First Report of Leaf Spot of *Datura metel* Caused by *Alternaria tenuissima* in Korea

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In June 2013, we collected leaf spot disease samples of *Datura metel* from Gangneung, Gangwon Province, Korea. The symptoms observed were small circular to oval dark brown spots with irregular in shape or remained circular with concentric rings. We isolated the pathogen from infected leaves and cultured the fungus on potato dextrose agar. We examined the fungus morphologically and 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), glycerol-3-phosphate dehydrogenase (G3PDH) and the RNA polymerase II second largest subunit (RPB2) gene sequence revealed that the causal agent was *Alternaria tenuissima*. To the best of our knowledge, this is the first report of leaf spot of *D. metel* caused by *A. tenuissima* in Korea as well as worldwide. 

**Keywords** : *Alternaria tenuissima*, Devil’s trumpet, Leaf spot, Pathogenicity

*Datura metel* L. (devil’s trumpet) is a small perennials, usually grown as an annuals herbaceous plant with white flowers, belonging to the family Solanaceae and it is considered as one of the most important medicinal plants throughout the world. The main active constituents of the plant are the medicinally important tropane alkaloids such as hyoscyamine and scopolamine (Cusidó et al., 1999). Due to the presence of bioactive compounds, the plant was widely used in traditional medicine to cure diseases such as asthma, cough, wound treatment, convulsion, headache, insanity, hemorrhoids, and rheumatism (Ali et al., 2004; Dabur et al., 2004). Aqueous and alcoholic extract of the plant possesses good antibacterial (Siva et al., 2011), antifungal (Kagalea et al., 2014), nematocidal (Moosavi, 2012), antitumor (Islam et al., 2008) and anticancer (Nazeema et al., 2014) activities also. Asia as the geographic origin of *D. metel* and recognized its distribution in Asia, Africa, and the tropical and subtropical regions of America (Fuentes, 1980; Satina and Avery, 1959).

In June 2013, serious leaf spots symptoms were observed in *D. metel* plants in Gangneung, Gangwon Province, Korea. The symptoms observed were small circular to oval dark brown spots with an average diameter of 1–5 μm (Fig. 1A). The spots gradually enlarged in size and later became irregular in shape or remained circular with concentric rings (Fig. 1B). To identify the causative agent associated with leaf spot observed on *D. metel*, based on mycological characteristics, molecular phylogenetics, and pathogenicity.

For pathogen isolation, small pieces of infected leaves were sterilized by immersion in 0.1% sodium hypochlorite (NaOCl) for 1 min, rinsed three times with sterile distilled water, and incubated on potato dextrose agar (PDA; Difco, USA) for 5 days at 20±2°C in dark. Afterwards, they were constantly exposed to the fluorescent light for 2 days. The fungus produced gray colonies (65–70 mm diameter) with olive green peripheries and cottony mycelium (Fig. 1D and E). The conidiophores (n=20) were branched, straight measuring 23.7–40.2 μm long and 3.6–5.0 μm thick (Fig. 1H). Short conidia chains consist of 3–7 or more conidia, occasionally (uncommonly) branched (Fig. 1G) were found. The conidia (n=50) were dark brown in color and size of conidia varied from 12.5–48.0 μm in length and 8.1–14.4 μm in width with short taper beak (1.6 to 3.2 μm) or no beak was observed (Fig. 1F). Horizontal and vertical septa of conidia varied from 1–6 and 0–2, respectively. Under light regime, conidia were germinated (Fig. 1I). A representative isolate (A003) was deposited in Gangneung-Wonju National University and used for further studies. Morphological and cultural characters of the isolate were deposited in Gangneung-Wonju National University.
were consistent with those of *A. tenuissima* (Simmons, 2007; Yu, 2001).

Molecular characteristics of the studied isolate were determined by DNA extraction from 100 mg mycelia using Plant DNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA). ITS1 and ITS4 primers (White *et al.*, 1990) was used to amplify the internal transcribed spacer region (ITS) region containing ITS1-5.8S-ITS2 of nuclear ribosomal DNA (rDNA), and glycerol-3-phosphate dehydrogenase (G3PDH) (Berbee *et al.*, 1999), the RNA polymerase II second largest subunit (RPB2) (Liu *et al.*, 1999) two nuclear protein-coding genes were sequenced. PCR was performed in a total volume of 25 μl by using 0.5 μl of dNTP, 2.5 units of Taq DNA polymerase (0.5 μl of 5 U/μl enzyme; Bioneer, Daejeon, Korea), 2.0 μl of genomic DNA, 2.5 μl of 10× PCR reaction buffer and 5 pmol/l of each primer (0.5 μl each). The reaction was performed in Eppendorf Mastercycler Gradient (Eppendorf, Germany). The PCR amplification conditions were as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 35 sec, 52°C for 35 sec, and 72°C for 1 min, with a final extension step at 72°C for 10 min (for ITS). For RPB2 gene fragments, 94°C for 5 min (1 cycle); 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s (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. DNA was purified using QIAquick PCR Purification Kit (Qiagen) following the instructions of the producer. Sequence was performed by Bioneer Sequencing Service (Bioneer, Daejeon, Korea) with the same primers used for the PCR amplifications. The resulting 540 bp ITS, 602 bp G3PDH and 733 bp RPB2 gene nucleotide sequence were deposited in GeneBank with accession number KP731980, KT955743 and KT955744, respectively. The obtained nucleotide sequences were searched by using BLASTn available from the

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**Fig. 1.** Leaf spot disease of *Datura metel* caused by *Alternaria tenuissima*. A: Symptoms on leaves, B: Close-up view of a diseased leaf, C: Symptoms on artificially induced leaves 15 days after inoculation, D and E: One-week-old colony on PDA, F: Conidia, G: Conidial chain, H: Conidiophore, I: Germinating conidium. Scale bars: 20 μm (F, H and I), 40 μm (G).
GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences identified based on ITS, G3PDH and RPB2 gene alignment were 100% similar to *A. tenuissima* species (AF347032, AY278809, KC584435). For phylogenetic analysis, combined ITS, G3PDH and RPB2 gene sequence of some species belongs to the *Alternaria* were retrieved from GeneBank. *Pleospora herbarum* was used as an outgroup taxon. Maximum parsimony trees were constructed for the combined datasets of ITS, G3PDH and RPB2 gene sequences using MEGA6 (Tamura et al., 2013) program. Bootstrap analysis using 1000 replications was performed to assess the relative stability of the branches. The phylogenetic relationship using combined ITS, G3PDH and RPB2 gene sequence showed that one isolate clustered with *A. tenuissima*, distinct from other species in the *Alternaria* in a maximum parsimony trees (Fig. 2).

To determine the fungal pathogenicity, inoculum was prepared by harvesting conidia from 2-week-old cultures on PDA. A conidial suspension (5 \times 10^5 conidia/ml) was sprayed onto healthy leaves of three potted *D. metel* plants. Another three potted plants were sprayed with sterilized water, serving as controls. After inoculation, plants were transfer into humid chamber for 2 days (25°C and 80–100% RH) and placed in the greenhouse. The first foliar lesions developed on leaves 15 days after inoculation (Fig. 1C), whereas control plants remained symptomless. The pathogenicity test was carried out twice with similar results. The pathogen was successfully re-isolated from inoculated leaves, fulfilling Koch’s postulates.

*A. tenuissima* species are important plant pathogens. The leaf spot disease has been reported on *D. metel* in the USA by *A. crassa* and *A. solani* (Farr and Rossman, 2015). A variety of crops other than the *D. metel* are infected with *A. tenuissima*, including eggplant leaf spot in Malaysia (Nasehi et al., 2012), and potato leaf blight in China (Zheng and Wu, 2013), pepper leaf spot and fruit rot in China (Li et al., 2011), and blueberry leaf spot in Australia (You et al., 2014). However, this is the first report that *A. tenuissima* is a causal pathogen of the leaf spot disease of *D. metel* in Korea as well as worldwide.

### References

Ali, A. N., Ali, N., Al-rahwil, K. and Lindequeist, U. 2004. Some medicinal plants used in Yemeni herbal medicine to treat Malaria. *Afr. J. Tradit. Compl. Med.* 1: 72–76.

Berbee, M. L., Pirseyedi, M. and Hubbard, S. 1999. Cochliobolus phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehydes-3-phosphate dehydrogenase gene sequences. *Mycologia* 91: 964–977.

Cusidó, R. M., Palazón, J., Piñol, M., Bonfill, M. and Morales, C. 1999. *Datura metel*: in vitro production of tropane alkaloids. *Planta Medica* 65: 144-148.

Dabur, R., Ali, M., Singh, H., Gupta, J. and Sharma, G. 2004. A novel antifungal pyrrole derivative from *Datura metel* leaves. *Pharmazie* 59: 568–570.

Farr, D. F. and Rossman, A. Y. 2015. Fungal databases. Systematic Mycology & Microbiology Laboratory, ARS, USDA, Retrieved June 22, 2015, from http://nt.arsgrin.gov/fungaldatabases/

Fuentes, V. 1980. Solanaceas de Cuba I *Datura L.* *Rev. Jard. Bot. Nat. Cuba* 1: 61–81.

Islam, M. S., Rahman, M. M., Alam, M. J., Nurunnahar, Parvez, M. S.,
Anisuzzaman, M. and Alam, M. F. 2008. Screening of antitumor activity of *Datura metel* L. using potato disc bioassay. *Plant Environ. Dev.* 2: 87–92.

Kagalea, S., Marimuthua, T., Thayumanavanb, B., Nandakumara, R. and Samiyappana, R. 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae pv. oryzae*. *Physiol. Mol. Plant Pathol.* 65: 91–100.

Li, Y., Zhang, D., Xu, W., Wu, Z., Guo, M. and Cao, A. 2011. *Alternaria tenuissima* causing leaf spot and fruit rot on pepper (*Capsicum annuum*): first report in China. *New Dis. Rep.* 24: 3.

Liu, Y. J., Whelen, S. and Hall, B. D. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol. Biol. Evol.* 16: 1799–1808.

Moosavi, M. R. 2012. Nematicidal effect of some herbal powders and their aqueous extracts against *Meloidogyne javanica*. *Nematropica* 42: 48–56.

Nasehi, A., Kadir, J. B., Abidin, M. A. Z., Wong, M. Y. and Mahmodi, F. 2012. First report of *Alternaria tenuissima* causing leaf spot on eggplant in Malaysia. *Plant Dis.* 96: 1226.

Nazeema, B. B., Julie, J., Abirami, J., Kumareasan, R., Muthukumaran, T., Rajasree, S., Jeya, J. K. and Kumaran, S. 2014. Anti-cancer activity of *Datura metel* on MCF-7 cell line. *Asian J. Pharmaceut. Clinic. Res.* 7: 181–183.

Satina, S. and Avery, A. G. 1959. A review of the taxonomy history of Datura. In: Blakeslee: the Genus *Datura*, eds. by A. G. Avery, S. Satina and J. Rietsema, pp. 16–47. Ronald Press, New York.

Simmons, E. G. 2007. *Alternaria*: An identification manual. CBS Fungal Biodiversity Centre, Utrecht, the Netherlands, 775 pp.

Siva, S. S., Saranraj, P. and Geetha, M. 2011. Antibacterial evaluation and phytochemical screening of *Datura metel* leaf extracts against bacterial pathogens. *Int. J. Pharm. Bio. Arch.* 2: 1130–1136.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30: 2725–2729.

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, eds. by M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, pp. 315–322. Academic Press Inc, New York, USA.

You, M. P., Lanoiselet, V., Wang, C. P. and Barbetti, M. J. 2014. First report of *Alternaria* leaf spot caused by *Alternaria tenuissima* on blueberry (*Vaccinium corymbosum*) in Western Australia. *Plant Dis.* 98: 423.

Yu, S. H. 2001. Korean species of *Alternaria* and *Stemphylium*. National Institute of Agricultural Science and Technology, Suwon, pp. 100–106. (In Korean)

Zheng, H. H. and Wu, X. H. 2013. First report of *Alternaria* blight of potato caused by *Alternaria tenuissima* in China. *Plant Dis.* 97: 1246.