RESEARCH NOTE

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Occurrence of Sooty Blotch and Flyspeck Disease on Sweet Persimmon in Korea

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Sooty blotch and flyspeck (SBFS), a disease caused by a complex of fungi, results in substantial economic losses for commercial growers of sweet persimmon (*Diospyros kaki* L.) in Korea. However, many species causing SBFS in Korea have not been identified and sources of inoculum are uncertain. Based on mycological characteristics, pathogenicity, and molecular data, the causal fungi were identified as *Dissocoenium* sp. and *Zygophiala wisconsinensis*. This is the first report of SBFS of sweet persimmon in Korea.

KEYWORDS : *Dissocoenium* sp., Sooty blotch and flyspeck, *Zygophiala wisconsinensis*

A complex of fungi have been known to cause sooty blotch and flyspeck (SBFS) disease as a result of colonization on the surface of the wax layer of fruits [1]. Because their hyphae, fruiting bodies, and survival structures are darkly pigmented, symptoms of SBFS appear as blemishes on fruits. SBFS fungi cause no serious damage to the fruits; however, because the quality of infected fruit is downgraded from premium grade, they have been regarded as diseases by growers and plant pathologists. However, many species causing SBFS in Korea have not been identified and sources of inoculum are uncertain. In August 2010, sweet persimmon fruit exhibiting signs of SBFS were sampled from supermarkets in Changwon, Jinju, Jinyoung, and Sacheon, Southern Korea (Fig. 1). Here, we report on a new disease of sweet persimmon caused by SBFS fungi based on symptoms, mycological characteristics, molecular identification, and pathogenicity test.

Isolation, purification, and microscopic observation.

Colonies consisted of olive green-to-black mycelial mats with a few sclerotium-like bodies; infections ranged in severity from scattered spots to nearly complete coverage of the fruit surface. Colonies were transferred directly from the sweet persimmon fruit onto oatmeal agar (OA) and cultured at 22°C in darkness. Microscopic examination of the freshly isolated fungi on OA was performed. Detailed microscopic examinations of a representative

specimen were performed using a light microscope (Axioplan; Carl Zeiss, Jena, Germany). Conidophore cells were dark brown hyaline and had two phialide. Conidia were fusiform to obclavate with smooth, thick walls and a single septate was observed in the conidia (Fig. 2).

Pathogenicity test. To fulfill Koch's postulates, each of the 10 isolates was used in inoculation of three sweet persimmon fruits that had previously been washed under tap water and disinfested with 70% ethanol. For preparation of inoculum, one-month-old cultures growing on OA were comminuted into a suspension of mycelial fragments and conidia (10^5 ~ 10^6 CFU/mL) in a blender with sterile distilled water (SDW). Using cotton swabs, each isolate was inoculated on three sweet persimmon fruits. As controls, two surface-disinfested sweet persimmon fruits were swabbed with SDW. After incubation of the inoculated sweet persimmon fruits in a moist chamber at 22°C for one month, all inoculated sweet persimmon fruits exhibited signs of SBFS similar to those of the original colonies on sweet persimmon fruit, however, the controls did not (Fig. 1).

DNA extraction, amplification, and sequence analysis.

The Exgene Plant-Fungal SV mini kit (Geneall Biotechnology Co., Seoul, Korea) was used for extraction of genomic DNA from colonies grown on 2% OA, following the manufacturer's instructions. The primers first internal transcribed spacer region (ITS1) (5'-TCCGTAGGTGAAC-

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Fig. 1. Appearance of the sooty blotch and flyspeck complex on sweet persimmon fruit. Flyspeck: A, Naturally infected fruit; B, Flyspeck on sweet persimmon peel; C, One-month-old colony on oatmeal agar. Discrete speck: D, Naturally infected fruit; E, Discrete speck on sweet persimmon peel; F, Two-month-old colony on oatmeal agar (scale bars = 0.5 cm).

Fig. 2. *Zygophiala wisconsinensis* on oatmeal agar. A–C, Conidiophores; D–F, Conidia (scale bars = 5 μ m).

CTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [2] were used to amplify the ITS of the nuclear ribosomal RNA operon, including: the 3' end of the 18S rRNA gene, the ITS1, the 5.8S rRNA gene, the second ITS (ITS2), and the 5' end of the 28S rRNA gene. Part of the large subunit 28S rRNA (LSU) gene was amplified using primers LR0R (5'-ACCCGCTGAACCTTAAGC-3') and LR5 (5'-TCCTGAGGGAACTTCG-3') [3]. The PCR reaction was performed in a mixture with 0.5 units *Taq* polymerase (Takara, Tokyo, Japan), 1 $\times$ PCR buffer, 0.5 mM MgCl₂, 0.2 mM of each dNTP, 5 pmol of each primer, and approximately 10–15 ng of fungal genomic DNA, with the total volume adjusted to 50 μ L with sterile water. Reactions were performed on an Astec PC 802 thermal cycler (Astec, Fukuoka, Japan) with cycling conditions consisting of 5 min at 98°C for primary denaturation, followed by 30

cycles at 98°C (30 sec), 55°C (30 sec), and 72°C (30 sec), with a final 4 min extension step at 72°C to complete the reaction. Following agarose gel electrophoresis, extraction of PCR amplicons were performed using a gel extraction kit (Geneall Biotechnology Co.). Purified PCR products were cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced with primers M13F (5'-GTAA-AACGACGGCCAGT-3') and M13R (5'-GCGGATAACA-ATTTACACAGG-3'). A Bigdye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) was used in performance of sequencing, following the manufacturer's instructions.

GenBank accession number. Amplification of the ITS region generated a 566 bp sequence (GenBank accession No. JX415826), and the LSU region was 1,383 bp (GenBank accession No. JX415827).

Alignment and phylogenetic analyses. Newly generated sequences were subjected to a Blast search of the NCBI databases; sequences with high similarity were downloaded from GenBank and comparisons were made based on the alignment of the obtained sequences. MEGA4 with the neighbor-joining method and the Tajima-Nei distance model were used in performance of phylogenetic analysis [4]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The ITS and LSU trees were rooted using sequences of *Agaricus bisporus* as an outgroup. Blast of the ITS rDNA sequences showed 91% identity

Fig. 3. Phylogenetic trees using internal transcribed spacer region (ITS) (A) and large subunit 28S rRNA (LSU) (B) sequences. DNA sequences from the National Center for Biotechnology Information (NCBI) nucleotide database were aligned using ClustalW. Numbers above the branches indicate the bootstrap values. Bars indicate the number of nucleotide substitutions per site. Our isolate, DA001, infecting sweet persimmon is shown in bold font. Classes are shown to the left of the tree and families, where known, to the right. The tree was rooted to a sequence of *Agaricus bisporus*.

with *D. aciculare* (GenBank accession No. AY639404), infecting Eucalyptus (Fig. 3A). Blast of the LSU DNA sequences showed 98% identity with *D. aciculare* (GenBank accession No. EU019266), infecting Black Medic (*Medicago*

lupulina) (Fig. 3B). In the phylogenetic tree (Fig. 3), the isolates were placed within a clade comprising reference isolates of *Dissoconium* spp.

Based on symptoms, mycological characteristics,

molecular data, and pathogenicity to the host plant, these fungi were identified as *Dissoconium* sp. Flyspeck disease on sweet persimmon fruits caused by *Z. wisconsinensis* has been recorded previously [5], however, discrete speck disease caused by *Dissoconium* sp. has not been reported in Korea [6]. To the best of our knowledge, this is the first report of the presence of *Dissoconium* sp. on sweet persimmon in Korea. This disease is highly dependent upon weather conditions of the growing season. Recent occurrence of the disease suggests the possibility that SBFS may be widespread. SBFS, a disease caused by a complex of fungi, results in substantial economic losses for commercial growers of sweet persimmon fruits in Korea.

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