Phylogenetic Placement and Morphological Characterization of *Sclerotium rolfsii* (Teleomorph: *Athelia rolfsii*) Associated with Blight Disease of *Ipomoea batatas* in Korea

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**Abstract** In this study, we aimed to characterize fungal samples from necrotic lesions on collar regions observed in different sweetpotato growing regions during 2015 and 2016 in Korea. Sclerotia appeared on the root zone soil surface, and white dense mycelia were observed. At the later stages of infection, mother roots quickly rotted, and large areas of the plants were destroyed. The disease occurrence was monitored at 45 and 84 farms, and 11.8% and 6.8% of the land areas were found to be infected in 2015 and 2016, respectively. Fungi were isolated from disease samples, and 36 strains were preserved. Based on the cultural and morphological characteristics of colonies, the isolates resembled the reference strain of *Sclerotium rolfsii*. Representative strains were identified as *S. rolfsii* (teleomorph: *Athelia rolfsii*) based on phylogenetic analysis of the internal transcribed spacer and large subunit genes along with morphological observations. To test the pathogenicity, sweetpotato storage roots were inoculated with different *S. rolfsii* strains. ‘Yulmi’ variety displayed the highest disease incidence, whereas ‘Pungwonmi’ resulted in the least. These findings suggested that morphological characteristics and molecular phylogenetic analysis were useful for identification of *S. rolfsii*.

**Keywords** Fungal morphology, *Ipomoea batatas*, Molecular phylogeny, Pathogenicity, *Sclerotium rolfsii*

Sweetpotato (*Ipomoea batatas*) is a common plant of the Convovulaceae family and is ranked as the seventh most important food crop in terms of production; in developing countries, sweetpotatoes rank fifth in economic value production, sixth in dry matter production, seventh in energy production, and ninth in protein production and have many applications as foods, feeds, and industrial products [1, 2]. Roughly 80% of the world’s sweetpotatoes are grown in Asia. In addition to China and Vietnam, sweetpotatoes play important roles in the rural economy in many other parts of Asia, including the Philippines, India, Korea, Taiwan, some eastern islands of Indonesia (Bali and Irian Jaya) and Papua New Guinea [3, 4]. In Korea, the sweetpotato is widely cultivated and consumed, and the total area of cultivation reached as much as 22,000 ha of land in 2015, which was 27% higher than that in 2000 [5].

Numerous fungal diseases have been reported on sweetpotatoes from different regions worldwide. Sclerotial blight is a major fungal disease caused by *Sclerotium rolfsii* during the early cultivation period and has been observed in plant beds, mature plants as circular spots, and tubers as a post-harvest disease. *S. rolfsii* Sacc. (teleomorph *Athelia rolfsii* [Curzi] C. C. Tu. & Kimbr.) is a serious disease-causing fungal pathogen that affects diverse crops grown around the world, particularly tropical and subtropical countries [6]. Because of its broad host range, *S. rolfsii* is considered one of the most destructive pathogens worldwide; indeed, about 500 plant species from 100 families, including tomatoes, potatoes, chili peppers, carrots, cabbage, sweetpotatoes, common beans, and ground nuts [7, 8], are affected by this pathogen. The fungus generally infects the collar region or stems near the soil surface and spreads over the whole plant or fruits and remains in soil surface [9, 10]. *S. rolfsii* produces sclerotia on hosts, survives and overwinters for a long time and infects the same host crop or other nearby...
Various molecular approaches have been employed to identify *S. rolfsii* and other fungal populations [12-15]. The internal transcribed spacer (ITS) is a common genomic target used to measure fungal diversity at the molecular level [6]. The level of divergence between species or strains or within the species examining by ITS rDNA sequences is low; however, this method shows improved sensitivity at the genus level [16]. The ITS and large subunit (LSU) show slight phylogenetic differences among species that are closely related to *S. rolfsii* (e.g., *S. coffeicola* and *S. delphinii*) [17]. However, no studies have performed molecular analysis along with morphological characterization of *S. rolfsii* in sweetpotatoes in Korea. Therefore, in the present study, we aimed to evaluate disease occurrence on sweetpotatoes by *S. rolfsii*, analyze the molecular data by ITS and LSU sequences and morphological characteristics of the pathogen, and test their pathogenicity on different variety.

**MATERIALS AND METHODS**

**Disease symptom and incidence.** Initially sudden wilting of the stalks appeared in plant beds followed by deaths (Fig. 1A). In high humid conditions the disease spreads and infection was initiated near the collar regions and soon after infections, necrotic lesions were observed along with aerial cottony, coarse, white mycelia (Fig. 1B and 1C). Mycelium covers the soil surface and produced mycelial mats. After that sclerotia appeared large in numbers on the mycelial mats, mats and sclerotia were found on the surface layer of the newly grown storage roots. Then, disease incidence was measured as the proportion of a plant community that is diseased. Field surveys were conducted in farmers’ fields in 6 different locations of Yeouju, Nonsan, Iksan, Gimje, Yeongam, and Haenam (Table 1). During the survey of disease incidence, we inspected 45 and 84 farmlands in 2015 and 2016, respectively, and monitored the presence of disease and the affected land area. After obtaining data from different locations, we calculated the disease incidence by the following formula [18].

\[
I (\%) = \frac{n_i}{N} \times 100
\]

Where, ‘I’ is the disease incidence, ‘ni’ is the total number of affected plants, and ‘N’ is the total number of evaluated plants.

**Pathogen isolation and culture maintenance.** Leaves, roots, stems, and storage roots affected by *Sclerotium* blight disease were collected and were transported to the laboratory. Samples were washed under running tap water and dried in a laminar air flow chamber. After surface sterilization [19], samples were cut into small pieces, dried, and transferred to potato dextrose agar (PDA) supplemented with rifampicin to stop bacterial growth. PDA petri dishes were then incubated at 25°C for 3–7 days. Plates were checked on a regular basis by the naked eye for hyphal growth. Hyphae were then transferred to PDA, and pure cultures of *Sclerotium*-like fungi were prepared. A total of 36 strains were obtained after confirming pure cultures by growing on PDA several times. Fungi were then assigned identification numbers (Table 2), maintained in PDA slant tubes and 20% glycerol stock solution, and deposited in the culture collection of the Sweetpotato Laboratory, Bioenergy Crop Research Institute, Rural Development Administration (RDA),

| Province   | Location | Farm surveyed | Disease incidence (%) |
|------------|----------|---------------|-----------------------|
|            |          | 2015          | 2016                  | 2015 | 2016 |
| Gyeong-gi  | Yeouju   | 6             | 8                     | 11.7 | 10.6 |
| Chungnam   | Nonsan   | 8             | 15                    | 8.8  | 7.0  |
| Jeonbuk    | Iksan    | 7             | 14                    | 20.0 | 3.9  |
|            | Gimje    | 12            | 11                    | 7.9  | 3.2  |
| Jeonnam    | Yeongam  | 3             | 12                    | 1.7  | 5.8  |
|            | Haenam   | 9             | 24                    | 20.8 | 10.8 |
| Total      |          | 45            | 84                    | 11.8 | 6.8  |
Table 2. Isolates collected from different sweetpotato growing regions in Korea during 2015 and 2016

| Species               | Strain number | Host                      | Location      | Accession No. |
|-----------------------|---------------|---------------------------|---------------|---------------|
|                        |               |                           |               | **ITS**       | **LSU**       |
| Athelia sp.           | SPL15001      | Stem, Sweetpotato         | Yeoju, Korea  | KY446394      | KY446368      |
| Athelia sp.           | SPL15002      | Stem, Sweetpotato         | Iksan, Korea  | KY446393      | KY446369      |
| Athelia sp.           | SPL15004      | Root zone soil, Sweetpotato | Haenam, Korea | KY446392      | KY446370      |
| Athelia sp.           | SPL15005      | Storage roots, sweetpotato | Haenam, Korea | KY446391      | KY446371      |
| Athelia sp.           | SPL15009      | Storage roots, sweetpotato | Gimje, Korea  | KY446389      | KY446372      |
| Athelia sp.           | SPL15011      | Storage roots, sweetpotato | Yeongam, Korea| KY446388      | KY446373      |
| Athelia sp.           | SPL16001      | Stem, Sweetpotato         | Haenam, Korea | KY446387      | KY446374      |
| Athelia sp.           | SPL16005      | Stem, Sweetpotato         | Haenam, Korea | KY446386      | KY446375      |
| Athelia sp.           | SPL16006      | Root zone soil, Sweetpotato | Haenam, Korea | KY446385      | KY446376      |
| Athelia sp.           | SPL16011      | Storage roots, sweetpotato | Yeongam, Korea| KY446384      | KY446377      |
| Athelia sp.           | SPL16020      | Stem, Sweetpotato         | Gimje, Korea  | KY446383      | KY446378      |
| Athelia sp.           | SPL16023      | Stem, Sweetpotato         | Nonsan, Korea | KY446382      | KY446379      |
| Athelia sp.           | SPL16057      | Leaf, Sweetpotato         | Gimje, Korea  | KY446381      | KY446380      |
| A. rolfsii            | AFTOL-ID 664  | N/A                       | USA           | DQ484062      | AY635773      |
| A. rolfsii            | 09-044        | N/A                       | Korea         | JN017199      |               |
| A. rolfsii            | KACC 47819    | Balsam pear               | Korea         | KP257582      |               |
| A. rolfsii            | MHGNU F117    | Melon                     | Korea         | U760983       |               |
| A. rolfsii            | SR 23         | Groundnut                 | India         | KT878487      |               |
| A. rolfsii            | LP3           | Loblab purpureus          | India         | K002765       |               |
| A. rolfsii            | KACC 47820    | Hooker chives             | Korea         | KP257581      |               |
| A. rolfsii            | FSR-052       | Lily                      | Taiwan        | AY684917      |               |
| A. rolfsii            | SCG5          | Syagrus oleracea          | Brazil        | KU096992      |               |
| A. rolfsii            | A             | Stevia rebaudiana         | Colombia      | KP784421      |               |
| A. rolfsii            | BOScR-1       | Butterfly orchid          | China         | KJ546416      |               |
| A. rolfsii            | BLH-02        | Mackaeya cordata          | China         | KT428156      |               |
| A. rolfsii            | CBS 132553    | Snake bean                | Lao PDR       | JX566993      |               |
| A. rolfsii            | CBS 132553    | Snake bean                | Lao PDR       | JX566993      |               |
| A. rolfsii            | 13M-0091      | N/A                       | Japan         | KT222898      |               |
| A. rolfsii            | 13M-0093      | N/A                       | Japan         | KT222902      |               |
| A. rolfsii            | 3092          | Arachis hypogaea          | China         | JN241562      | FJ213239      |
| A. rolfsii            | ATCC 201126   | N/A                       | Argentina     | AF499018      | AF499019      |
| A. rolfsii            | Sr2           | Arachis hypogaea          | Georgia, USA  | FJ213232      |               |
| A. rolfsii            | 3098          | Lens culinaris            | Pakistan      | FJ213233      |               |
| Agaricus aridicola    | LAPAG589      | N/A                       | Spain         | K951331       |               |
| Agaricus augustus     | WC414         | PSUMC                      | USA           | -             | AY484672      |
| Amyloaethlia crassuscula | GB/K169-796 | N/A                       | Sweden        | -             | DQ144610      |
| Aureosbasidium pullulans | ATCC 62921 | N/A                       | Belgium       | -             | AF505239      |
| Botryotinia fuckeliana | AFTOL-ID 59 | N/A                       | USA           | -             | AY546561      |
| Ceratorhiza oryzae-sativa | CBS 235.91 | Oryza sativa              | Japan         | -             | FJ213231      |
| C. oryzae-sativa      | CBS 577.81    | N/A                       | Italy         | FJ231392      | FJ213235      |
| Coprinus comatus      | AFTOL-ID 626  | N/A                       | USA           | -             | AY635772      |
| Ceratobasidium cornigerum | RoK 210   | N/A                       | Germany       | -             | AY52405       |
| Ceratobasidium cornigerum | CBS 139.82 | N/A                       | Pittosporum sp. | USA         | AJ302006      |
| C. goodyearae-repentis | UAMH 61440   | Desert orchid             | USA           | -             | AY435232      |
| Calvatia cratiformis  | Steinke001017 | N/A                       | Germany       | -             | DQ142625      |
| Cortinarius viridibasalis | TUB 011490 | N/A                       | Germany       | -             | AF539717      |
| Chloroscypha enterochroma | AFTOL-ID 67 | N/A                       | USA           | U92312        | AY544735      |
| Lepiota proceria      | DSH 96-038    | N/A                       | USA           | -             | AF518628      |
| Montagnea candollei   | EK7           | N/A                       | USA           | -             | AF261481      |
| Monilia fructicola    | AFTOL-ID 279  | N/A                       | USA           | -             | AF546833      |
| Macrozypha fistulosa  | TUB 011490   | N/A                       | Germany       | -             | DQ071735      |
| Phlyctenops nidalans  | TUB 011567   | N/A                       | Germany       | -             | DQ071736      |
| Phyllophthora nidalans | HMJAU7272 | N/A                       | China         | GQ142019      |               |
| Pseudohydnum gelatinosum | AFTOL-ID 1875 | N/A                       | Germany       | -             | DQ520094      |
| S. delphini           | CBS672.71     | N/A                       | USA           | JN241578      |               |
| S. delphini           | CBS272.30     | N/A                       | N/A           | JN241577      |               |
Table 2. Continued

| Species                  | Strain number | Host            | Location           | Accession No. |
|--------------------------|---------------|-----------------|--------------------|---------------|
| *S. delphini*            | 3089          | *Malus domestica* | China              | - FJ212327    |
| *S. delphini*            | ATCC 15200    | *Iris* sp.      | NB, USA            | - FJ212326    |
| *S. coffeicola*          | CBS 521.92    | *Vitis* sp.     | Venezuela          | - FJ212345    |
| *S. coffeicola*          | CBS115.19     | *Coffee* sp.    | Costa Rica         | AB075319      |
| *S. coffeicola*          | CBS 668.85    | *Coffeea* sp.   | Costa Rica         | - FJ212346    |
| *S. rhizodes*            | CBS 276.69    | *Glyceria maxima* | Germany          | FJ231393      |
| *S. perniciosum*         | CBS 274.93    | *Allium* sp.    | USA                | FJ212357      |
| *S. hydrophilum*         | CBS 201.27    | *Victoria regia* | Netherlands        | FJ231396      |
| *S. denigrans*           | CBS 118.43    | *Convallaria majalis* | Germany     | FJ212349      |
| *S. cepivorum*           | CBS 189.82    | N/A             | Egypt              | - FJ212339    |
| *Stypella papillata*     | KHL 11751     | N/A             | Finland            | EU118672      |
| *Thanatephorus cucumeris*| AG4-HG-III    | N/A             | USA                | - AF354076    |
| *Sclerotinia sclerotiorum*| CBS 499.50    | N/A             | Germany            | AF431951      |

Bold letters indicate the experiment conducted in the present study and assigned accession numbers.

ITS, internal transcribed spacer; LSU, large subunit; SPL, Sweet Potato Lab., Muan, Korea; N/A, not applicable; -, not found; AFTOL, Assembling the Fungal Tree of Life, Germany; KACC, Korean Agricultural Culture Collection; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ATCC, American Type Culture Collection, USA; TUB, Herbarium Tubingense, Germany.

Morphological characterization. Fungal strains were grown on PDA and malt extract agar (MEA) at 25°C in the dark to examine morphological characteristics. Small discs (0.5 mm diameter) were cut from the margins of developing colonies, transferred to the center of PDA and MEA plates, and incubated at 25°C. We observed the colony growth rate (speed/day), the production of clamp connections on mycelia, and evaluated aerial mycelium, sclerotia formation and sclerotial size, shape, and color for each of the strains using an AX10 microscope (Zeiss, Gottingen, Germany) with an Artcam 300 MI digital camera (Artray, Tokyo, Japan). Colors were named using the Mycological Colour Chart [20]. Morphological characteristics of the isolates were then compared with previous descriptions.

DNA extraction, PCR, and sequencing. All the selected fungal isolates were grown on PDA for 7 days. Mycelial tufts of each colony were transferred to 1.8 mL eppendorf tubes. Genomic DNA was extracted with Solgent Genomic DNA Prep Kit (Solgent Co. Ltd., Daejeon, Korea) following the manufacturer's instructions. A portion of the ITS and LSU regions of rDNA was used for PCR amplifications with the following primer pairs ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3')/ITS4 (5'-TCC TCC GCT Tat TGA Tat GC-3') [21] and LROR (5'-ACC CGC TGA ACT TAA GC-3')/LR5 (5'-TCC TGA GGG AAA CTT CG-3'); and the products generated by PCR was used for sequencing [22]. PCR amplification was carried out using a BIO-RAD PCR System T100 thermo cycler (BIO-RAD Laboratories, Hercules, CA, USA) with a 50 µL reaction volume containing 5 µL 10× e–Taq reaction buffer (Solgent Co. Ltd.), 1 µL 10 mM dNTP mix, 1 µL each primer, 1 µL template DNA solution, 1.5 µL U Taq-DNA polymerase and sterilized distilled water. PCR was carried out under the following conditions: initial denaturation at 94°C for 95 sec, followed by 35 cycles of denaturation at 94°C for 35 sec, annealing at 49°C for LSU and 55°C for ITS for 60 sec, and the final extension at 72°C for 2 min. PCR products were then purified using a Wizard PCR Preparation Kit (Promega, Madison, WI, USA) and sequenced by a commercial sequencing service provider (Macrogen, Daejeon, Korea). The resulting sequences were deposited in GenBank and assigned accession numbers (Table 2).

Phylogenetic analysis. All nucleotide sequences generated in the present study and retrieved from the GenBank (Table 2) were assembled using PHYDIT program ver. 3.2 [23] and initially aligned with the Clustal X v.1.83 program [24]. The sequences were then imported into BioEdit v. 5.0.9.1 program for manual adjustment [25], if necessary. Phylogenetic relationships were estimated by a maximum parsimony (MP) analysis using MEGA 6 program [26]. Bootstrap analysis using 1,000 replications was performed to assess the relative stability of the branches.

Pathogenicity test. Six selected isolates of *Sclerotium* (SPL15001, SPL15004, SPL15011, SPL16001, SPL16023, and SPL16057) were tested on six different varieties of sweet potato storage roots with three replications. Varieties inoculated with pathogens were Juhwanchi, Dahomi, Yulmi, Pungwonmi, Sinjami, and Jinhongmi. Three storage roots from each variety were washed in running tap water and sterilized as previously described methods [27] with minor modifications. Samples were then washed three times with distilled water to remove surface sterilizing agents. Hyphal disks of each colony (5 mm), previously grown on PDA,
were placed on storage roots by generating a wound with a 5-mm cork borer. Four wounds were made in a single storage root; one was used as a control, and the others were inoculated with *Sclerotium* isolates. Tubers were then transferred to a sterilized plastic mesh platform in moistened clean boxes. To develop disease, the boxes were incubated up to 10 days at 25°C in the dark. After incubation, the diameters of necrotic lesions were measured and scored using a 5-point rating system, where 0 = no symptoms, 1 = diameter less than 5 mm, 2 = diameter of 5–10 mm, 3 = diameter of 10–15 mm, 5 = diameter greater than 15 mm. All controls were treated with nonfungal blank PDA disks. To fulfill Koch's postulates, fungi were re-isolated from artificially inoculated tubers to confirm the presence of *Sclerotium* species. The re-isolated fungi were cultured on PDA, colony characteristics were observed, and sclerotia formation was monitored and compared with that of the original isolates.

### RESULTS

**Disease symptom and incidence.** During our survey, blight disease was observed on the basal parts of crops during their early stages of growth. Small and necrotic lesions were observed at early stage and later stages of growth, and symptoms were enlarged and spread over the plants and fields (Fig. 1). White, dense, fluffy, aerial, cotton-like mycelium remained in the soil surface in heavily affected fields. During 2015 and 2016, 45 and 84 farmlands were visited, respectively, to monitor sclerotial disease. The results revealed that the disease rates were 11.8% and 6.8%, respectively. Yeou and Haenam farmers experienced higher disease rates of 10.8% and 10.6%, respectively, in 2016 (Table 1), whereas Gimje and Iksan farmlands showed lower disease rates of 3.2% and 3.9%, respectively. The average disease rate in 2015 (11.8%) was higher than that in 2016 (6.8%). The highest disease incidence was recorded in Haenam in 2015 (20.8%).

**Morphological characterization.** Colonies of all isolates were white to pale olive buff in color (Fig. 2). The growth rates of the colonies were recorded for 5 days and the average colony growth rate was 1.2–1.5 mm/day. The isolate SPL15001 showed the highest colony growth rate (1.5 mm/day) among all isolates. After 10 days of incubation, spherical sclerotia started to grow and all the isolates produced clamp connections. During the early stage of sclerotial growth, the color was white to pale light brown; however, the color changed to cinnamon brown or dresden brown over time.

Sclerotial size varied from 0.5 to 2.0 μm (Table 3). Some isolates showed larger (max. 2.0 μm) sclerotia, and some produced smaller sclerotia (0.50–1.0 μm). The sizes of sclerotia in isolates SPL15004, SPL15009 on PDA and SPL16001, SPL16023 on MEA.

### Table 3. Morphological characteristics of eight representative isolates of *Sclerotium rolfsii* obtained from different locations in Korea

| Isolate ID | Colony  | Colony color                | Growth speed (mm/day) | Characteristics of sclerotia | Clamp connection |
|------------|---------|----------------------------|-----------------------|------------------------------|------------------|
| SPL15001   | Compact | White to pale olive buff   | 1.5                   | 248 Spherical                | Cinnamon brown   |
|            |         |                            |                       | 0.5–1.0                      | Present          |
| SPL15004   | Compact | White to pale olive buff   | 1.4                   | 106 Spherical to irregular   | Cinnamon brown   |
|            |         |                            |                       | 0.5–2.0                      | Present          |
| SPL15009   | Fluffy  | White to pale olive buff   | 1.4                   | 2 Spherical to irregular     | Cinnamon to dresden brown |
|            |         |                            |                       | 1.0–2.0                      | Present          |
| SPL15011   | Compact | White to pale olive buff   | 1.3                   | 64 Spherical                 | Cinnamon brown   |
|            |         |                            |                       | 0.5–1.0                      | Present          |
| SPL16001   | Fluffy  | White to pale olive buff   | 1.2                   | 64 Spherical                 | Cinnamon to dresden brown |
|            |         |                            |                       | 0.5–1.0                      | Present          |
| SPL16006   | Fluffy  | White to pale olive buff   | 1.2                   | 0 Spherical                  | Cinnamon to dresden brown |
|            |         |                            |                       | 0.5–1.0                      | Present          |
| SPL16023   | Fluffy  | White to pale olive buff   | 1.2                   | 35 Spherical                 | Cinnamon to dresden brown |
|            |         |                            |                       | 1.0–2.0                      | Present          |
| SPL16057   | Fluffy  | White to pale olive buff   | 1.3                   | 0 Spherical                  | Cinnamon to dresden brown |
|            |         |                            |                       | 1.0–2.0                      | Present          |
Molecular characterization. A total of 13 isolates were sequenced, and all sequences of ITS and LSU gene showed 99–100% similarity with Athelia rolfsii (anamorph: S. rolfsii) sequences from GenBank by BLAST search analysis. Parsimony analysis of ITS sequences showed three species of Sclerotium (S. rolfsii, S. delphinii, and S. coffeicola) produced a single group with a high (99%) bootstrap value. For construction of a phylogenetic tree, we used sequences of S. rolfsii collected worldwide, along with S. delphinii and S. coffeicola. S. delphinii and S. coffeicola produced a separate subgroup, supporting that the isolates identified in this study were different from these two species (Fig. 3). MP analysis of the LSU sequences resulted in two equally most parsimonious trees. A tree was selected to illustrate the phylogenetic location of the present isolates. Parsimony analysis (MP) of all sequences produced three major clusters, designated as S1, S2, and S3. S. denigrans, S. cepivorum, S. sclerotiorum, and S. perniciosum were included in the S1 cluster together with Monilinia fructicola and Botryotinia fuckeliana, which was under sclerotiniaceae (Helotiales, Ascomycota). S. hydrophilum, S. rhizodes, Thanatephorus cucumeris, and Ceratorhiza oryzae-sativaec clustered together as the S2 group within Cantharellales (Basidiomycota). S. rolfsii, S. delphinii, and S. coffeicola clustered together with a maximum bootstrap value (100%) in the S3 group (Fig. 4).

Pathogenicity test. For different varieties of storage roots, lesions of S. rolfsii occurred after inoculation with mycelial disks. The six different isolates exhibited similar pathogenicities on the tested storage roots; however, the pathogenicity range varied among isolates. Isolate SPL16001 produced higher disease severity than isolate SPL16004. In every case, the ‘Yulmi’ variety showed higher susceptibility to S. rolfsii, whereas ‘Pungwonmi’ showed better resistance to S. rolfsii (T1, T2, and T3 in Fig. 5). No symptoms were observed on control storage roots C in Fig. 5. Pathogens from infected storage roots were re-isolated, and their identity was confirmed.
Sclerotial blight on *Ipomoea batatas* caused by *S. rolfsii* is a common plant disease found worldwide. In sweetpotatoes, disease develops during the seedling stage after transplanting into contaminated soil. When environmental conditions are humid, cotton-like mycelia develop around the basal part of the plants and spread through the soil. Disease incidence becomes severe at later stages of growth. The pathogen also attacks sweetpotato storage roots during storage, leading to circular spot disease [1], which appears in fields before harvesting. Lesions are circular and whitish or yellowish brown in color. Kim *et al.* [28] surveyed stem rot in sweetpotatoes caused by *S. rolfsii* from 2007 to 2009 but the survey was limited to one location, which revealed that 23.1%, 26.7%, and 34.5% of seedlings were affected by the pathogen in 2007, 2008, and 2009, respectively. In the present study, we surveyed six sweetpotato growing regions and found that the disease rates were 11.8% and 6.8% in 2015 and 2016, respectively. The disease incidence was decreased because of the use of resistant and disease-free sweetpotato varieties by the farmers. Notably, variations in disease incidence caused by *S. rolfsii* have been observed worldwide. For example, in stem rot of tomatoes in Korea, the disease rate was 8.2% [29]; in contrast, disease rates have been reported to be as high as 22% in pumpkin fields in India [10], 30% in candy leaf plants in Italy [30], 10% in Chinese *Macleaya cordata* [31], and 30–35% in common beans in India [32]. Moreover, wild coffee plants in India have been reported to have a high disease incidence of 47.36% [33].

Morphological studies are essential for characterizing *S. rolfsii* isolates from different geographical locations. In the present study, morphological variability was observed among *S. rolfsii* isolates, with variations in mycelial growth rates, colony morphology, production, and arrangement of sclerotia, and number, size, and color of sclerotia. Both silky-white mycelia and fluffy or compact colony type were observed.
when isolates were cultured on PDA and MEA. Of eight isolates, SPL15009, SPL16001, SPL16006, SPL16023, and SPL16057 were fluffy on PDA, and others (SPL15001, SPL15004, and SPL15011) were compact; in contrast, SPL16001 and SPL16023 were compact on MEA, and the others showed fluffy colony morphologies, consistent with other studies [11, 34, 35].

Sclerotia formation was observed after 10–16 days of incubation at 25°C. Spherical (rarely spherical to irregular) sclerotia were whitish at the beginning and turned cinnamon brown or cinnamon to dresden brown over time. Additionally, variations in sclerotia production were observed, consistent with a previous report by Akram et al. [34]. Indeed, sclerotic production can vary among isolates, and same isolate can show different colony arrangements or distribution patterns were mostly central, spread over the whole petri dish; few isolates showed peripheral arrangements, distinguishing these isolates from S. coffeicola and S. delphinii [36], which show ring-like sclerotial distributions or peripheral and ring-like structures, respectively.

Sclerotial size is an important characteristic feature for separating S. rolfsii from the other two neighboring species. In the present study, the size of the sclerotia varied with isolates. Average sizes of the isolates were 0.5–2.0. Similar sizes have been reported from S. rolfsii in different crops in Korea. For samples, sclerotial sizes from S. rolfsii were 1.0–3.0, 1.0–2.7, 1.0–3.0, and 1.0–3.0 mm in Convallaria keiskei [37], corn [38], Capsicum annuum [39], and Allium sativum [40], respectively. Mahadevakumar and Janardhana [33] characterized the morphology of S. rolfsii and showed that sclerotia size varied from 0.5 to 2.5 mm, similar to the present study results. The fluffy nature of sclerotia on PDA, their small sizes, and their colors distinguished this fungal species from S. delphinii and S. coffeicola (3.0–5.0 mm; S. delphinii Ochraceous: tawny to buckthorn brown, S. coffeicola: yellow ocher to buckthorn brown) [41]. The morphological observations of the isolates completely matched with the description of S. rolfsii [42]; therefore, the fungal strains in this study were identified as S. rolfsii Sacc. (teleomorph: Athelia rolfsii (Curzi) Tu and Kimbrough).

Phylogenetic analysis of all ITS sequences of the isolates from the present study revealed that the references sequences of A. rolfsii, A. coffeicola, and A. delphinii clustered together in a group and suggested that these three species were closely related to each other. S. delphinii and S. coffeicola produced a separate subgroup in the same cluster, which separated these two species from S. rolfsii. Similar ITS phylogeny described earlier on S. rolfsii and explained the same as of the present study [10, 14, 16, 32-35]. They showed the similarity among the above mentioned three species of Sclerotium species and narrated slight differences of S. rolfsii with the others [43-45]. Harlton et al. [36] described that S. rolfsii and S. delphinii clustered together and S. coffeicola were separated by different closely related cluster, whereas Adandonon et al. [43] mentioned that the ITS sequences could separate S. rolfsii from the other two related species [43]. Hence, all the isolates obtained in the present study were identified as S. rolfsii on the basis of ITS sequence analysis.

Phylogenetic data analysis and their placement confirmed that the LSU sequences of 13 different isolates of Sclerotium species isolated from sweetpotatoes in Korea and their reference species obtained from GenBank produced three distinctive groups, represented as S1, S2, and S3; the isolates from the present study belonged to the group S3. Three morphologically similar species, i.e., S. rolfsii, S. delphinii, and S. coffeicola, were grouped into S3. Xu [17] explained that these three species are phylogenetically and morphologically very similar and produce similar clades in phylogenetic analysis [45, 46]. In group S3, there were three subgroups, for which the present study isolates formed separate subgroups, and the three reference sequences of S. delphinii and two reference sequences of S. coffeicola were separated from A. rolfsii (teleomorph: S. rolfsii) and from each other with a high bootstrap value. All of the ITS sequences showed similar results in the molecular data analysis. Thus, the molecular identification supported the morphological observations.

Irani et al. [47] tested the pathogenicity of S. sclerotiorum on detached rapeseed and found variations in pathogenicity and aggressiveness. Adandonon et al. [43] evaluated
pathogenicity on cowpea and showed variations in pathogenicity and aggressiveness in Benin and South Africa. Our pathogenicity study revealed that there might present different races of *S. rolfsii* in Korean sweetpotato isolates. Our further study would focus on the races. Thus, along with these previous studies, our current results suggested that morphological characteristics along with molecular phylogenetic analysis are useful tools to identify and distinguish *Sclerotium* species.

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