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Sclerotium rolfsii causes stem rot on *Ixeridium dentatum* in Korea

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Abstract

Stem rot was observed on *Ixeridium dentatum* in Jinan-gun and Dangjin-gun, Korea during the growing seasons of 2016 and 2017. The first symptom was water-soaked lesions on the basal parts of stems. Lower leaves became wilted and blighted before the plants eventually died. White cottony mycelial mats and brown spherical or irregular sclerotia formed on the basal stem and adjacent soil surfaces. The optimal temperature for in vitro colony growth and sclerotia germination were 30 °C and 25 °C, respectively. The in vitro and morphological characteristics of the fungus were identical with those described for *Sclerotium rolfsii*. Phylogenetic analysis based on the internal transcribed spacer (ITS) region revealed that two isolates isolated from *I. dentatum* formed a monophyletic group with reference isolates of *S. rolfsii*. Koch's postulates were satisfied for the same two *S. rolfsii* isolates, thereby confirming that the pathogen causes stem rot of *I. detatum* in Korea.

Keywords *Ixeridium dentatum* · New report · *Sclerotium rolfsii* · Stem rot

Ixeridium dentatum, a perennial herb belonging in the family Asteraceae, is widely distributed throughout East Asia including Korea, Japan, and China (Yook 1997). The plant is well known as a medicinal crop with anti-cancer, anti-oxidative, and anti-allergic activities (Yi et al. 2002; Lee et al. 2014). Cucumber mosaic virus (CMV disease), Tomato spotted wilt virus (TSWV), and *Puccinia lactucae-debilis* (rust) have been reported to be pathogenic to *I. dentatum* in Korea (Anonymous 2009).

Infected portions of the stem as well as sclerotia around the stem base of affected *I. dentatum* plants were collected from several areas of Jinan-gun and Dangjin-gun, Korea during the growing seasons of 2016 and 2017. Initial symptoms of infection were water-soaked lesions on the stems near the soil line. Infected plants became wilted and blighted and a white mycelium mat and abundant, spherical, brown sclerotia appeared on stems at the soil line. Ultimately the plants rotted and died (Fig. 1). The infected tissues and sclerotia were surface-sterilized with a 1% NaOCl solution for 1 min, rinsed three times with sterilized distilled water, and placed on water agar at 25 °C for two days. The fungal hyphae growing from the tissue and sclerotia were transferred to potato dextrose agar (PDA, Difco, Becton Dickinson) plate. Five (5) isolates of the fungus obtained from rotting stem of *Ixeridium dentatum* were stored in 30% glycerol at −70 °C at the Herbal Crop Research Division Collection (HCRD). Two isolates (HCRD 16076 and HCRD 16077) used in this study also was deposited in the Korean Agricultural Culture Collection (KACC), Korea (KACC48476 and KACC48477).

To observe colony morphology, agar block colonized by hyphae of two isolates (KACC48476 and KACC48477) were placed on PDA in 9-cm-diameter petri dishes and incubated at 10, 15, 20, 25, and 30 °C for seven days in the dark. To assay germination of sclerotia, the sclerotia were surface-sterilized with a 1% NaOCl solution for 1 min, rinsed three times with sterilized distilled water, and placed on PDA plate at 10, 15, 20, 25, and 30 °C for seven days. Colonies growing on PDA were white, cottony, often with fan-shaped sections and hyphae were 4.0–8.5 μm in diameter with clamp connections. Sclerotia started to develop in the colonies after 5 days at 30 °C, initially white then becoming brown with age, spherical or irregular, and 1.0 to 3.5 mm in diameter. The optimal temperature for colony growth and sclerotial germination was 30 °C and 25 °C,

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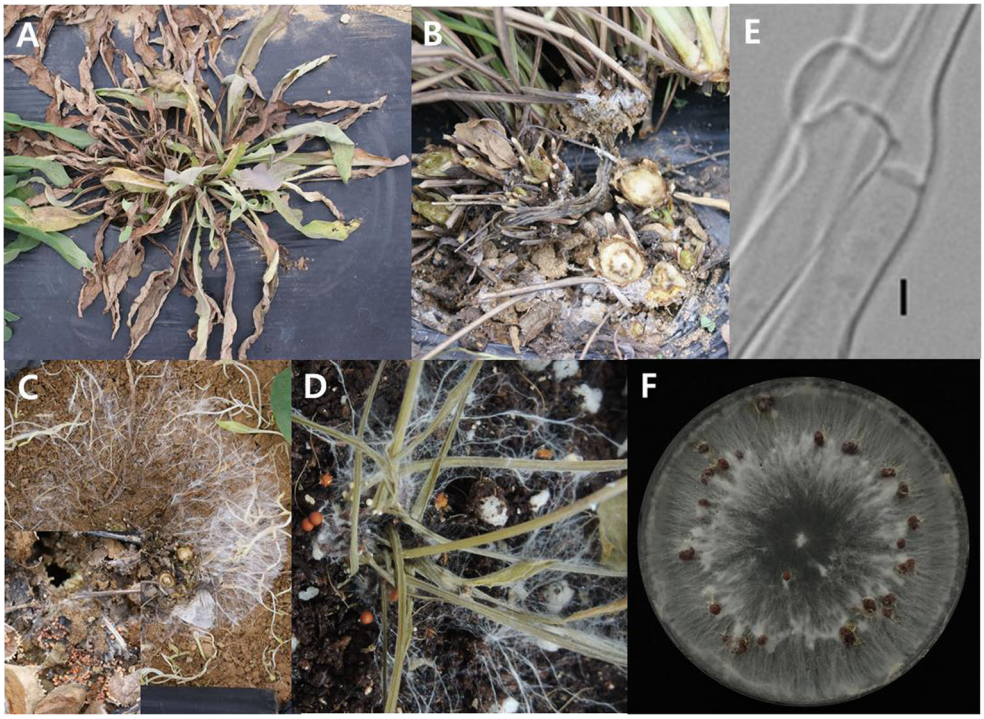

Fig. 1 Symptoms of stem rot of *Ixeridium dentatum* caused by *Sclerotium rolfsii* and morphological features of the pathogen. **a**, **b**: Infected plants, **c**: Mycelial mats and sclerotia, **d**: Symptoms induced by artificial inoculation, E: A clamp connection in the hyphae (scale bars - 10 μm), F: Mycelium mat and sclerotia produced on PDA after 14 days at 30 °C

respectively. In vitro, all isolates were morphologically similar to description of *S. rolfsii* provided by Mordue (1974).

Genomic DNA of two isolates (KACC48476 and KACC48477) was extracted using a modified cetyl trimethylammonium bromide extraction protocol (Rogers and Bendich 1994). The ITS region was amplified using the primers ITS1F and ITS4 (White et al. 1990) following previously described methods (Park et al. 2015). DNA sequencing was performed at Macrogen (Seoul, Korea) using an ABI Prism 3700 Genetic Analyzer (Life Technologies, Gaithersburg, MD, USA). The resulting sequences were edited using MEGA ver. 5.0 (Tamura et al. 2011). Reference sequences were downloaded from the GenBank and multiple alignments were performed using the default settings of MAFFT v7 (Katoh and Standley 2013). Neighbor-joining (NJ) trees were constructed with MEGA 5 using Kimura 2-parameter model and 1000 bootstrap replicates (Kimura 1980). The sequences of HCRD 16076 and HCRD 16077 were deposited in GenBank (accession nos. MG645250 and MG645251). Based on the ITS sequences, KACC48476 and KACC48477 isolated from infected tissues formed a monophyletic group with *S. rolfsii* with 100% bootstrap values. The isolates showed sequence similarity of 100% to *S. rolfsii* (Fig. 2). Based on these morphological and sequence analyses, all Korean isolates obtained from infected *I. dentatum* plants were identified as *S. rolfsii*.

To fulfill Koch's postulates, pathogenicity tests were conducted on the lower stem of three healthy *I. dentatum* 60-day-old plants by placing 6-mm-diameter mycelium plugs obtained from 2-day-old cultures. Non-inoculated plants were used as a control. All plants were kept in a dew chamber at 25 °C and relative humidity of >95%. Every inoculated plants showed symptoms such as water-soaked spots on the stem within two days followed by rotting, wilting, blighting, and

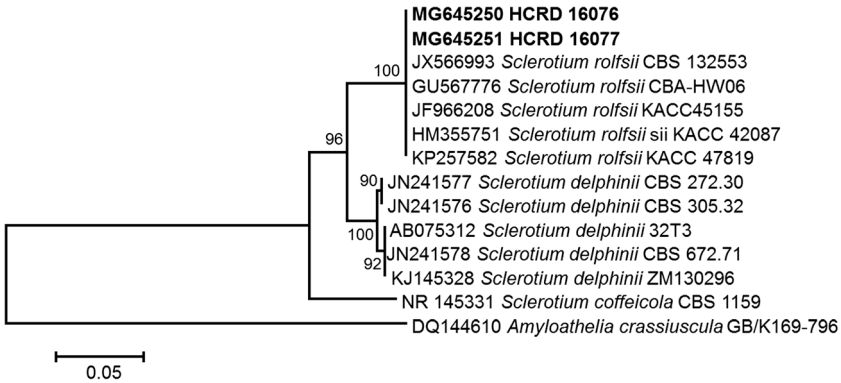

Fig. 2 Neighbor joining tree inferred from the internal transcribed spacer (ITS) sequences of *Sclerotium* species. Bootstrap scores >50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. Strains isolated from *Ixeridium dentatum* are indicated in bold

eventually death, whereas non-inoculated plants remained healthy. Sclerotia developed on the basal stem and soil surfaces within six days (Fig. 1). The pathogenicity tests were similar to those reported by Kwon et al. (2017). *S. rolfsii* was consistently re-isolated from the symptomatic tissue to complete Koch's postulates.

Based on these morphological features, sequence analysis, and the pathogenicity test, the Korean isolates KACC48476 and KACC48477 responsible for stem rot of *I. dentatum* were identified as *S. rolfsii*. Therefore, we conclude that *S. rolfsii* is the causal agent responsible for stem rot of *I. dentatum* in Korea.

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