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First Report of Foliar Blight on *Dendropanax morbifera* Caused by *Alternaria panax*

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Leaf spot and blight disease was observed on two-year-old seedlings of *Dendropanax morbifera* (Korean name: Hwangchil tree) during July of 2008 in Jindo Island, Korea. Symptoms included yellow-brown to dark brown irregularly enlarged spots frequently located along the veins of leaves. The lesions were often surrounded by chlorotic haloes. Severe leaf blight and subsequent defoliation occurred when conditions favored disease outbreak. The causal organism of the disease was identified as *Alternaria panax* based on morphological characteristics and sequence analysis of the internal transcribed spacer region of rDNA. *A. panax* isolates induced leaf spots and blight symptoms not only on *D. morbifera* but also on the other members of Araliaceae tested. This is the first report of foliar blight caused by *A. panax* on *D. morbifera*.

KEYWORDS : ***Alternaria panax,*** **Araliaceae,** ***Dendropanax morbifera*****, Identification, Pathogenicity**

Dendropanax morbifera Lev. is a subtropical, broad-leaved evergreen tree belonging to the family Araliaceae. Lacquer derived from *Dendropanax* has a golden color and is an excellent varnish for use in woodworking and metalworking [1]. The lacquer is called Hwangchil in Korea, Koshiabura in Japan, and Jin-qi or Huang-qi in China [2]. Parts of *D. morbifera* can also be used in folk medicine for the treatment of migraine headaches and dysmenorrhea [3]. Further, *D. morbifera* is endemic to Korea but is naturally found only in the southern part [4, 5]. It has also been cultivated on Jindo Island, Suncheon, and Wando Island in Korea.

Severe leaf spots and blight disease of *D. morbifera* resulting in defoliation was observed on two-year-old seedlings in Jindo nurseries during July of 2008. A species of *Alternaria* was repeatedly isolated from diseased leaves of the plant and was suspected as the causal agent. However, the etiology of the disease has not been previously reported. The study reported here was initiated to determine the etiology of the disease.

Materials and Methods

Isolation of the fungus. Diseased leaves of *Dendropanax morbifera* were obtained from two-year-old seedlings in Jindo nurseries during July of 2008. Leaves with necrotic spots and blight symptoms were cut, placed in Petri dishes with moist filter paper, and incubated for 2~3 days at 25°C to achieve sporulation of the causal organism. *Alternaria* isolates were obtained by single spore isolation from leaf spot symptoms and were deposited in the Culture Collection of Chungnam National University.

Morphological characteristics. Two isolates, CNU085017 and CNU085031, were used to determine morphology of the pathogen and were cultured on potato dextrose agar (PDA, Difco™, Detroit, MI, USA) for 3~5 days. Plugs (3 mm diameter) were taken from the edge of the colonies and transferred to PDA and V8 juice agar (V8A). After 7 days of incubation at 25°C, colony characteristics (color, growth rate, and pigment) were determined. Conidia produced on V8A (treated with 12/12 hr near-ultraviolet [NUV] light/darkness cycle) were mounted in lactophenol and measured using as light microscope (BX-50; Olympus, Tokyo, Japan) and Artray Artcam 300MI digital camera (Artray Inc., Tokyo, Japan). Identification of the fungus was conducted according to the description of Simmons [6, 7] and Yu [8].

DNA extraction, PCR amplification, and sequence analysis. The isolates were grown in potato dextrose broth for 5~7 days at 25°C. Mycelia were collected and freeze-dried for further extraction. Genomic DNA was extracted by the method described previously [9]. To identify the fungus, the internal transcribed spacer (ITS) region of rDNA was amplified using primers ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) [10]. PCR amplification of the ITS gene was performed in a 50-μL reaction mixture using a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The reaction was carried out with an initial denaturation for 3 min at 95°C, fol-

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lowed by 25 cycles of denaturation for 40 sec at 94°C, annealing for 1 min at 50°C, extension for 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were purified using a PCR Clean-up System purification kit (Promega, Madison, WI, USA) and sequenced using BigDye terminator cycle sequence kits (Applied Biosystems) with a ABI Prism 310 genetic analyzer (Applied Biosystems). The sequences of this fungus were compared with the ITS sequences of related *Alternaria* species available in the GenBank database by BLAST search. Sequences generated from the materials in this study and those retrieved from GenBank were initially aligned using the CLUSTAL X program [11], and the alignment was refined using PHYDIT program ver. 3.2 [12]. A neighbor-joining tree was reconstructed with Kimura's 2-parameter distance model method [13] using PHYLIP 3.57c package [14]. Bootstrap analysis using 1,000 replications was performed to assess the relative stability of the branches.

Pathogenicity tests. Inoculation experiments for the two isolates of *A. panax* (CNU085017 and CNU085031) were carried out with detached leaves of *D. morbifera* and two species of Araliaceae (*Aralia elata* and *Schefflera arboricola*). Leaves of the non-host plant *Glycine max* were used as controls. Inocula for the experiments were grown in 9-cm-diameter Petri dishes containing V8A for 14 days at 25°C under 12-hr photoperiods of NUV light. Sterile water (10 mL) was added to the plates, and the cultures were rubbed gently with a flamed glass rod. The resulting conidial suspensions were decanted into sterile test tubes and vigorously agitated with a vortex mixer for 30 sec before dilution and use. Young leaves were detached from each plant and surface-sterilized with 70% ethanol by gentle wiping. After the evaporation of ethanol, the leaves were inoculated at their center with 20 μL of conidial suspension (1×10^5 conidia/mL) and placed in plastic boxes with moistened paper towels. The boxes were sealed with parafilm and incubated at 22~25°C for 7 days and then observed for symptom development.

Results and Discussion

Symptoms. Initial symptoms on the leaves were observed as small, circular, and water-soaked spots. The spots were yellow-brown in the center with a broad dark brown border. The spots enlarged into lesions with irregular patterns frequently along the veins. A chlorotic halo often surrounded the lesions (Fig. 1A~1D). Severe leaf blight and subsequent defoliation occurred when the conditions favored disease outbreak.

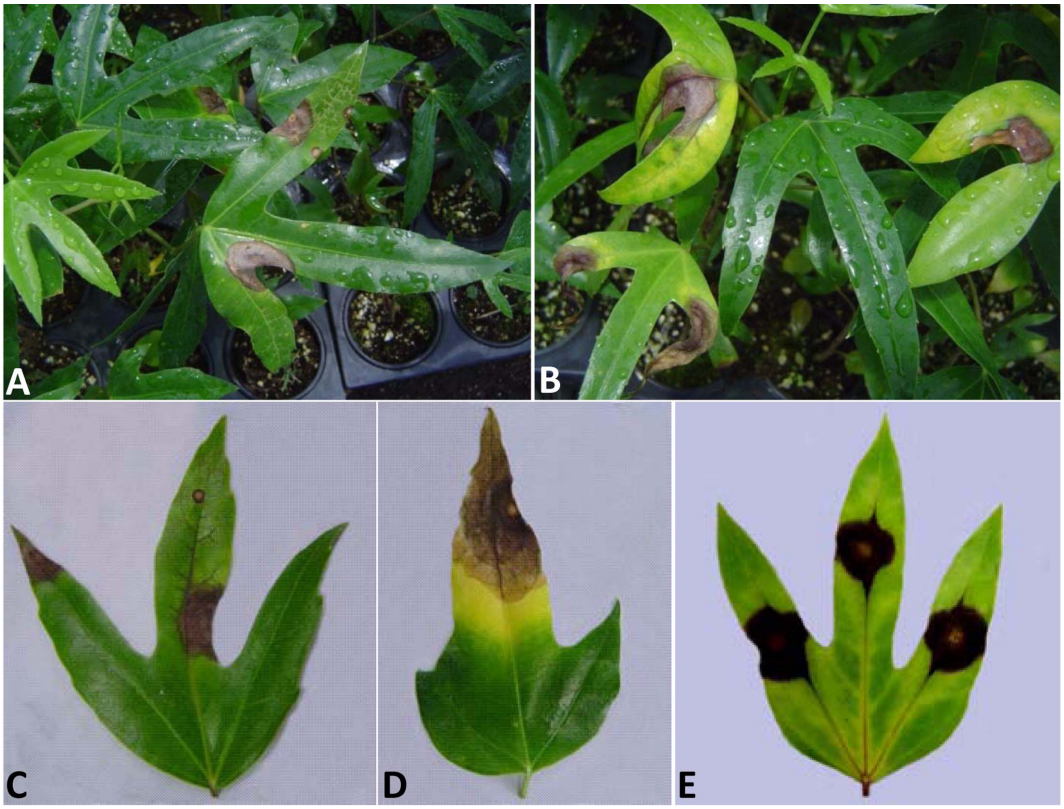

Fig. 1. Symptoms of foliar spots and blight on *Dendropanax morbifera* caused by *Alternaria panax*. A, B, Symptoms on naturally infected two-year-old seedlings; C, D, Lesions often advanced irregularly along with veins and were surrounded by a yellow halo; E, Symptoms on artificially induced leaves after 7 days of inoculation.

Morphological characteristics. Conidia of large-spored *Alternaria* sp. were abundantly produced on diseased leaves of *D. morbifera* incubated in moist chambers. Mostly large conidia with long beak were borne singly on the lesions, and others in chains of 2 to 3 formed in the moist chamber. Conidiophores were solitary or in clusters. The fungus can be isolated easily by selecting a single spore from the infected host material kept 2~3 days in a moist chamber.

Colonies were velvety, pale gray, 60~65 mm in diameter on V8A (Fig. 2A) and were cottony, white, 45~50 mm in diameter on PDA after 7 days at 25°C. The fungus produced diffusible yellow pigment in the PDA substrate.

Conidia sporulated abundantly on V8A under a 12/12 hr NUV light/darkness cycle (Fig. 2C and 2D). Conidiophores arose singly, terminally or laterally from hyphae, straight or curved, smooth-walled, septate, pale brown, usually with only one pigmented terminal conidiogenous site, slightly swollen at the apex, up to 150 μm long, 4~9 μm wide (Fig. 2E). Conidia solitary or 2~3 in small chains, straight or slightly curved, long elliptical or obclavate, golden-brown to dark brown in color, smooth-walled when juvenile, becoming verruculose to verrucose, excessive cellular swelling causing the conidium to be distorted, with 5~11 transverse septa and 1 to several longitudinal or oblique septa, slightly or strongly constricted at the transverse septa, conidial body 70~120 × 15~30 (~45) μm. Some conidia had a beak (rostrate), some had a secondary conidiophore (pseudorostrate). Rostra almost cylindrical, rigid, simple, and unbranched, blunt-tipped, concolorous with conidium body or slightly lighter than the body, variable in length up to 83 μm, 3~7 μm wide. Pseudorostra cylindrical, simple, as short as 20 μm or as long as the spore body, 4~9 μm wide. These morphological characteristics agreed well with the *Alternaria panax* Whetzel as described by Simmons [6, 7] and Yu [8] (Table 1).

Phylogenetic analysis. The ITS region sequences of CNU085017 and CNU085031 isolates of *A. panax* were

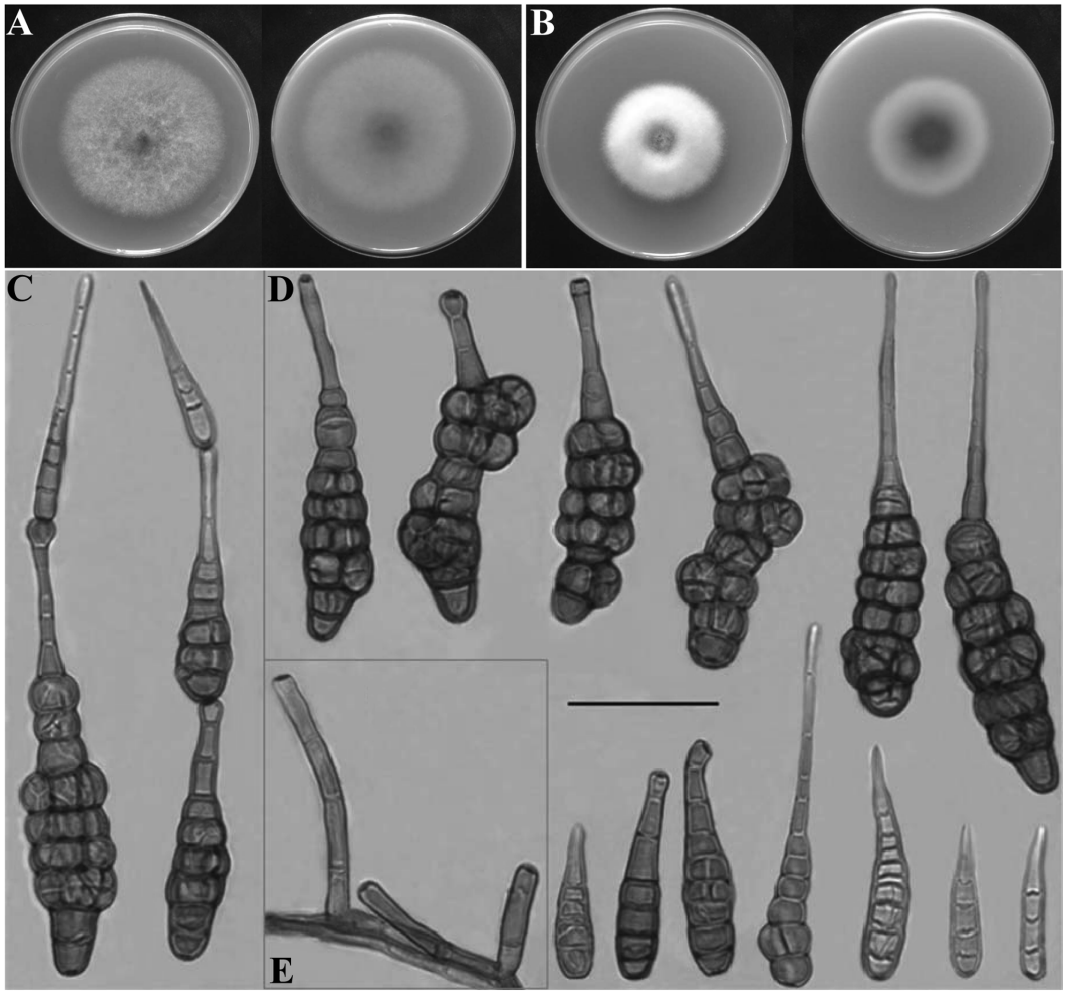

Fig. 2. Morphology of *Alternaria panax* from *Dendropanax morbifera*. Colonies on V8 juice agar (V8A) (A) and potato dextrose agar (B) after 7 days of incubation at 25°C; Conidia (C, D) and conidiophores (E) of the fungus produced on V8A (scale bar = 50 μm).

Table 1. Comparison of morphological characteristics of *Alternaria* isolates from *Dendropanax morbifera* and previously described *Alternaria panax*

Characteristics	Present isolate	*Alternaria panax* Whetzel	
		Simmons [7]	Yu [8]
Colony			
Color	White to pale gray	-	White to pale gray
Size	64 mm on V8A 47 mm on PDA	60 mm on V8A	45~55 mm on the media
Pigment	Yellow pigment in PDA substrate	-	Red pigment in PDA substrate
Conidia			
Shape	Long-elliptical or obclavate	Long elliptical to long obclavate	Long-elliptical, obclavate to broadly obclavate
Size (μm)	Well developed conidial body: 70~120 × 15~30 (~45); conidial beak: 40~90	Well-developed conidia on natural substrate: 150~160 × 12~20; conidial beak: 80~90	Conidial body: 35~90 × 10~25 (~40); conidial beak: up to 100 long
No. of septa	5~11	9~11	3~11

PDA, potato dextrose agar.

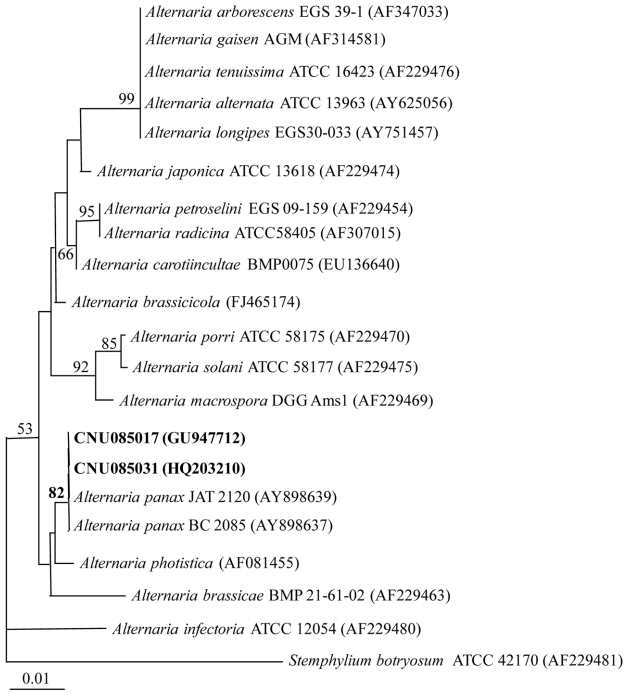

Fig. 3. Neighbor-joining tree based on sequence analysis of the internal transcribed spacer (ITS) region (ITS5/ITS4). Numbers close to the branch indicate bootstrap values obtained from 1,000 bootstrap replicates. Bar indicates the numbers of nucleotide substitutions per site. Accession numbers of GenBank are provided in parentheses.

deposited in GenBank (accession No. GU947712 and HQ203210). In a phylogenetic analysis using the neighbor-joining method, sequences of CNU085017 and CNU085031 isolates were found to be 100% identical to those of reference strains *A. panax* JAT 2120 and BC 2085 (GenBank accession No. AY898639 and AY898637), supported by a high bootstrap value (82%) (Fig. 3). They were also closely related to *A. photistica* (AF081455), as previously reported by Quayyum *et al*. [15]. The present phylogenetic analysis along with the morphological characteristics indicates that the causal fungus was *A. panax*.

Table 2. Pathogenicity of *Alternaria panax* on detached leaves of three species of Araliaceae and *Glycine max*

Test plants	Isolate of *A. panax*	
	CNU085017	CNU085031
Aralia elata	+[a]	+
Dendropanax morbifera	+	+
Schefflera arboricola	+	+
Glycine max	−	−

−, no symptoms developed; +, symptoms developed (lesion diameter > 15 mm).
[a]Lesion was observed at 7 days after inoculation.

Pathogenecity. The two isolates of the fungus were pathogenic to *D. morbifera, Aralia elata*, and *Schefflera arboricola* but not to *G. max* (Table 2). No significant differences were observed in pathogenicity between the two isolates, and the pathogen was frequently reisolated from the infected leaves. Symptoms appeared on *D. morbifera* leaves after 2~3 days of inoculation as small, dark, water-soaked spots. The spots enlarged into irregular lesions (Fig. 1E). These symptoms were identical to those observed on naturally infected plants.

To date, eleven hosts of *A. panax* have been reported [6-8, 16-18], and all are members of the Araliaceae family. These results provide the first documented evidence that *D. morbifera* is a new host of *A. panax*.

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References

1. Kim SH, Jang YS, Han JG, Chung HG, Lee SW, Cho KJ. Genetic variation and population structure of *Dendropanax morbifera* Lev. (Araliaceae) in Korea. Silvae Genet 2006; 55:7-13.
2. Lee BH, Choi JH, Kim HJ. Curing and thermal behaviors of Korean *Dendropanax* Lacquer made by acetone and wine spirit extraction methods. Prog Org Coatings 2005;52:241-5.
3. Bae KH. The medicinal plants of Korea. Seoul: Kyo-Hak Publishing Co Ltd.; 2000.
4. Han SH, Jung YH, Ko MH, Oh YS, Koh SC, Kim MH, Oh MY. Phytogenetic relationships of the *Dendropanax morbifera* and *D. trifidus* based on PCR-RAPD. Korean J Genet 1998;20:173-81.
5. Kim SH. Ecology and superior tree selection of *Dendropanax morbifera* Lev [dissertation]. Jinju: Gyeongsang National University; 1998.
6. Simmons EG. *Alternaria*: themes and variations. Mycotaxon 1982;14:17-43.
7. Simmons EG. *Alternaria*: an identification manual. Utrecht: CBS Fungal Biodiversity Centre; 2007. p. 38.
8. Yu SH. Korean species of *Alternaria* and *Stemphylium*. Suwon: National Institute of Agricultural Science and Technology; 2001. p. 100-6.
9. Park MS, Seo GS, Bae KS, Yu SH. Characterization of *Trichoderma* spp. associated with green mold of oyster mushroom by PCR-RFLP and sequence analysis of ITS regions of rDNA. Plant Pathol J 2005;21:229-36.
10. 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. San Diego: Academic Press; 1990. p. 315-22.
11. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876-82.
12. Chun J. Computer-assisted classification and identification of actinomycetes (Dissertation). Newcastle Upon Tyne (UK): University of Newcastle; 1995.
13. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
14. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783-91.
15. Quayyum HA, Dobinson KF, Traquair JA. Conidial morphology, virulence, molecular characterization, and host-parasite interactions of selected *Alternaria panax* isolates on American ginseng. Can J Bot 2005;83:1133-43.
16. Atilano RA. A foliar blight of Ming Aralia caused by *Alternaria panax*. Plant Dis 1983;67:224-6.
17. Forsberg LI. *Alternaria panax* on umbrella tree. Australas Plant Pathol 1983;12:7.
18. Garibaldi A, Gilardi G, Gullino ML. First report of *Alternaria* leaf blight of *Aralia japonica* caused by *Alternaria panax* in Europe. Plant Dis 2004;88:82.