Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Berkeleyomyces basicola* Causing Black Root Rot on Lisianthus (*Eustoma grandiflorum*) in China

Y.-S. Huang, X.-W. Xie, Y.-X. Shi, A.-L. Chai, L. Li,[†] and B.-J. Li[†]

Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

Y.-S. Huang and X.-W. Xie contributed equally to this paper.

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Lisianthus (*Eustoma grandiflorum* [Raf.] Shinn.) is an important ornamental plant ranking in the top 10 cut flowers worldwide (Xiao et al. 2018). In 2020 and 2021, black root rot was found as a major disease limiting lisianthus production in Yunnan Province, China. Black root rot was first observed in early July 2020 on lisianthus grown in a commercial flower-production plantation, with nearly 60% of plants infected. Symptoms appeared as coalescing necrotic lesions leading to black discoloration of the roots. Root damage induced by disease resulted in insufficient water and nutrient uptake by the plant, causing stunting and whole-plant wilting. The pathogen could not infect the intact endodermis, and vascular tissues below the discolored cortical tissue remained healthy. Symptomatic roots were surface sterilized using 1% NaClO for 1 min, rinsed three times in sterile water, placed onto potato dextrose agar (PDA), and incubated at 25°C for 7 days in the dark. The morphological characteristics were basically consistent: the colonies were white to gray in color, and the conidiophores were colorless to brown, solitary or clustered. Conidia were single-celled, colorless, rod-shaped, and obtuse at both ends. Chlamydospores were dark brown, clustered or solitary. The morphological characteristics of the pathogen were similar to those of *Berkeleyomyces basicola* (Berk. & Broome) W.J. Nel, Z.W. de Beer, T.A. Duong & M.J. Wingf. (Nakane et al. 2019). DNA was extracted from mycelia of representative isolate TB using the Plant Genomic DNA Kit (Tiangen, Beijing, China). The internal transcribed spacers (ITS), DNA replication licensing factor (MCM7), ribosomal large subunit (LSU), and 60S ribosomal

protein RPL10 (60S) regions were amplified with primer pairs ITS1/ITS4 (Groenewald et al. 2013), MCM7-for/MCM7-rev, LR0R/LR5, and 60S-506F/60S-908R, respectively (Nel et al. 2018). Phylogenetic analysis of multiple genes (Bakhshi et al. 2018) was conducted with the maximum likelihood method using MEGA7. The sequences of our isolate (TB) and three published sequences of *B. basicola* were clustered into one clade with a 100% bootstrapping value. The accession numbers of *B. basicola* reference sequences are MF952423 (ITS), MF967079 (MCM7), MF948658 (LSU), and MF967072 (60S) of isolate CMW6714; MF952428 (ITS), MF967088 (MCM7), MF948661 (LSU), and MF967073 (60S) of isolate CMW25440; and MF952429 (ITS), MF967102 (MCM7), MF948659 (LSU), and MF967075 (60S) of isolate CMW49352. The sequences of TB have been deposited in GenBank with accession numbers MZ351733 for ITS, MZ695817 for MCM7, MZ695816 for LSU, and MZ695815 for the 60S region. To verify the pathogenicity of the fungus, inoculations were performed on ten 2-month-old potted lisianthus plants by dipping the roots into a conidial suspension (10⁵ spores/ml) for 2 h. Ten plants were mock inoculated with distilled water as a control. Symptoms of black root rot were observed 30 days after inoculation, whereas the control roots remained healthy. The causal fungus has a host range of over 230 species and is a destructive pathogen of many crops and ornamental plants, including cotton (*Gossypium barbadense* L.), tobacco (*Nicotiana tabacum* L.), and mango (*Mangifera indica* L.) (Shukla et al. 2021; Toksoz and Rothrock 2009). This is the first report worldwide of *B. basicola* infecting lisianthus. This discovery is of great importance for Chinese flower growers because this fungus is well established in the observed area, and effective measures are needed to manage this disease.

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[†]Indicates the corresponding authors.

L. Li; caulilei@163.com, and B.-J. Li; libaojuivf@163.com