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Oxalis purpurea sclerotium rot caused by *Athelia rolfsii*

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Abstract

Over a 3-year period (2015–2017), sclerotium rot was observed on purple wood sorrel (*Oxalis purpurea*) in the exhibition field of Gyeongsangnam-do Agricultural Research and Extension Services, Jinju, South Korea. Infected plants exhibited blight and rot symptoms. White mycelial mats spread over lesions, and numerous sclerotia formed on the petiole near the soil line. Sclerotia were globoid in shape, 1–3 mm in size, and white to brown in color. The optimum temperature for mycelial growth and sclerotium formation on potato dextrose agar (PDA) was 30 °C and the hyphal width was 4–8 μm. Typical clamp connections were observed in the hyphae of fungi grown on PDA. Molecular identification was conducted by sequencing and analysis of the complete internal transcribed spacer (ITS) rDNA sequence of the causal fungus. On the basis of mycological characteristics, molecular identification, and pathogenicity to host plants, this fungus was identified as *Athelia rolfsii*. This is the first report of sclerotium rot on purple wood sorrel caused by *A. rolfsii*.

Keywords *Athelia rolfsii* · Sclerotium rot · *Oxalis purpurea* · Purple wood sorrel

Purple wood sorrel (*Oxalis purpurea*) is cultivated as an ornamental plant for use as a flowering groundcover in gardens, and for natural landscaping projects. It spreads by running roots and bulbs to form colonies (Dreyer and Makwarela 2000; Goldblatt and Manning 2000). All parts of the plant, including the flowers, leaves, stems, and bulbs are edible; however, it should not be eaten in large quantities due to its high concentration of oxalic acid. In Korea, the petals and leaves of purple wood sorrel are used as salad material for bibimbap.

Sclerotium rot is a serious disease affecting many plants. As the disease progresses, petioles of infected inflorescences turn brown, and then became watery, followed by the appearance of abnormal symptoms in the plant. Diseased plants slowly turn brown, wilt, and eventually die. White mycelia and small round sclerotia form in the stems of diseased plants. To date, there has been no report of sclerotium rot in purple wood sorrel (Farr and Rossman 2018).

Over a 3-year period (2015–2017), we observed sclerotium rot on purple wood sorrel that was cultivated at the roadside as landscaping in the exhibition field of Gyeongsangnam-do Agricultural Research and Extension Services, Jinju, South Korea. During this period, rainfall was frequent, temperature and humidity were constant, and the disease was well-developed.

Symptoms included water-soaking on the petioles near the soil line and rot. White hyphae were observed on lesions on the diseased plants, and small, round brownish sclerotia were produced over time (Fig. 1).

To isolate the pathogen from the lesions, diseased plants were sampled and border tissues between healthy and lesion plant materials were cut into 3 × 3 mm squares. Tissues were sterilized with 1% NaOCl solution for 1 min and then washed three times with sterilized water. Excess water was removed by placing the tissues on sterilized filter paper, and then on to water agar. After 2 days of incubation at 25 °C, water agar blocks containing newly grown mycelia were moved to potato agar medium (PDA). Fungal hyphae on the PDA were white and produced white sclerotia (1–3 mm in diameter), which turned brown as incubation time increased (Fig. 2a). Clamp connections were observed by scanning electron microscopy

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Fig. 1 Symptoms of sclerotium rot on purple wood sorrel (*Oxalis purpurea*) caused by the pathogenic fungus *Athelia rolfsii*. **a–b** Infected plants showing typical symptoms on petioles and leaves; **c** sclerotia produced on lesions

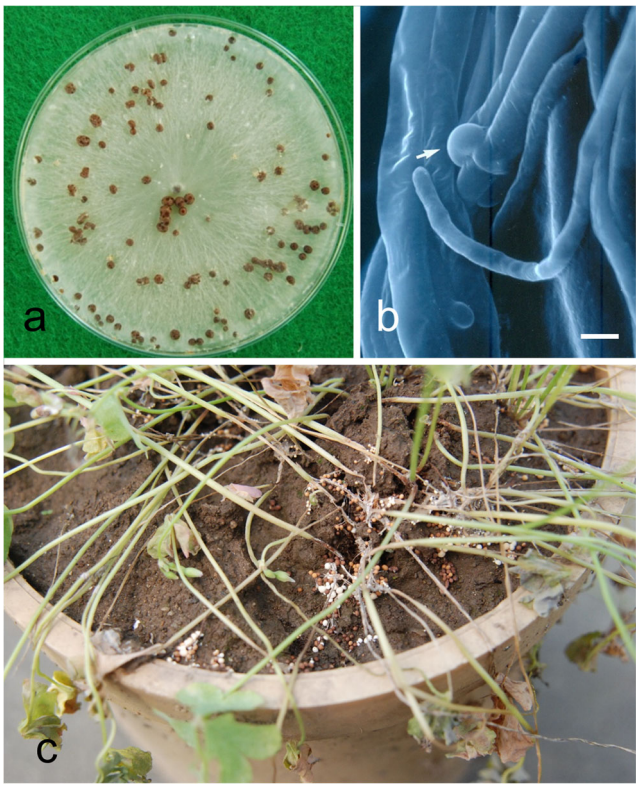

Fig. 2 Mycological characteristics of *Athelia rolfsii* isolated from *O. purpurea*. **a** Mycelial mats and sclerotia produced on PDA after 20 days; **b** scanning electron micrograph of clamp connection (arrow), bar = 5 μm; **c** symptoms induced by artificial inoculation

(SEM) (Fig. 2b). A representative isolate was deposited in the Korean Agricultural Culture Collection (KACC 48132).

Pathogenicity tests were conducted as follows. We purchased and seeded 30 healthy bulbs in Wagner pots (one bulb per pot). After 4 months, the plants were artificially inoculated with PDA blocks containing fungal mycelia and sclerotia. Five isolated fungi including KACC 48132 were cultivated on PDA medium for 7 days. Mycelial mats (1 cm^2) containing mycelia and sclerotia from the five fungal isolates were placed near each purple wood sorrel bulb, and plants were placed in a greenhouse. The plants slowly faded and died within 7 days of inoculation, exhibiting unusual vigorously growing mycelia. On the lesion, white mycelia and brown, round sclerotia formed (Fig. 2c). To fulfil Koch's postulates, the fungal pathogen was re-isolated from the lesions and its fungal morphological characteristics and ITS DNA sequences were confirmed.

To identify the pathogen, total DNA from the representative fungal strain KACC 48132 was extracted and primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3′) were used for polymerase chain reaction (PCR) as described by White et al. (1990). The PCR conditions were 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 nM dNTPs, 10 pmol primer, and 0.1 unit of rTaq DNA polymerase (TaKaRa). The PCR reaction conditions were predenaturation (98 °C, 2 min), denaturation (98 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 30 s) for a total of 30 cycles, and finalization (4 °C, 4 min). The amplified PCR products were electrophoresed on 0.8% agarose gel, stained with ethidium bromide, and confirmed by UV transilluminator. The confirmed bands were isolated and purified using the QIAquick PCR purification kit (Qiagen), cloned into the pGEM-T Easy cloning vector (Promega), and sequenced using M13F and M13R primers (Macrogen). The resulting 687-bp fragment of the ITS rDNA sequence was deposited in GenBank (Accession No. MH571744) and was analyzed using the BLASTN program for fungal molecular

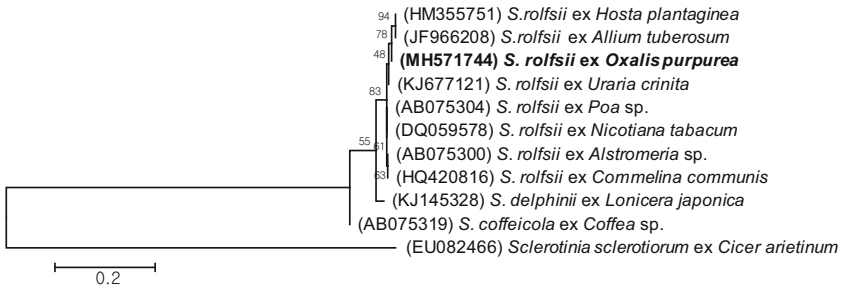

Fig. 3 Phylogenetic tree produced using internal transcribed spacer sequences to show the closest known relatives of *A. rolfsii*. Numbers above branches indicate bootstrap values. Bars indicate the number of nucleotide substitutions per site. The isolate studied in the present study is marked in bold

identification. The ITS rDNA region was 99% homologous with *A. rolfsii*, which causes rot in *Hosta plantaginea* (HM355751) and *Allium tuberosum* (JF966208) (Kwon et al. 2010). Phylogenetic analysis was performed using MEGA software (ver. 4.1) employing the neighbor-joining method and the Tajima–Nei distance model (Tamura et al. 2007). The nucleotide sequence of the obtained ITS rDNA region was compared with those of reported ITS sequences in the National Center for Biotechnology Information (NCBI) nucleotide database for reference. The sequence alignment of the ITS rDNA region was performed using ClustalW software. Previously published ITS sequences of *A. rolfsii* strains were included for reference, and *Sclerotinia sclerotiorum* (EU082466) was used as an outgroup. In the phylogenetic tree, the fungus isolated from purple wood sorrel was placed within a clade comprising reference isolates of *A. rolfsii* (Fig. 3).

As described above, the mycological characteristics, pathogenicity, and ITS nucleotide sequence analysis of the pathogens were consistent with *A. rolfsii* (Mordue 1974; Tu and Kimbrough 1978). Two diseases have been reported on purple wood sorrel in the United States Department of Agriculture (USDA) fungal databases (Farr and Rossman 2018): *Aecidium oxalidis* from South Africa (Doidge 1950) and *Puccinia oxalidis* from Canary Islands (Gjaerum and Sunding 1986). To our knowledge, the current paper is the first report of sclerotium rot on purple wood sorrel caused by *A. rolfsii*. This study was presented at the Korean Plant Pathology Conference on April 26, 2018 in Cheongju, South Korea (Kwon et al. 2018).

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