Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Necrosis of Balloon Flower (*Platycodon grandiflorus*) Caused by *Nigrospora sphaerica* in China

Ying Chen, Dankan Yan, Kelei Han, Chao Ma, Zhengliang Gao, Xianxun Bao, and Fang Wang[†]

Institute of Plant Protection and Agro-Products Safety, Anhui Academy of Agricultural Sciences, Hefei, Anhui 230031, China

Y. Chen and D. Yan contributed equally to this work.

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Balloon flower (*Platycodon grandiflorus* (Jacq.) A.DC.) is a perennial dicotyledonous herb. It is one of the most used medicinal plants in China, with broad applications. It is also a functional food rich in polysaccharides, minerals, and fiber (Lee et al. 2014). Field surveys conducted in August and September 2020 in major balloon-flower-growing regions of Anhui province (115°25' E, 33°08' N) in China revealed the occurrence of an unknown disease with characteristic necrosis symptoms. The disease occurred in 6 to 8% of the balloon flower leaves. Symptoms were characterized by leaf necrosis and plant withering. Infected leaves were cut into small pieces, surface sterilized with 2% NaOCl for 2 min, rinsed in sterile distilled water three times, and dried by blotting filter paper. Treated tissues were placed on potato dextrose agar (PDA) and incubated at room temperature (25 ± 2°C) for 2 days. Colonies grew rapidly and reached 8 cm in diameter after 15 days at 25°C. Pure cultures were obtained, and their colonies were initially white and eventually turned gray. Conidia were black, single-celled, smooth, spherical, 10 to 22 µm in diameter ($n = 30$), and borne singly on a hyaline vesicle at the tip of each conidiophore. Based on the characteristics of colony morphology, the fungus was identified as a *Nigrospora* sp. A pathogenicity test was conducted on healthy balloon flower plants grown under greenhouse conditions to complete Koch's postulates. The adaxial surface of each of five healthy leaves was pricked and inoculated with a disc

grown with the 6-day-old mycelium of a *Nigrospora* sp. Five leaves inoculated with PDA-only discs served as the controls. Treated leaves were wrapped with parafilm and treated plants were covered with polyethylene bags for 24 to 48 h. After 10 days of inoculation, typical necrotic lesions and stem necrosis developed on the inoculated leaves while no symptoms developed on the control leaves. The same fungus was reisolated from diseased leaves and its identity confirmed based on morphology and culture characteristics. To confirm the identification, total DNA was extracted and the internal transcribed spacer region (ITS), a partial sequence of the β -tubulin (*TUB*) gene, and the *translation elongation factor 1- α* (*TEF1*) gene were amplified and sequenced using the primers ITS1/ITS4 (5'-TCCGTAGGTGAACCTGCGG-3' and 5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990), Bt2a/Bt2b (5'-GGTAACCAATCGGTGCTGCTTTC-3' and 5'-ACCCTCAGTGTAGTGACCCTTGCC3') (Glass and Donaldson 1995), and EF1-728F/EF1-986R (5'-CATCGAGAAGTTCGAGAAGG3' and 5'-TACTTGAA GGAACCCCTTACC-3') (Carbone and Kohn 1999), respectively. The sequences (GenBank accession no. MW082789 for ITS, MW368916 for TUB, and MW368915 for TEF1) were aligned using Blastn in GenBank, revealing 100% identity (97% coverage) with *Nigrospora sphaerica* for ITS (MW081353), 100% identity (97% coverage) with *N. sphaerica* for TUB (MN719407), and 99% identity (98% coverage) with *N. sphaerica* for TEF1 (MN053315). Based on morphological, microscopic, and molecular characteristics, the associated fungal pathogen was identified as *N. sphaerica*. This is the first report of *N. sphaerica* causing necrosis disease on balloon flower in China. This study provides important information for the identification of the balloon flower fungal disease, which will help develop management strategies to reduce economic losses caused by the disease.

References:

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[†]Indicates the corresponding author.
F. Wang; wangfang1378@126.com