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Research Note

# The First Report of Postharvest Stem Rot of Kohlrabi Caused by *Sclerotinia sclerotiorum* in Korea

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**Abstract** In March 2014, a kohlrabi stem rot sample was collected from the cold storage room of Daegwallyong Horticultural Cooperative, Korea. White and fuzzy mycelial growth was observed on the stem, symptomatic of stem rot disease. The pathogen was isolated from the infected stem and cultured on potato dextrose agar for further fungal morphological observation and to confirm its pathogenicity, according to Koch's postulates. Morphological data, pathogenicity test results, and rDNA sequences of internal transcribed spacer regions (ITS 1 and 4) showed that the postharvest stem rot of kohlrabi was caused by *Sclerotinia sclerotiorum*. This is the first report of postharvest stem rot of kohlrabi in Korea.

**Keywords** Kohlrabi, Pathogenicity, Postharvest disease, *Sclerotinia sclerotiorum, Sclerotinia* stem rot

Kohlrabi (*Brassica oleracea* var. *gongylodes*) is a low, stout cultivar of the cabbage that will grow almost anywhere. It develops a thickened stem just above the soil line. Although this vegetable is often called a "turnip-rooted cabbage," the edible portion is an enlarged stem rather than root tissue. Kohlrabi originated in northern Europe in the 16th century.

*Sclerotinia sclerotiorum* (Lib.) de Bary is among the world's most successful and omnivorous fungal pathogens, affecting a wide range of vegetable and field crops in many countries. Diseases caused by *S. sclerotiorum* occur in all stages of plant growth, including in seedlings and mature plants, and in post-harvest products [1, 2]. It has been reported that the fungus attacks more than 400 different species of plants [3, 4], and in Korea alone, more than 59 plant species have been reported as being hosts for *S. sclerotiorum* [5]. Cool and humid conditions favor the occurrence of *Sclerotinia* rot in the field, during transit and in storage, and the optimal temperatures for fungal growth range from 15°C to 21°C [6]. In Korea, this fungus has been associated with *Sclerotinia* rot in various vegetable crops in the field, but has not been reported as a postharvest disease [7]. The objective of this study was to identify the causal agent associated with postharvest *Sclerotinia* stem rot disease in kohlrabi, based on culture characteristics, molecular phylogenetics, and pathogenicity.

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**Isolation of fungi and pathogenicity test.** In March 2014, symptoms of rot were observed on kohlrabi in the cold storage room of Daegwallyong Horticultural Cooperative, Pyeongchang, Gangwon Province, Korea (Fig. 1A). Specifically, white and fuzzy mycelial growth was seen on the stems. Infected stems were collected in sterilized plastic polythene bags and transported to the laboratory for pathogen isolation. They were then cut into small pieces, approximately 0.5 to 1 cm in size. The pieces were then surface-sterilized with 1% (v/v) sodium hypochlorite (NaOCl) for 1 min, washed 3 times with sterile distilled water, and then dried with sterilized filter paper. Next, the pieces were placed in Petri plates containing potato dextrose agar (PDA) medium (Difco, Detroit, MI, USA) and incubated at 20 ± 2°C for 5 days. For pure culture isolation, the mycelia grown on the PDA plates were used to inoculate fresh PDA plates.

To determine the pathogenicity of the fungus, 1 mycelial disc (7 days old, 6.5 mm) was placed into a small cut of kohlrabi stem and put in to a plastic box lined with moist filter papers. This was then incubated at 20 ± 2°C, under laboratory conditions. After 5 days, white mycelia were

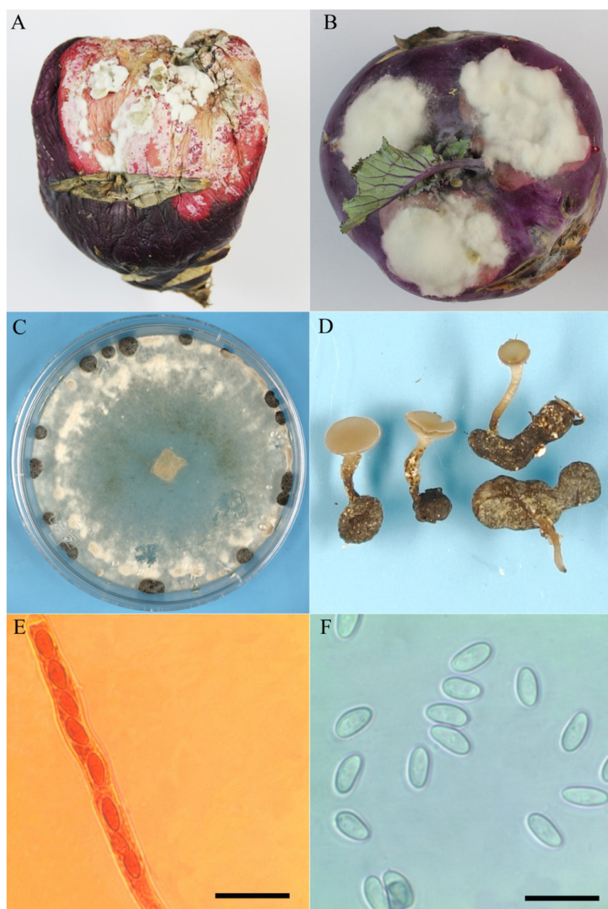


**Fig. 1.** A, Diseased kohlrabi showing fuzzy growths of mycelium on the stem; B, Kohlrabi inoculated with *Sclerotinia sclerotiorum* developed *Sclerotinia* stem rot symptoms after 5 days of incubation; C, Three-week-old colony and black sclerotia of *S. sclerotiorum* growing on potato dextrose agar medium; D, Apothecia; E, Ascus containing 8 ascospores; F, Ascospores (scale bars: E, F = 20 μm).

seen on the stem, along with symptoms of rot. The fungal pathogen was re-isolated from the disease lesions on the inoculated stem; this re-isolated pathogen exhibited the same morphological characteristics as those of the original isolates (Fig. 1B). Thus, the fungal pathogen fulfilled the criteria stipulated by Koch's postulates and was identified as the causal agent of postharvest Sclerotinia stem rot of kohlrabi.

**Sclerotia germination and sexual structure.** For sclerotia germination, sandy loam soil was placed in a Petri dish, and 30-day-old 10~15 sclerotia were washed, dried, and pressed down to a depth of approximately 2 mm from the top of the Petri dish. The Petri dish was then kept shaded from sunlight, at 20 ± 2°C, and watered 3 times a week with sterilized water. The plate was exposed to natural sunlight after the appearance of the first stipe initial [8, 9]. Fully developed apothecia were removed for further study. The asci and ascospores were examined microscopically and were photographed at 40× magnification.

**DNA extraction, polymerase chain reaction (PCR), and sequence analysis.** For DNA extraction, mycelia were grown in 250-mL flasks containing 100 mL of potato dextrose broth, which were incubated for 6 days at 25°C on a rotary shaker at 135 rpm. Mycelia were harvested by vacuum filtration through Whatman grade 1 filter paper and then lyophilized for 24 hr before grinding them to a fine powder. Next, 100 mg of the ground powder was transferred to a 1.5-mL Eppendorf tube, and DNA was extracted using the CTAB extraction method [10].

The extracted DNA was used for PCR sequencing of rDNA genes by using universal primers for internal transcribed spacer (ITS) 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) [11]. The amplification was performed in a 25 μL reaction mixture containing 0.5 μL of each primer, 0.5 μL of *Taq* DNA polymerase (Bioneer, Daejeon, Korea), 0.5 μL of each dNTP, 2.5 μL of 10× PCR reaction buffer, 18.5 μL of distilled water, and 2.0 μL of template DNA. The reaction was performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) under the following conditions: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 35 sec, annealing at 52°C for

**Table 1.** Morphological and culture characteristics of *Sclerotinia sclerotiorum* isolated from kohlrabi

| Characteristics | | Study isolate | *S. sclerotiorum*[a] |
|---|---|---|---|
| Colony | Color | White to gray | White to gray |
| Sclerotium | Shape | Initially cushion-like or globularor irregular, dark brown, finally black | Initially cushion-like or short-cylindrical and white, finally black |
| Apothecium | Shape | Cup-shaped with yellowish brown color | Cup-shaped with yellowish brown color |
| | Size (cm) | 0.8~1.4 | 0.5~2.0 |
| Ascus | Shape | Cylindrical, hyaline, 8-spored | Cylindrical, hyaline, 8-spored |
| | Length (μm) | 103.5~142.7 | 110~160.0 |
| | Width (μm) | 5.7~7.7 | 6.0~10.0 |
| Ascospore | Shape | Hyaline, ellipsoid to ovoid | Hyaline, ellipsoid to ovoid |
| | Length (μm) | 8.9~11.2 | 9.0~14.0 |
| | Width (μm) | 4.4~5.3 | 4.0~6.0 |

[a]Described by Kohn [12].

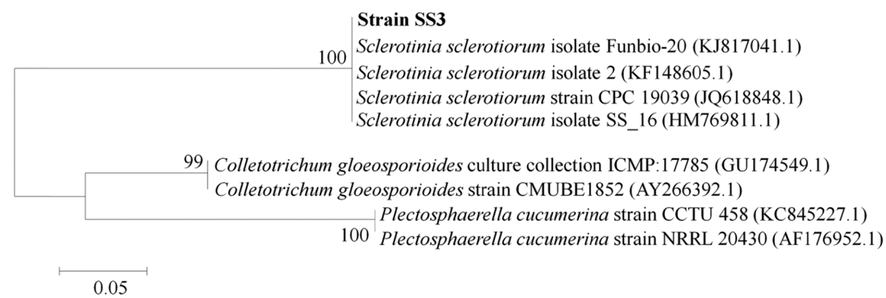


**Fig. 2.** Neighbor-joining phylogenetic tree of *Sclerotinia sclerotiorum* strain SS3 and related species identified by the internal transcribed spacer gene sequences from the GenBank database. Numbers at the nodes indicate bootstrap values from a test of 1,000 replicates. The scale bar indicates the number of nucleotide substitutions. Evolutionary analyses were conducted using the MEGA5 program [13].

55 sec, and elongation at 72°C for 1 min, followed by a final extension at 72°C for 10 min [14]. The obtained nucleotide sequences were used in a BLASTN search of the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analysis of *S. sclerotiorum* was performed using the MEGA5 program, with the neighbor-joining method [13]. Sequence data were deposited in GenBank (accession No. KM656466).

**Identification and characterization of *S. sclerotiorum*.** In total, 7 fungal strains were obtained from the kohlrabi with *Sclerotinia* rot, and of these, strain SS3 was examined for identification. Strain SS3 was identified as *S. sclerotiorum* by analyzing the morphological characteristics of the isolated fungus and performing rDNA sequencing analysis. The fungus produced white to gray colonies on PDA medium incubated at 20 ± 2°C for 7 days, which after 3 wk showed small sclerotia that were produced on the peripheries of the plates (Fig. 1C). These sclerotia were black in color and globose, cylindrical, or irregular in shape. Apothecia were obtained from germinating sclerotia on sand medium after 5~6 wk incubation at 20 ± 2°C (Fig. 1D). Eight elliptical ascospores of uniform size were observed in each ascus under the microscope (Fig. 1E and 1F), which is a typical characteristic of *S. sclerotiorum* [15]. The morphological characteristics of the identified species are summarized in Table 1. The ITS sequences obtained in this study were compared to GenBank database sequences by using the NCBI BLAST search tool. The sequences identified based on rRNA-ITS alignment were 100% similar to those of several *S. sclerotiorum* species (accession Nos. KJ817041.1, KF148605.1, JQ618848.1, and HM769811.1). Thus, *S. sclerotiorum* was identified as the causative agent of postharvest *Sclerotinia* stem rot of kohlrabi in this study (Fig. 2), which constitutes the first report of this disease in kohlrabi in Korea.

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