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First Report of Gray Mold Disease of Sweet Basil (*Ocimum basilicum*) Caused by *Botrytis cinerea* in Korea

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ABSTRACT : In August 2015, we collected samples of gray mold from sweet basil growing in Sachunmeon, Gangneung, Gangwon Province, Korea. Symptoms included extensive growth of mycelia with gray conidia on young leaves, stems, and blossoms. The pathogen was isolated from infected leaves and blossoms and the fungus was cultured on potato dextrose agar. For identification of the fungus, morphology and rDNA sequencing analysis of the fungus were performed, which confirmed its pathogenicity according to Koch's postulates. The results of morphological examinations, pathogenicity tests, and the rDNA sequences of the internal transcribed spacer regions (ITS1 and ITS4) and the three nuclear protein-coding genes G3PDH, HSP60, and RPB2 showed that the causal agent was *Botrytis cinerea*. This is the first report of gray mold caused by *Botrytis cinerea* on sweet basil in Korea.

KEYWORDS : ***Botrytis cinerea*, Gray mold, Pathogenicity, Sweet basil**

Sweet basil (*Ocimum basilicum* L.) is a popular culinary perennial herb, native of central Asia and northwest India belonging to the mint family (Lamiaceae). It grows in many countries of the world as a spice, medicinal, and aromatic plant. Its essential oil has been used for many years to flavor foods, as an ingredient of dental and oral health care products, and in fragrances [1, 2].

Botrytis cinerea (teleomorph *Botryotinia fuckeliana*), a necrotrophic ascomycete fungal pathogen with a broad host range [3], causes gray mold on a variety of dicotyledonous plants, including many vegetables, fruits, ornamental flowers, and greenhouse plants [4]. The disease is manifested by necrotic areas with extensive fungal growth, which result in the characteristic appearance of gray mold. *Botrytis* infections are favored by a cool, rainy spring season and summer temperatures of approximately 15°C (60°F). Gray mold can be particularly damaging when rainy and/or damp weather occurs over several days. Association of basil with *B. cinerea* has been reported in Greece, Hungary, Italy, Chile, and Poland [5] but has not been previously reported in Korea. The objective of the current study was to identify the causal agent associated with gray mold observed on sweet basil in Korea, based on culture characteristics, molecular phylogenetics, and pathogenicity.

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Fungal isolation and pathogenicity test

In August 2015, we observed gray mold symptoms on sweet basil plants growing in Sachunmeon, Gangneung, Gangwon Province, Korea. The disease symptoms included dark brown lesions covered with gray spore masses on the leaf, stem, and blossoms of infected plants (Fig. 1A). For pathogen isolation, small pieces of infected leaves and blossoms were sterilized by immersion in 0.1% sodium hypochlorite (NaOCl) for 1 min, rinsed three times with sterile distilled water, and cultivated on potato dextrose agar (PDA; Difco, Detroit, MI, USA) for 7 days at 20 ± 2°C.

For performance of the pathogenicity test for this fungus, a conidial suspension (2×10^6 conidia/mL) was prepared by harvesting conidia from 2-week-old *B. cinerea* cultures and sprayed onto three healthy potted basil plants

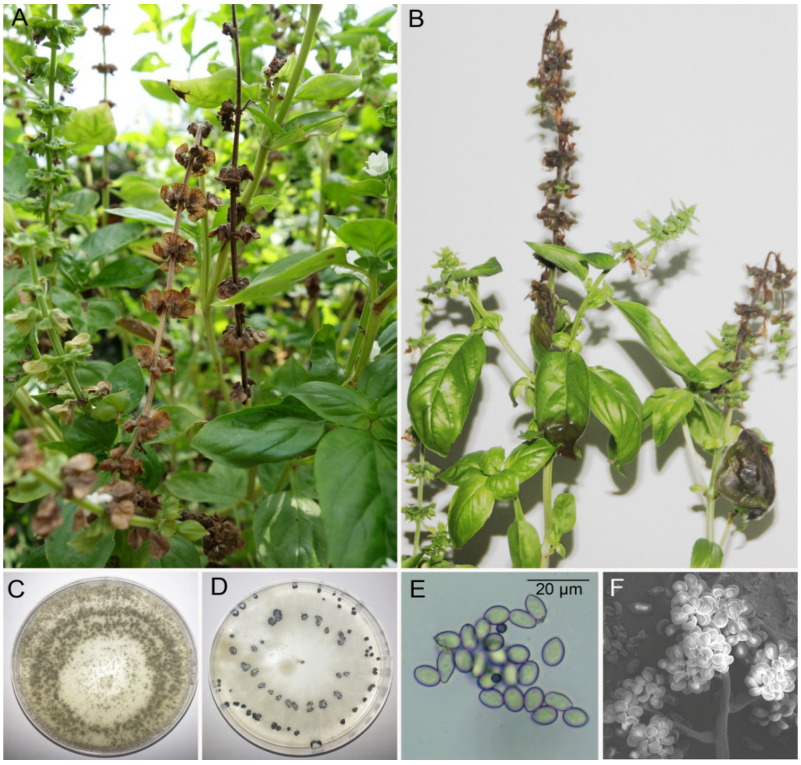

Fig. 1. Gray mold caused by *Botrytis cinerea* on sweet basil (*Ocimum basilicum* L.). A, Flower blossoms damaged by gray mold; B, Gray mold was observed 7 days after artificial inoculation; C, Two-week-old colony of *B. cinerea* on potato dextrose agar (PDA); D, Three-week-old colony of *B. cinerea* showing black sclerotia on PDA; E, Conidia; F, Scanning electron microscopy of *B. cinerea*.

(73 days old, flowering stage). Another three plants were sprayed with sterilized water, serving as controls. Plants were covered with plastic bags for 3 days after inoculation to maintain high relative humidity and were placed in a growth chamber at 20 ± 2°C. The first disease lesions developed on leaves and blossoms 7 days after inoculation (Fig. 1B), whereas control plants remained symptomless. The pathogenicity test was performed twice with similar results. The fungal pathogen was re-isolated from the disease lesions of the inoculated plants; the morphological characteristics of the re-isolated pathogen were identical to those of the original isolates. In other words, the fungal pathogen fulfilled the criteria stipulated by Koch's postulates and was identified as the causal agent of gray mold on sweet basil.

DNA extraction, polymerase chain reaction, and sequence analysis

Genomic DNA of the strain was extracted using a DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. To confirm the identity of the causal fungus, the entire internal transcribed spacer region (ITS) rDNA was amplified and sequenced using universal primers for ITS1 and ITS4 [6]. To confirm the species identification, three nuclear protein-coding genes were sequenced: glyceraldehyde-3-phosphate dehydrogenase (G3PDH); heat-shock Protein 60 (HSP60); and DNA-dependent RNA polymerase subunit II (RPB2) [7]. Polymerase chain reaction (PCR) was performed in a 25-uL reaction mixture containing 0.5 μL of each primer, 0.5 μL of TaqDNA 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 a Mastercycler Gradient (Eppendorf, Hamburg, Germany). For ITS, the conditions were pre-denaturation at 94°C for 5 min, followed by 35 cycles each of denaturation at 94°C for 35 sec, annealing at 52°C for 55 sec, and elongation at 72°C for 1 min, and a final extension step at 72°C for 10 min. For HSP60 and RPB2 gene fragments, 94°C for 5 min (1 cycle); 94°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec (35 cycles), and then 72°C for 10 min (1 cycle). The same program with an annealing temperature of 64°C was used for G3PDH gene fragments. The obtained nucleotide sequ-

Table 1. Morphological characteristics of the gray mold fungus isolated from sweet basil

Characteristic		Present isolate	*Botrytis cinerea*[a]
Colony	Color	Grayish brown	Grayish brown
Conidia	Shape	Ellipsoidal or ovoid	Ellipsoidal or ovoid
	Size (μm)	5.3~10.3 × 4.6~7.3	6.0~18.0 × 4.0~11.0
	Color	Dark brown	Pale brown
Conidiophore	Size (μm)	13.2~27.6	16~32
Sclerotia	Shape	Flat or irregular	Flat or irregular
	Color	Black	Black

[a]Described by Ellis and Waller [9].

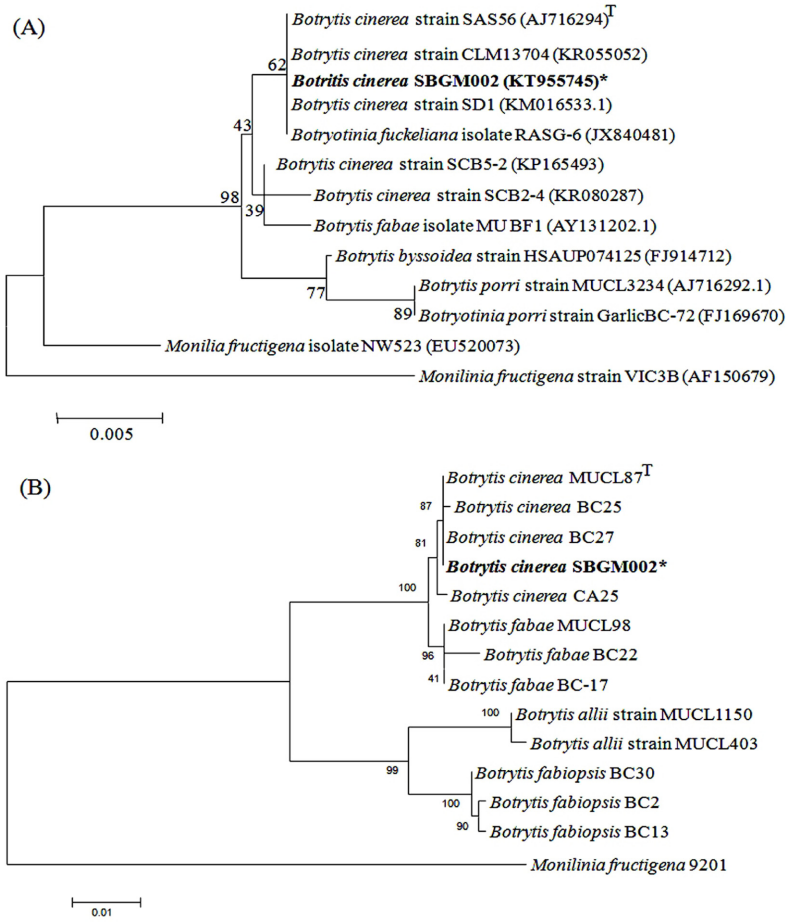

Fig. 2. Phylogenetic analysis of *Botrytis cinerea* strain SBGM002, constructed using the neighbor-joining method based on internal transcribed spacer (ITS) region (A) and combined G3PDH, HSP60, and RPB2 gene sequence data (B). *Monilinia fructigena* was used as the outgroup. The numbers at the nodes indicate bootstrap values from a test of 1,000 replicates. The scale bar indicates the number of nucleotide substitutions. [T], Standard isolate; *, Present isolate.

ences were searched using BLASTN available from the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analysis of *Botrytis cinerea* was performed using the MEGA5 program with the neighbor-joining method [8].

Identification and characterization of *B. cinerea*

Three fungal strains were obtained from sweet basil with gray mold and one of these strains, SBGM002, was examined. Based on morphological examination of the isolated fungus and the results of rDNA sequencing ana-

lysis, this strain was identified as *B. cinerea*. The fungal colonies were grayish brown and produced abundant conidia after 14 days of cultivation on PDA at 20 ± 2°C (Fig. 1C). The conidia (n = 50) were single-celled, ellipsoidal or ovoid in shape, dark brown, and 5.2 to 7.6 × 5.4 to 9.8 μm on naturally infected blossoms and 5.3 to 10.3 × 4.6 to 7.3 μm on PDA (Fig. E). The conidiophores were solitary, cylindrical, terminally branched, 13.4~27.5 μm wide, grayish to olivaceous gray, and smooth (Fig. 1F). After three weeks, the fungus formed several black sclerotia near the edge of the petri dish (Fig. 1D). A representative isolate (SBGM002) was deposited in Gangneung-Wonju National University and used for further studies. The morphological characteristics of the identified species are summarized in Table 1. Morphological characteristics were consistent with those of *B. cinerea* Pers.: Fr. Ellis and Waller [9].

The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) and three nuclear protein-coding G3PDH, HSP60, and RPB2 gene sequences were compared with the GenBank database sequences using the NCBI BLAST search tool. BLAST analysis of the ITS sequence (KT955745) was 100% identical to that of *B. cinerea* (KP 900730). The G3PDH, HSP60, and RPB2 sequences (KT 955746, KT955747, and KT955748, respectively) were 100% identical to those of *B. cinerea* (KJ937074, KJ937067, and KR052881, respectively). Thus, we confirmed *B. cinerea* as the causal agent of gray mold on sweet basil in Korea (Fig. 2). To the best of our knowledge, this is the first report of gray mold caused by *B. cinerea* on sweet basil in Korea.

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