Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Southern Blight on *Polygonatum sibiricum* Caused by *Sclerotium delphinii* in China

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Polygonatum sibiricum Delar. ex Redoute is used for medicine and food. Its rhizomes have potential medicinal values such as enhancing immunity, antiaging, antitumor, and antibacterial, as well as improving memory and reducing blood lipids and sugar. Rhizomes can also be used as raw materials for drinks, preserves, and health products (Su et al. 2018). Annual demand for *P. sibiricum* is ~3,500 to 4,000 t in China, and the demand and price continue to rise (Su et al. 2018). In August 2019, there was an outbreak of southern blight in the *P. sibiricum* planting fields (N30°04'06", E115°39'47") of Luotian County in Hubei province of China. Approximately 30% of plants were affected in many fields (333.33 ha). We observed that the surface of the infected rhizome and the surrounding soils were covered with white hyphae and sclerotia. Hyphae gradually extended downward to the rhizomes, causing rhizome rot and leaf yellowing and wilting. Mycelial fragments and sclerotia from 10 symptomatic rhizomes were collected in the fields and incubated directly on PDA containing 50 µg/ml of kanamycin at 27°C. The fungal colonies were transferred to PDA after 2 days of cultivation. The white colonies were formed with fluffy aerial mycelia, which grew radially with an average growth rate of 20.54 ± 0.52 mm/day ($n = 10$). Sclerotia were milky white at first and then gradually turned to beige and yellow-brown. After a 2-week incubation, sclerotia became dark brown. Most of the sclerotia were spherical or nearly spherical, with round bulges on the surface. The number of mature sclerotia produced per plate ranged from 8 to 23 ($n = 10$), and the size ranged from 2.5 × 3.0 mm to 7.5 × 13.0 mm (5.95 ± 2.34 × 7.51 ± 2.88 mm; $n = 50$). Clamp connections were observed under the microscope. For molecular identification, genomic DNA was

extracted from isolate HJ-1 using the CTAB method (Mahadevakumar et al. 2018). The internal transcribed spacer (*ITS*) regions of rDNA were amplified with primers ITS1/ITS4 (White et al. 1990). The resulting *ITS* sequence (MW049362) showed 99.66% homology with *Sclerotium delphinii* according to GenBank. The second largest subunit of RNA polymerase II gene (*RBP2*) and part of the elongation factor 1- α (*EF1- α*) gene were also amplified by using the primers RPB26F/RPB2-7CR (Liu et al. 1999) and EF595F/EF1160R, respectively (Wendland and Kothe 1997). *RBP2* gene sequence was deposited in GenBank (MW415935) and had 99.53% identity to *Athelia rolfsii* isolate MSB5-1. *TEF-1 α* sequence was deposited in GenBank (MW415934) and had 91.35% similarity to *S. delphinii* strain Sd_405. Because there are few reference sequences of *RBP2* genes from *S. delphinii* in GenBank to compare with, we choose the *ITS* and *TEF-1 α* gene sequences to construct the concatenated phylogenetic tree by the neighbor-joining method (Tamura et al. 2013). Results showed that HJ-1 clustered with *S. delphinii* isolates selected from NCBI database. Based on morphological and molecular characteristics, the fungus was identified as *S. delphinii* Welch (teleomorph *Athelia rolfsii* [Curzi] C.C. Tu & Kimbr). Pathogenicity tests were performed on healthy leaves, roots, stems, and plants ($n = 3$) of *P. sibiricum*. Each sample was inoculated with one sclerotium produced from a 15-day-old colony and there was on-wound treatment. Inoculated and control samples (treated with sterile water) were incubated in a moist chamber (25 ± 2°C, RH 85%) (Mahadevakumar et al. 2018). Typical disease symptoms were apparent on leaves, stems, rhizomes, and plants at 4, 6, 5, and 15 days postinoculation, respectively. Fulfilling Koch's postulates, the fungal pathogens were isolated and purified from the inoculated site and were reconfirmed as *S. delphinii* based on the morphological features. This is the first report of *S. delphinii* causing southern blight on *P. sibiricum* in China. *S. delphinii* has a wide host range worldwide and often causes crop yield reduction. This study will be helpful for the prevention and control of *P. sibiricum* southern blight.

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