Diseases Caused by Fungi and Fungus-Like Organisms

Leaf Spot Disease Caused by *Alternaria alternata* on *Rehmannia glutinosa* in China

Jia-fang Du,¹ Wen-kai Nian,¹ Zhang-jin Zhou,¹ Tao Dou,¹ Guo-hong Song,¹ Li Gu,² Ming-jie Li,² Min Wang,¹ Yue-hua Geng,¹ Ying Zhao,¹ Feng-qing Wang,¹ Mo Wang,³ Zhong-yi Zhang,² and Xiang-yang Liu^{1,†}

¹ Henan Agricultural University, Zhengzhou 450002, China

² Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China

³ College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China

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Rehmannia glutinosa Libosch. is a perennial herbaceous plant used as one of the basic herbal medicines in 50 traditional Chinese medicines (Zhang et al. 2016). From 2017 to 2019, outbreaks of leaf spot disease occurred on *R. glutinosa* plants in Jiaozuo, Henan Province, China, with the incidence ranging from 20 to 40%. The disease mainly occurred in late July and August. Symptoms included small water-soaked pale brown at the early stage and circular or irregular spots with black or dark brown mildew center sometimes surrounded by pale yellow halos in the late stage, measuring 3 to 10 mm in diameter. Infected leaf sample tissues (5 × 5 mm) collected from the field were surface sterilized with 75% ethanol for 30 s and 2% sodium hypochlorite for 90 s, rinsed four times with sterilized distilled water, and incubated on potato dextrose agar (PDA) containing 50 µg/ml of cephalosporin. The plates were incubated at 25°C for 6 days with a 12-h photoperiod. The fungal colonies were olivaceous to dark olive with whitish mycelium borders and abundant aerial mycelia. Conidiophores were septate and measured 1.9 to 4.1 µm in width. Conidia that produced in single or branched chains were obclavate or ovoid, measuring 7.5 to 46.7 × 6.2 to 18.3 µm ($n = 50$) with two to five transverse septa and zero to three longitudinal septa. Based on the morphological characteristics (Simmons 2007), the fungus was identified as *Alternaria alternata*. Molecular identification of the fungus was performed by sequencing the internal

transcribed spacer, glyceraldehyde-3-phosphate dehydrogenase, RNA polymerase II second largest subunit, translation elongation factor 1- α gene, major allergen Alt a 1 gene, endopolygalacturonase gene, and anonymous gene region amplified by primers ITS1/ITS4 (White et al. 1990), Gpd-1/Gpd-2 (Berbee et al. 1999), RPB2-5F2/RPB2-5R (Sung et al. 2007), EF-728F/EF-986R (Carbone and Kohn 1999), Alt-for/Alt-rev (Hong et al. 2005), EPG-specific/EPG-3b (Peever et al. 2005), and OPA 10-2R/OPA 10-2L (Andrew et al. 2009) from the total genomic DNA that was extracted with the CTAB method (Stenglein and Balatti 2006). BLAST analysis showed 99 to 100% similarity with previously submitted sequences of *A. alternata* (MH084279, MK637438, MK605900, KY094928, JQ595557, MH975219, and MH003611). Phylogenetic analysis based on multigenes indicated that the isolate and other *A. alternata* strains formed one clade. Then sequences of the isolate were deposited in GenBank (MN512447, MN900903, MN900904, MN900905, MN900906, MN900907, and MN900908). To test the pathogenicity of the isolate, mycelial plugs (4 mm in diameter) from 6-day-old cultures on PDA were placed on the leaf surface of 24 plants. PDA only plugs inoculated on 12 plants served as the control. Treated plants were put in a growth chamber at 28°C with 80 to 85% relative humidity under a 12-h photoperiod for 6 to 8 days. The inoculated leaves developed dark brown lesions similar to those observed on the leaves with initial symptoms in the field, whereas the control leaves remained symptomless. The fungus was reisolated from the diseased leaves and confirmed as *A. alternata* based on morphological and molecular characteristics, which satisfied Koch's postulates. To the best of our knowledge, this is the first report of *A. alternata* causing leaf spot on *R. glutinosa* in China.

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[†]Indicates the corresponding author.

E-mail: liuxiangyang@henau.edu.cn (X.-yL.)