. Ten days after inoculation, black necrotic lesions identical to those observed in the field were evident on the roots of all inoculated plants. Using the same methods described above, fungi with identical morphologies as described above were isolated from lesions caused by each of the three isolates. Control plants remained healthy, and no fungus was reisolated. This completed Koch's postulates. Based on morphological characteristics and molecular identification, the pathogen was determined to be *D. torresensis*. To our knowledge, this is the first report of *D. torresensis* causing root rot of *B. striata* in Yunnan, China. It is important to further study the impacts of this new disease on *B. striata* production in China.

References:

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