Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium oxysporum* Causing Root Rot on *Pleione bulbocodioides* in China

Zhonghui Hu,[1] Wei Zhang,[1] Yufan Yang,[2] and Shibao Zhang[1,†]

[1] Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[2] Zhaotong Yunsheng Agricultural Technology Development Limited Company, Zhaotong 657109, China

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The members of *Pleione* (family Orchidaceae) are popular worldwide because of their beautiful flowers and medicinal value. In October 2021, we observed the typical symptoms of root rot such as yellow or brown leaves, rotted root, and plant death on *P. bulbocodioides*. Nearly 30% of the plants showed disease symptoms in the farm in Zhaotong City, Yunnan Province, China. Three fresh root samples with typical symptoms were collected from the plants of *P. bulbocodioides* in the field. The root sections (3 × 3 mm) from the border of the symptomatic tissue were cut, sterilized with 75% ethanol for 30 s, followed by 3% sodium hypochlorite (NaClO) for 2 min, and then rinsed three times with sterile water. The sterilized root tissues were inoculated on potato dextrose agar (PDA) at 28°C in the incubator for 3 days. Colonies were obtained and subcultured from the hyphal tip on the new PDA for further purification. The colonies grew on PDA at 28°C for 1 week, the hyphal color from white turned to purple, and the center of the colonies became brick red. The colonies produced abundant microconidia, macroconidia, and chlamydospores, but no sporodochia were observed. Microconidia were oval or irregularly oval, zero to one septate, and 2.0 × 5.2 to 4.1 × 12.2 µm (n = 20). Macroconidia were falcate, slender, with a distinct curve to the latter half of the apical cell, three to five septate, and 4.0 × 15.2 to 5.1 × 39.3 µm (n = 20). Morphological characterization showed that the three isolates were similar and appeared to be *Fusarium oxysporum* (Leslie and Summerell 2006). For molecular identification, total genomic DNA of the two representative isolates DSL-Q and DSL-Y was extracted with the CTAB method, and the PCR amplification was performed. The sequence of partial translation elongation factor 1-alpha (*TEF1-α*) gene was amplified using the primer pair EF-1/EF-2 (O'Donnell et al. 1998). The sequence of β-tubulin (*TUB2*) gene was amplified using the primer pair T1/T22 (O'Donnell and Cigelnik 1997). The sequences from the two isolates were obtained and sequenced. Clustal2.1 searches indicated that the sequences of the three loci of the two isolates revealed 97.8 to 100% similarity to *F. oxysporum* strains and were deposited in GenBank (accession nos. OP150481 and OP150485 for *TEF1-α*; OP150483 and OP186426 for *TUB2*). A pathogenicity test was performed to confirm Koch's postulates. Inoculum was obtained from the two isolates by culturing in 500 ml of potato dextrose broth on a shaker at 25°C. After 10 days, the hyphae grew into a cluster. The six individuals of *P. bulbocodioides* were divided into two groups. Three individuals grew in the bark substrate containing hyphae cluster, while another three individuals grew in the bark substrate containing sterile agar medium. The plants were kept in a greenhouse (constant temperature at 25°C, day and night for 12 h). After 20 days, the group inoculated with *F. oxysporum* isolates showed the same disease symptoms observed on the plants in the field, whereas the control plants remained disease free. *F. oxysporum* was reisolated from the infected tissues. Phylogenetic dendrograms of *F. oxysporum* were grouped by *TEF1-α* and *TUB2* sequences. The results confirmed that this fungus was identical to those identified by colony morphology, phylogenetic relationship, and *TEF1-α* and *TUB2* sequences. To our knowledge, this is the first report of *F. oxysporum* causing root rot on *Pleione* species in China. This is a pathogenic fungus in the production of *Pleione* species. Our study is helpful for the identification of root rot on *Pleione* species and the development of disease control strategy for cultivation.

References:

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[†]Indicates the corresponding author.
S. B. Zhang; sbzhang@mail.ac.cn