

First Record of *Alternaria simsimi* Causing Leaf Spot on Sesame (*Sesamum indicum* L.) in Korea

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Abstract Leaf spot disease was observed in sesame (*Sesamum indicum* L.) during 2009 and 2010 in Korea. The pathogen was identified as *Alternaria simsimi* based on morphological and cultural characteristics. The morphological identification was well supported by phylogenetic analysis of the ribosomal DNA-internal transcribed spacer region. *A. simsimi* isolates caused spot symptoms on leaves and stems of sesame plants 2 wk after artificial inoculation, which were similar to those observed in the field. This is the first record of leaf spot disease in Korea caused by *A. simsimi*.

Keywords *Alternaria simsimi*, Identification, Leaf spot, Pathogenicity, Sesame

Sesame (*Sesamum indicum* L.) is one of the most important oil-seed crops in the world, and has been cultivated in Korea, Japan, China, and other East Asian countries since ancient times for use as a traditional healthy food. Sesame seeds and oil are primarily used for commercial products [1]. *Alternaria sesami* and two unidentified fungal species, *Alternaria* sp. (1) and *Alternaria* sp. (2) were reported to cause leaf blight and leaf spot disease on sesame [2]. The fungus, *A. simsimi*, was presumed to be one of the two previously reported *Alternaria* species observed in Korea; however, this was never confirmed nor reported. The purpose of this study was to identify the fungus causing leaf spot disease on sesame and to confirm its pathogenicity on host.

Alternaria isolates were collected from sesame leaf, stem, and seed tissues from commercial fields in seven different locations (Gyeongbuk Pohang, Gyeongbuk Uljin, Gyeonggi

Yangpyeong, Gyeonggi Yeosu, Jeonbuk Iksan, Jeonnam Hampyeong, and Jeonnam Naju) in Korea during 2009 and 2010 (Fig. 1A). Symptoms of disease were brown to dark fuscous, oval to irregular spots with pale margin and yellow halo. Spots were primarily observed on leaves; however, in severely infected plants, spots developed on stems and capsules. On severely affected leaves, several spots coalesced to form large necrotic lesions, leading to blight and defoliation, and death of the plant. The disease was most severe under moist conditions following wet weather.

Surface-sterilized tissue pieces were placed in Petri dishes with moist sterilized blotting paper, and incubated for 2~3 days at 25°C under 12 hr NUV light/12 hr darkness conditions. Seed-borne fungi were isolated by the blotter method [3]. Isolates were obtained by single spore isolation and cultured on potato dextrose agar (PDA) and V8 juice agar to assess cultural characteristics. Colonies were characterized after 7 days of culture at 25°C. On PDA, colonies were raised, vinaceous buff, and 47~54 mm in diameter, with regular margins and immersed or partly superficial mycelia (Fig. 1C). On V8 juice agar, colonies were effuse, fuscous black, and 52~60 mm in diameter with circular margins and immersed or partly superficial mycelia (Fig. 1D). Mycelia grew on both PDA and V8 juice agar between 5~35°C, with an optimum temperature between 25~30°C.

Sporulation pattern, conidiophores and conidial morphology were examined on V8 juice agar media under a compound light microscope (Olympus BX50; Olympus, Tokyo, Japan). Conidiophores borne solitary or in small fascicles, simple or branched, smooth-walled, pale to light brown in color, with a single, small, and swollen, terminal, pigmented conidiogenous site at the apex or proliferating sympodially and geniculate with 1~3 pigmented former conidium

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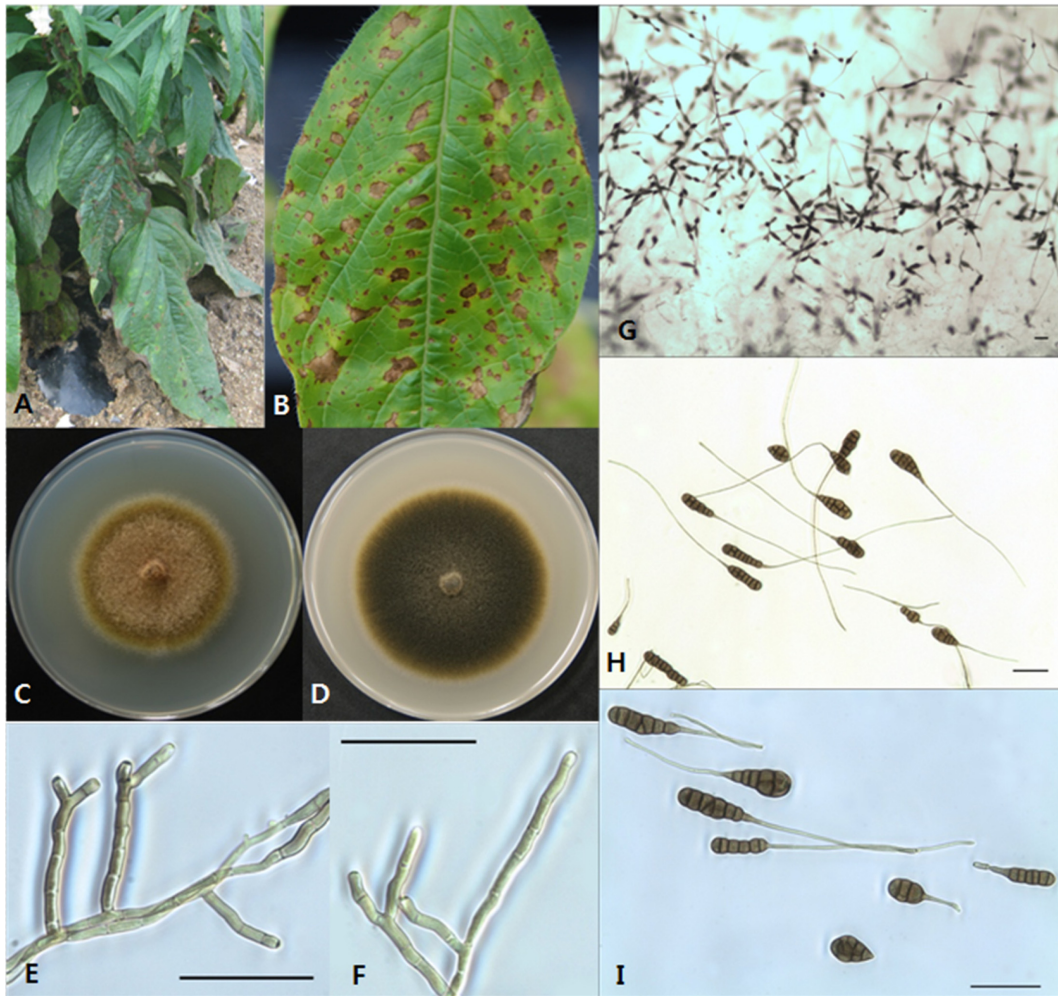


Fig. 1. A~B, Typical symptoms observed on sesame in field. Symptoms observed after artificial inoculation; C~D, Colony characteristics, as observed on potato dextrose agar (C) and V8 juice agar (D); E~I, Morphological characteristics of *Alternaria simsimi*: Conidiophores (E, F); sporulation pattern (G); conidia (H, I) (scale bars: E, F, H, I = 50 μ m).

attachment sites, up to 90 μ m long, and 3~7 μ m wide (Fig. 1E and 1F). Conidia were highly variable in shape; subcylindrical, obclavate, ovoid or narrow ellipsoid, rostrate or without a beak, tapering spore body to smooth or abrupt beak, pale to medium brown with eusepta in darker contrast, a size range of 29~60.2 μ m long (avg. 43.8 μ m), 7.8~14.2 μ m wide (avg. 11.7 μ m), with 2~7 transverse septa and 1~2 longitudinal septa in a few of the transverse divisions, the wall ornamentation is smooth, and produced solitary or in short chains of 6 conidia. The beak, when present was either long or short, narrowly filamentous, simple and not branched, pale to medium brown in color, variable in length, up to 480 μ m long (avg. 210 μ m), and 1.1~2.7 μ m wide (average, 1.9 μ m) (Fig. 1G~1I). The morphological characteristics of this isolate were similar to those of *Alternaria simsimi* described by Simmons (Table 1) [4].

The sequence of the ribosomal DNA-internal transcribed spacer (rDNA-ITS) region was analyzed to confirm the species identification. ITS5 and ITS4 were used as primers for amplification of the target region, and PCR was

conducted as previously described [5]. The sequence of the ITS region was aligned with sequences of representative isolates of *Alternaria* species by using the PHYDIT program (ver. 3.2) [6]. Maximum parsimony analysis was calculated using PAUP v.4.0b10 [7] and were estimated using heuristic searches consisting of random addition order and tree bisection-reconnection branch swapping. Bootstrap analysis was performed with 1,000 replicates to assess the relative stability of the branches. The resulting sequence of the CNU 104101 isolate of *A. simsimi* (accession No. JF780938) was deposited in GenBank of National Center for Biotechnology Information (NCBI). In a phylogenetic analysis, the sequence of CNU 104101 was found to be 100% identical to that of type strain *A. simsimi* EGS 13.110 (accession No. JF780937) in the section *Dianthicola* [8], supported by a strong bootstrap value (96%) (Fig. 2). The phylogenetic tree inferred from the sequence of the rDNA-ITS region correlated well with the species identified by morphological characteristics.

To determine pathogenicity, two isolates of the species

Table 1. Comparison between the morphological characteristics of the present isolates with those of a reference isolate of *Alternaria simsimi*

Characteristic	Present isolates	Simmons [4]
Sporulation	Solitary or short chains up to 6 chains	Chains of two or more conidia abundant
Conidiophores		
Shape	Simple or branched, solitary or in small fascicles	Simple or branched, have a few geniculate apical growth extensions
Length (µm)	Up to 90	Up to 80
Width (µm)	3.0~7.0	3.0~5.0
Conidial body		
Shape	Variable shape, subcylindrical, obclavate, ovoid or narrow ellipsoid	Usually narrow ellipsoid
Size (µm)	29~60×7.8~14.2 (avg. 43.8×11.7) ^a	45~90×12~18
No. of septa		
Transverse	2~7 (avg. 4.98) ^a	7~10
Longitudinal	0~2 (avg. 0.78) ^a	1~2
Color	Pale to medium brown with eusepta in darker contrast	Medium brown with eusepta in darker contrast
Ornamentation	Smooth	Lack ornamentation
Conidial beak		
Shape	Narrowly filamentous, simple and not branched	Short or long beak
Size (µm)	Up to 480×1.1~2.7 (avg. 210×1.9) ^a	275~325×2.5

^aFifty conidia were randomly selected to measure conidial body and beak size.

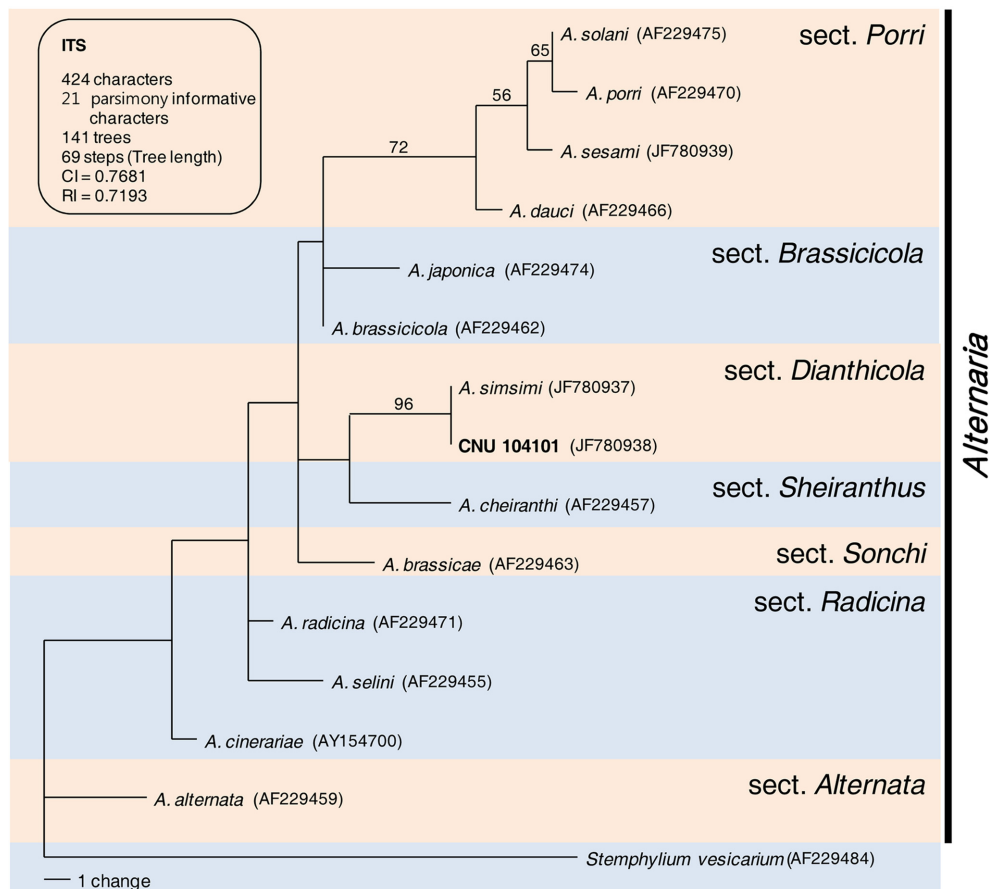


Fig. 2. One of the parsimonious trees inferred from sequence of internal transcribed spacer (ITS) region. Numbers above the branches are bootstrap values in 1,000 bootstrap replicates. Only bootstrap values higher than 50 are shown. The scale bar indicates the number of nucleotide substitutions. GenBank accession Nos. are represented in parentheses. CI, consistency index; RI, retention index.

and 2-mon-old sesame plants (cultivar, Hwang-Beak) were grown in a greenhouse at $25 \pm 2^\circ\text{C}$. Inoculation was performed by spraying conidial suspensions of 1.5×10^4 spores/mL on plants, without wounding. The conidia were scraped from the 10-day cultures on V8 agar plates grown at 25°C with a 12 hr photoperiod of NUV light. Control plants were sprayed with sterile water. The inoculated plants were first kept in humid plastic bags for 2 days at 25°C , then the cover was removed and the plants were transferred to a glasshouse at $25 \pm 2^\circ\text{C}$. Symptoms first appeared about 2 days after inoculation; lesions with chlorotic halos were developed and enlarged over time (Fig. 1B). These symptoms were similar to those observed in naturally infected plants. The same fungus was consistently isolated from diseased areas, but not from healthy controls. Thus, we concluded that the isolate is pathogenic to sesame. Inoculation experiments were conducted twice and provided similar results.

Based on conidial morphology, the pathogen was identified as *Alternaria simsimi* [9]. There were some differences in conidial size compared with that of Simmons (Table 1) [9], but were considered to be within natural variation, depending on maturity or culture conditions. This identification was well supported by phylogenetic analysis and the pathogenicity test. This is the first report of leaf spot disease caused by *A. simsimi* on sesame in Korea and appears to be the first confirmation of its pathogenicity on the sesame leaf.

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