Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Binucleate *Rhizoctonia* AG-L Causing Root and Stem Rot of Wishbone Flower (*Torenia fournieri*) in Taiwan

C. X. Chen,¹ Y. F. Wu,² H. H. Gong,¹ Y. J. Lin,¹ and C. Y. Chen^{1,†}

¹Department of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan

²Tainan District Agricultural Research and Extension Station, Council of Agriculture, Sinhua, Tainan 71246, Taiwan

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Wishbone flower (*Torenia fournieri* L.) is a common ornamental plant for flower beds in Taiwan. In August 2018, root and stem rot of wishbone flower occurred on the flower bed in the campus of National Chung Hsing University, Taichung city, with 100% incidence. Symptoms were dark brown discoloration of basal stems and brown necrosis of roots. Lesions from the base of stems were excised into 5-mm-long fragments, which were then surface sterilized in 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water, dried on filter paper, and thereafter placed onto 2% water agar. After 24 h, hyphae characteristic of *Rhizoctonia* (Sneh et al. 1991) appeared and dominated in every isolation. Hyphal tips were transferred to potato dextrose agar (PDA). After 5 days of incubation at 28°C, characteristic brown colonies of *Rhizoctonia* (Sneh et al. 1991) were observed. Hyphal width was 4.29 ± 0.52 µm. No sclerotia were visibly present after 21 days of growth on PDA at 28°C. Hyphae were stained by 0.3% safranin-O and 1% KOH. There were two nuclei in each hyphal compartment, suggesting a binucleate *Rhizoctonia* fungus. The ITS sequence has been used as the best tool to identify the specific anastomosis group (AG) of *Rhizoctonia*, as shown by Sharon et al. (2006, 2008). The ITS sequence was amplified using the primers Bd1a and ITS4 (Goka et al.

2009; White et al. 1990). BLAST search analysis of this acquired sequence (accession no. LC498494) revealed the highest similarity (98.75 to 99.83%) with the reference sequences (accession nos. AB286934, AB286933, and AB196653) of binucleate *Rhizoctonia* AG-L, namely *Ceratobasidium* sp. AG-L. A pathogenicity test was carried out using seedlings of 4-week-old wishbone flower, each grown in a pot of 6.35 cm diameter. To prepare the inoculum, a PDA block (6 mm in diameter) excised from the growing front of 5-day-old colony was transferred into a flask with 200 ml of potato dextrose broth (PDB) and incubated in a shaker at 26°C and 120 rpm for 6 days. The PDB was then blended into a slurry. Ten pots, each with a seedling, were inoculated by pouring 50 ml of slurry onto the potting medium. Five pots served as the controls by pouring PDB only. Pots were maintained in a greenhouse (26 to 33°C). Three days after inoculation, all inoculated plants exhibited symptom of root and stem rot. The same fungus was reisolated and confirmed by sequencing the rDNA-ITS. This is the first report of root and stem rot of wishbone flower caused by binucleate *Rhizoctonia* AG-L in Taiwan and in the world. Although this is the second case, since Wang and Hsieh (1993), for binucleate *Rhizoctonia* AG-L to be pathogenic, this study shows that this fungus has the potential to cause damage and is worth further investigation.

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The author(s) declare no conflict of interest.

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[†]Indicates the corresponding author.

C. Y. Chen; chiyu86@dragon.nchu.edu.tw