Mycobiology

Research Note

First Report of Black Spot Disease Caused by *Alternaria alternata* on Sweet Persimmon Fruits

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Abstract Black spot of sweet persimmon, caused by *Alternaria alternata*, occurred in an orchard in Gyeongnam province, Korea in 2012. The symptom was appearance of 0.5 to 4 cm black spots on the surface of fruit. The pathogen was isolated from flesh of disease lesions. The causal agent was identified as *A. alternata* by morphological characteristics and sequencers of the internal transcribed spacer (ITS) 1 and ITS4 regions of rRNA. Artificial inoculation of the pathogen resulted in development of disease symptoms and the re-isolated pathogen showed characteristics of *A. alternata*.

Keywords *Alternaria alternata*, Black spot, Sweet persimmon

Sweet persimmon (*Diospyros kaki* L.), belonging to the genus Diospyros, is native to China. China, Japan, and Korea are major producing countries of persimmon. Anthracnose (*Colletotrichum gloeosporioides*), circular leaf spot (*Mycosphaerella nawae*), and angular leaf spot (*Cercospora kaki*) are generally known as the main diseases of sweet persimmon. *Penicillium expensum*, *Penicillium* spp. [1].

Botrytis sp., *Cladosporium* sp., *Pestalotia* sp. and *Phompsis* sp. [2] are known agents of postharvest decay in sweet persimmon. In addition, *Alternaria alternata* was reported as a postharvest pathogen in sweet persimmon [3]. However, occurrence of pre-harvest disease by *A. alternata* has not been reported. In this study, we were able to identify and verify the pathogenicity of the causal agent of black spot disease occurrence as a pre-harvest disease in sweet persimmon fruit.

DNA extraction and internal transcribed spacer (ITS) sequencing analysis. The pathogen was isolated from disease lesions of sweet persimmon fruit using a surface sterilization method. The pathogen was maintained at 4°C on potato dextrose agar (PDA). Mycelium for DNA extraction was grown in 5 mL of potato dextrose broth in a rotary shaker at 160 rpm for 48 hr at 28°C. Total genomic DNA of the pathogen was extracted using the CTAB extraction method [4].

The ITS region was amplified using the primers ITS1 and ITS4 [5]. The amplification was performed in a 20 μL reaction mixture containing 10 pmol of each primer, two units of *Taq* DNA polymerase (Takara, Tokyo, Japan), 1 μL of each dNTP, 2 μL of 10× PCR reaction buffer, and 50 ng of template DNA. PCR conditions were as follows: pre-denaturation at 94°C 5 min; 30 cycles of denaturing at 94°C for 30 sec; annealing at 55°C for 45 sec and extension at 72°C for 40 sec; and final extension at 72°C for 10 min. Sequences from the amplified ITS PCR product were deposited in GenBank (accession No. KC752593.1). Phylogenetic analysis of *Alternaria alternata* was performed using the MEGA5 program with the neighbor-joining method [6]. For pathogenicity testing, *A. alternata* was incubated for one week on PDA at 28°C. A spore suspension adjusted to 1×10^6 spores/mL, then 10 μL, was inoculated on the surface of sweet persimmon fruit using a needle. Control fruits were treated with sterilized water and the inoculated sweet persimmon fruits were kept in 90% relative humidity at 28°C for two wk.

Disease symptom and pathogenicity test. We observed symptoms of black spot on sweet persimmon fruit from the orchard located in Jinju city, Gyeongnam province, Korea in 2012. The symptoms were similar to those of sweet persimmon anthracnose disease and the spot sizes ranged from 0.5~4 cm. The potential causal agent of disease was isolated and purified. When the isolated fungi were

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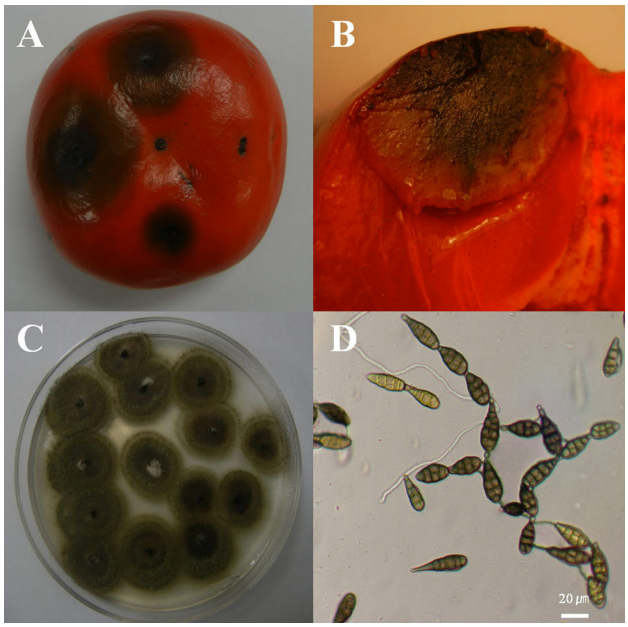

Fig. 1. Symptoms and morphological characteristics of persimmon (*Diospyros kaki* L.) by *Alternaria alternata*. A, Black spots of persimmon; B, Vertical section plan of the lesion; C, Colony on potato dextrose agar; D, Conidia.

inoculated artificially, the symptoms were observed on the surface of fruit at 14 days after inoculation. Small, black spots were observed on the inoculated fruit at four days after treatment. After two wk, these spots developed into large lesions (Fig. 1A). Vertical sections showed black and hard sponge symptoms (Fig. 1B), providing convincing evidence of infection through wound in the postharvest as well as the pre-harvest. The disease symptoms were similar to those of disease lesions observed on pre-harvested persimmon fruit in orchards. The fungal pathogen was re-isolated from disease lesions of inoculated fruit and the re-isolated pathogen exhibited the same morphological characteristics compared with the original isolates.

Mycological characteristics and phylogenetic analysis. The optimum temperature for mycelial growth was 25°C on PDA (data not shown). Mycelial colonies were typical for *Alternaria* (Fig. 1C). Conidiophores were simple, straight, bent, or sometimes branched. Conidia were brown, obpyriform to ellipsoid (22~39 × 8~15 μm), with both transverse and longitudinal septa (Fig. 1D). The pathogen of sweet persimmon black spot was identified as *Alternaria alternata* based on morphological characteristics (Table 1) [7].

The ITS sequence was compared to the GenBank database using the NCBI BLAST. The sequences determined from the rRNA-ITS were 100% similar to those of several *A. alternata* species of accession Nos. JF835809.1, JN005702.1, FJ717733.1, FJ717733.1, and JN673372.1; as a result, the causal fungus was identified as *A. alternata* (Fig. 2).

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Table 1. Comparison of morphological characteristics of the pathogen isolated from sweet persimmon with *Alternaria alternate*, described previously

Characteristics		Present isolate	*Alternaria alternata*[a]
Colony	Color	Grayish white, olive-green to sooty black	Olivaceous black to sooty black
Conidia	Shape	Brown, obpyriform to ellipsoid	Ellipsoid to ovoid, obclavate to obpyriform
	Size (μm)	22~39 × 8~15	15~35 × 8~14
	Septa	3~5 transverse, 1~2 longitudinal	3~5 transverse, 1 (rarely 2) longitudinal
Conidiophores	Shape	Simple, straight, bent or sometimes branched	Solitary or in small fascicles
	Size (μm)	34~100 × 4	35~110 × 3.4

[a]Described by Ellis [7].

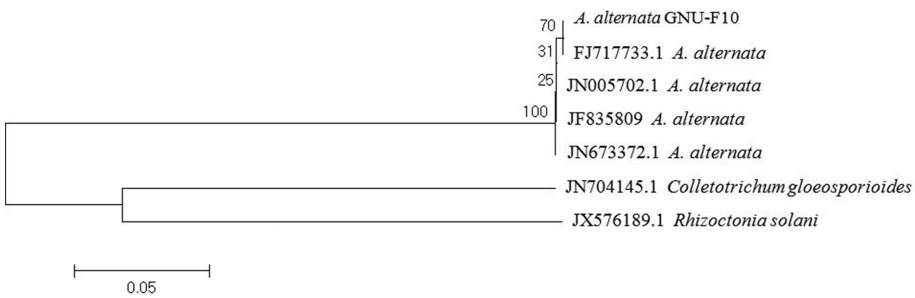

Fig. 2. Phylogenetic relationships of *Alternaria alternata* based on internal transcribed spacer rDNA sequences. Numerical values on branches are the bootstrap values as percentage of bootstrap replication from 1,000 replicate analyses. A phylogenetic tree was constructed using the MAGA 5 program and phylogenetic distances were calculated using the neighbor-joining method. Bar = 0.05 genetic distance between samples.

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REFERENCES

1. Kurt S, Soylu EM, Soylu S. First report of black spot disease caused by *Alternaria alternata* in persimmon fruits in Turkey. Plant Dis 2010;94:1069.
2. Kwon JH, Park CS. Sooty mold of persimmon (*Diospyros kaki*) caused by *Cladosporum cladosporioides*. Plant Pathol J 2003;19:266-8.
3. Palou L, Taberner V, Guardado A, Montesinos-Herrero C. First report of *Alternaria alternata* causing postharvest black spot of persimmon in Spain. Aust Plant Dis Notes 2012;7:41-2.
4. Graham J, Marshall B, Squire GR. Genetic differentiation over a spatial environmental gradient in wild *Rubus ideaus* populations. New Phytol 2003;157:667-75.
5. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315-22.
6. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596-9.
7. Ellis MB. Dematiaceous Hyphomycetes. Kew, Surrey: Commonwealth Mycological Institute; 1977.