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RESEARCH NOTE

First Report of *Sclerotinia sclerotiorum* Causing Sclerotinia Rot on *Ixeridium dentatum* in Korea

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Abstract

Sclerotinia rot was observed on *Ixeridium dentatum* cultivated as a succeeding crop in a garlic field in Seosan-si, Korea during the growing season in 2016 and 2017. Symptoms progressed from the initial irregular, water-soaked spots on main stems to wilting and eventually to plant death. White, cottony mycelia and black, irregular sclerotia formed on the basal stem and on soil surfaces. The optimal temperature of hyphal growth and sclerotia germination were 20°C and 25°C, respectively. Sequence analysis of the internal transcribed spacer (ITS) regions revealed that the three strains isolated from *Ixeridium dentatum* are grouped with *Sclerotinia sclerotiorum.* Three strains were identified as *Sclerotinia sclerotiorum* based on morphological features, ITS sequence, and pathogenicity test. To the best of our knowledge, this work is the first report of *Sclerotinia sclerotiorum* causing sclerotinia rot on *Ixeridium dentatum* in Korea.

Keywords: *Ixeridium dentatum*, New report, Sclerotinia rot, *Sclerotinia sclerotiorum*

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Ixeridium dentatum Tzvelev (family Asteraceae) is a perennial herb native to and widely distributed in Korea, Japan, and China [1]. The plant is well known for its anti-cancer, anti-oxidative and anti-allergenic activity [2, 3]. It is cultured in some areas, including Seosan, Dangjin, and Jinan. Sclerotinia rot symptoms were occasionally observed on *Ixeridium dentatum* cultivated as a succeeding crop in garlic fields in Seosan-si during the growing season of 2016 and 2017. Infected plants formed initially irregular, water-soaked spots on the base of the stems, wilted, then blighted and eventually died. Spherical or irregular sclerotia (2 to 7 mm in size) formed on the basal stem and on the soil surface (Fig. 1A, 1B). Cucumber mosaic virus (CMV disease), tomato spotted wilt virus (TSWV) and *Puccinia lactucae-debilis* (Rust) have been reported to be associated with diseases of *Ixeridium dentatum* in Korea [4]. The aim of the present study was to identify the causal

agent of sclerotinia rot on *Ixeridium dentatum,* based on morphological features, sequence analysis of the internal transcribed spacer (ITS) region and pathogenicity test.

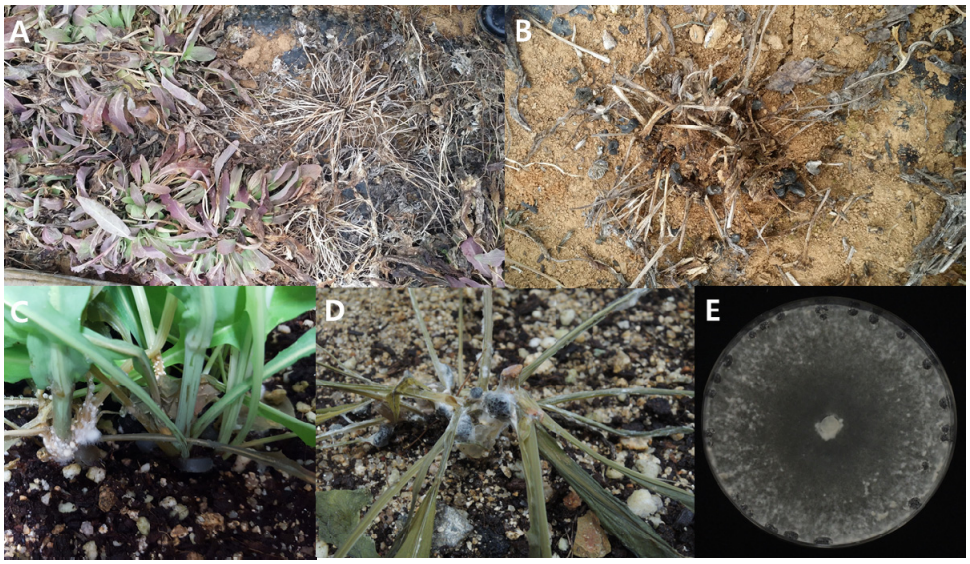

Fig. 1. Symptom of sclerotinia rot on *Ixeridium dentatum* caused by *Sclerotinia sclerotiorum* and culture features. A, B, Infected plants; C, D, Symptoms induced by artificial inoculation; E, Mycelial mat and sclerotia produced on potato dextrose agar after 10 days at 20°C.

Sampling and Isolation

Infected tissues and sclerotia on the basal stem of *Ixeridium dentatum* were collected from several sites, where it was cultivated as succeeding crop in garlic fields at Seosan-si during 2016 and 2017. The tissues and sclerotia were surface-sterilized by dipping in 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 margins of each fungal hyphae growing from the tissue and sclerotia were transferred to potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates. The isolated strains were maintained in a sterilized distilled water stock with 30% glycerol at −70°C for identification and pathogenicity tests in Herbal Crop Research Division (HCRD).

DNA extraction, PCR, sequencing, and phylogenetic analysis

Genomic DNA extraction and PCR amplification of the ITS region were performed using previously described methods [5]. DNA sequencing was performed at Macrogen (Seoul, Korea), using an ABI Prism 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were assembled, proofread, edited using MEGA ver. 5.0 [6]. The ITS sequences of the present isolates were aligned with reference sequences downloaded from GenBank using the default settings of MAFFT v7 [7]. Neighbor-joining (NJ) trees were

constructed with MEGA 5 using Kimura 2-parameter model [8] and 1,000 bootstrap replicates. All the sequences were deposited in GenBank (accession nos. MF270175~MF270177). HCRD 16078, HCRD 16080, and HCRD 16084 isolated from infected tissues formed a monophyletic group with *Sclerotinia sclerotiorum* with 56% bootstrap value. The sequences of the three strains were identical to those of strains previously identified as *Sclerotinia sclerotiorum* (Fig. 2).

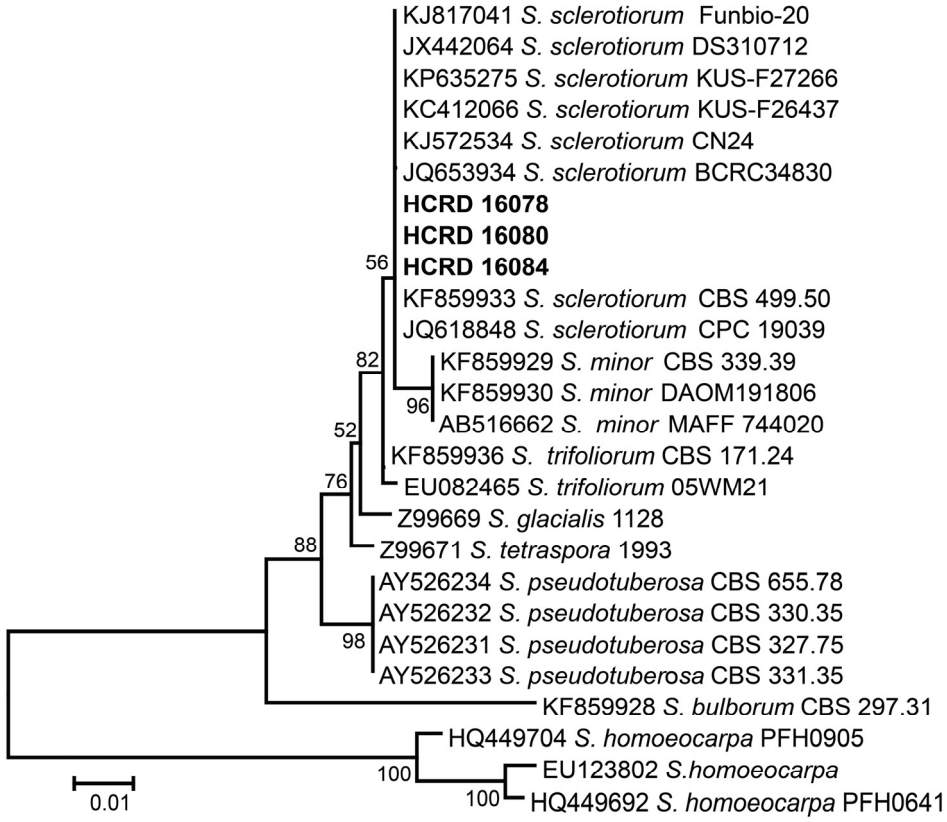

Fig. 2. Neighbor joining tree inferred from the internal transcribed spacer sequences of *Sclerotinia* species. Bootstrap values greater than 50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. The isolates originated from *Ixeridium dentatum* are indicated in bold.

Culture and morphological characteristics

Pure cultures on PDA grew rapidly and formed aerial, white mycelia and turned gray to chocolate after 3 to 4 days. Fungal cultures formed spherical or irregular, dark-black sclerotia on the peripheral edges of the PDA, which ranged from 3 to 8 mm in size (Table 1). The optimal temperature for hyphal growth and sclerotia formation was 20°C, whereas that for sclerotia germination was 25°C. The morphological characteristics of the strains from sclerotinia rot of *Ixeridium dentatum* were similar to those of previously reported *S.*

sclerotiorum [9].

Table 1. Comparison between morphological features of the strains obtained from sclerotinia rot of *Ixeridium dentatum* and previously reported as *Sclerotinia sclerotiorum*

Characteristics		Present isolate	*Sclerotinia sclerotiorum*[a]
Colony	Color	white, gray to chocolate	White, grey to chocolate
Sclerotia	Size (mm)	2.5~6.0 × 2.6~8.5	3.0~10.0
	Shape	spherical or irregular	spherical or irregular
	Color	dark black	dark brown to black

[a]Described by Kohn [9].

Pathogenicity test

Pathogenicity tests were conducted by placing five agar plugs (6 mm) of mycelium near the base of the stems of three healthy plants at soil line level. Non-inoculated plants were used as controls. All plants were kept in a dew chamber at 25°C and over 95% relative humidity. After three days, all inoculated plants showed symptoms, such as water soaked spots on the stem, covered with white mycelia, and then rotting, followed by wilting, blighting and eventually, death (Fig. 1C, 1D), whereas non-inoculated plants remained symptomless. *Sclerotinia sclerotiorum* was consistently re-isolated from the symptomatic tissue in full compliance with Koch's postulates.

Three Korean strains (HCRD 16078, HCRD 16080 and HCRD 16084) related to sclerotinia rot on *Ixeridium dentatum* were identified *S. sclerotiorum*, based on these morphological characteristics, sequence analysis, and pathogenicity test. This is the first report of *S. sclerotiorum* causing sclerotinia rot on *Ixeridium dentatum* in Korea.

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