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RESEARCH NOTE

New Record of Anthracnose Caused by *Colletotrichum liriopes* on Broadleaf Liriope in Korea

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Abstract

In 2015, the leaves of broadleaf liriope, *Liriope muscari*, showing anthracnose symptoms were found in a field of Korea. The fungus was isolated and identified as *Colletotrichum liriopes* based on the morphological characteristics and nucleotide sequence data of the glyceraldehyde-3-phosphate dehydrogenase, internal transcribed spacer and actin genes. To the best of our knowledge, this is the first report of *C. liriopes* isolated from *L. muscari* in Korea.

Keywords: Actin, *Colletotrichum liriopes*, Glyceraldehyde-3-phosphate dehydrogenase, Internal transcribed spacer, *Liriope muscari*

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Liriope muscari, also called “Maekmundong” in Korea, is an evergreen perennial herb, which grows in the mountainous regions of Korea[1]. Its tuberous roots are used as a traditional medicine in Korea[1]. Several fungi, such as *Colletotrichum liliacearum* (anthracnose), *Glomerella cingulata* (anthracnose), *Puccinia iwakuniensis* (rust), and *Stenellopsis liriopes* (red leaf spot), have been known to infect *L. muscari* in Korea[1-3]. Among these, anthracnose is an economically important disease of *L. muscari*. The genus *Colletotrichum* is known to occur worldwide, and has been described as both plant pathogen and saprophyte, causing anthracnose disease in more than ca. 200 plant species including crops, weeds, and trees[4, 5]. In the summer of 2015, a suspected anthracnose disease was observed in Maekmundong plants in a field of Chungcheongnam-do (Cheongyang), Korea. The symptoms were characterized by small patches with reddish brown speckles on leaves at an early stage of infection, followed by the subsequent spread and irregular enlargement of spotted lesions with dark brown discoloration and coalescence of spots throughout the leaves, resulting in black acervuli at a later stage.

Fresh leaf specimens were collected from the infected plants in the field. The leaves showing the typical disease symptoms were cut into small fragments (5 mm), which were surface-sterilized by dipping in 1% NaOCl (sodium hypochlorite) for 3 min and rinsed thrice with sterilized distilled water[6]. After drying, the surface-sterilized samples were placed onto the 90 mm petri dishes containing blotter paper and incubated at 25 ± 2°C in a

growth chamber with a 12 hr light/dark photoperiod. The petri dishes were monitored every day to check the formation of spore layer or cluster. After 2 days, the spore layers were isolated using an autoclaved toothpick or glass stick, and were mixed with distilled water containing streptomycin (300 ppm) in tubes. The spore suspensions were then spread on water agar media containing streptomycin (300 ppm)[7]. After incubating the culture for 3 days at 25°C, a single hyphal tip of the emerging fungus was transferred to a new plate containing potato dextrose agar (PDA; Difco, Detroit, MI, USA) to obtain a pure culture.

Pathogenicity of the isolated organism was confirmed on healthy leaves by the pin-prick method[8]. A spore suspension of a 7-day-old culture grown on V8 juice media at 25°C under the black light was prepared in sterilized distilled water. The pinpricked leaves were spray-inoculated with spore suspension (10^6 conidia/mL) of the pathogen. Leaves sprayed with sterilized distilled water served as a control. After 7 days of inoculation, reddish brown speckles similar to those observed in the field were developed on the inoculated leaves; however, no symptoms were observed on the control leaves (Fig. 1A, 1B). The pathogenicity tests were performed in 3 replicates, and similar results were obtained. The causal fungus was re-isolated from the inoculated leaves and compared with the original pathogen to satisfy Koch's postulates in each test[9].

For morphological observation, a small portion of the mycelium was removed from the fungal culture and mounted in a drop of lacto phenol[10]. On PDA, dark brown colonies of the pathogen with abundant setae and irregularly shaped black sclerotia were observed (Fig. 1C, 1D). After 7 days, hyphae grew on PDA with a growth rate of 55.0~60.0 μm at 25°C. Colonies growing on V8 juice agar media were pale smoke-grey on the front side and clearly pale yellow on the reverse side (Fig. 1E, 1F). Conidia were fusiform, falcate, colorless, smooth-walled, tapered gradually to each end, and 21.5~27.0 × 3.0~4.0 μm in size (Fig. 1G, 1H). The acervuli on necrotic lesions were dark brown and 75.0~150.0 μm in size. Setae abundant, septate (2~3), rigid, brown to dark brown, 50~90 μm long, base conical to slightly inflated, 3.5~7 μm in diameter, with acute tip (Fig. 1I). Appressoria were dark brown, aseptate, smooth-walled, elliptical-to-circular or irregularly shaped, and 8.5~13.0 × 6.0~8.5 μm in size (Fig. 1J). On the basis of the above-mentioned morphological characteristics, the isolated fungus matched well with the description of *C. liriopes* (Table 1)[9].

To validate the molecular identification of the isolated fungus, the genomic DNA was extracted from the fungal mycelia using Cenis's method[11]. For amplification of the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, internal transcribed spacer (ITS) rDNA regions, and actin (ACT) gene were amplified using the primers GDF and GDR[12], ITS1 and ITS4[13] and ACT512-F and ACT783-R[14], respectively. PCR amplifications were performed under the conditions described by Prihastuti et al. (2009)[7]. The PCR products were purified and directly sequenced with the same primers. The obtained sequence data was compared with all fungal sequences available in the NCBI GenBank database using the basic local alignment search tool (BLAST) program[15]. Compared with the sequence of *C. liriopes* strain CBS119444 in GenBank[16], the amplification products showed 99% homology with the GAPDH (GenBank No. GU228196),

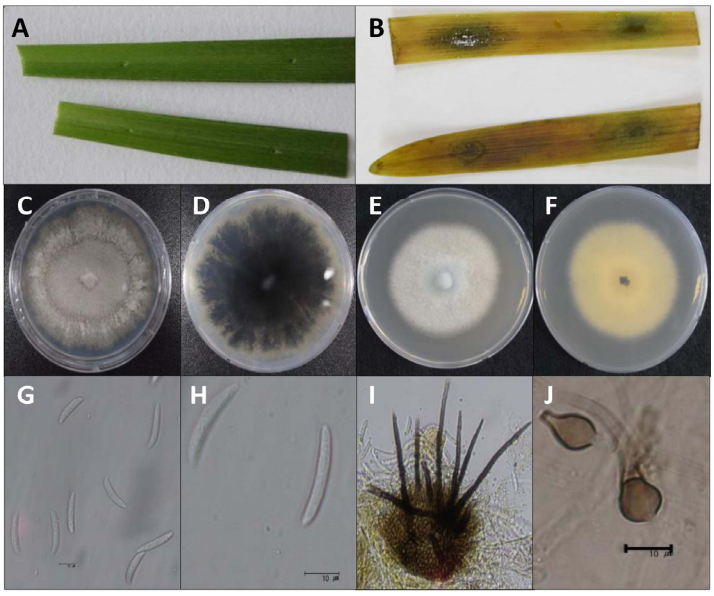

Fig. 1. Anthracnose disease caused by *Colletotrichum liriopes* on broadleaf liriope (*Liriope muscari*) leaves. A, Pathogenicity of the causal fungus of anthracnose on liriope leaves 7 days after inoculation. No symptoms on control leaves; B, Symptoms produced on inoculated leaves. The pathogenicity tests were repeated 3 times with similar results; C, D, Cultural and morphological characteristics of *C. liriopes* on *L. muscari*. Fungal colony on potato dextrose agar plates (C, front view; D, back view). E, F, Fungal colony on V8 juice plates (E, front view; F, back view). G, H, Round to slightly acute apex and truncate base conidia; I, Acervuli and dark brown setae; J, Clavate to circular or irregular shaped appressoria (scale bars: G, H, J = 10 μm).

Table 1. Comparison of the morphological characteristics of the study isolate with respect to the previously reported *Colletotrichum liriopes*

Characteristics	Study isolate *C. liriopes* CNU 151101	*Colletotrichum liriopes*[a]
Conidia		
Shape	falcate, fusiform, single cell	falcate, fusiform
Color	hyaline, colorless	colorless
Size	21.5~27 × 3~4.0 μm	21~26 × 2~3 μm
Setae		
Shape	rigid, acute tip, conical base, septate (2~3)	not described
Color	brown to dark brown	
Size	50~90 × 3.5~7 μm	
Appressoria		
Shape	elliptical to circular or irregularly shaped	clavate to circular
Color	dark brown	dark brown
Size	8.5~13 × 6~8.5 μm	8~12 × 6~8 μm
Acervuli		
Color	pale brown to dark brown	not described
Size	75~150 μm	

[a]Sources of description and illustration[9].

ITS (GenBank No. GU227804) and ACT (GenBank No. GU227902) sequences. The nucleotide sequence of GAPDH has been deposited in NCBI GenBank under the accession no. CNU151101 (GenBank no. KX545212). Nine reference sequences of *Colletotrichum* spp. were taken from the GenBank database to figure out the phylogenetic relationship. These sequences were manually aligned with the closely related strains using the ClustalW2 program (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_ phylogeny/). The reliability of the tree was evaluated with 1,000 bootstrap replications for branch stability. Phylogenetic tree (Fig. 2) was constructed by the distance-based neighbor-joining (NJ) method using the MEGA7.0 software[17]. Accordingly, the molecular analysis confirmed the morphological identification of the fungal pathogen. Therefore, on the basis of the observed symptoms, pathogenicity, morphology, and molecular characterization, this fungus was identified as *C. liriopes*[16].

This species has been known not only from the two duplicate strains isolated from *L. muscari* in Mexico, but also the result of a quarantine interception in Houston, USA[16], while it has been found on *Rohdea japonica* in Korea[9]. Generally, *C. liriopes* is similar to *C. liliacearum* in its morphological characteristics[1]. However, no detailed information about the gene sequence of *C. liliacearum* is available in the GenBank database, although the fungus was recorded as a pathogen of *L. muscari* (big blue lilyturf) in Korea[1]. Moreover, previous reports suggested that *C. liliacearum* and *C. lilii* are indeed identical, with the former being the non-pathogenic form and the latter the pathogenic form[18-21]. Based on the combined dataset of ITS, GAPDH, and ACT gene sequences, *C. liriopes* and *C. lilii* formed two different groups, with a signifiant genetic divergence among strains (Fig. 2). Therefore, there have been no reports of the occurrence of the anthracnose disease caused by *C. liriopes* on *L. muscari* in Korea. To the best of our knowledge, this is the first report of anthracnose caused by *C. liriopes* in *L. muscari* in Korea.

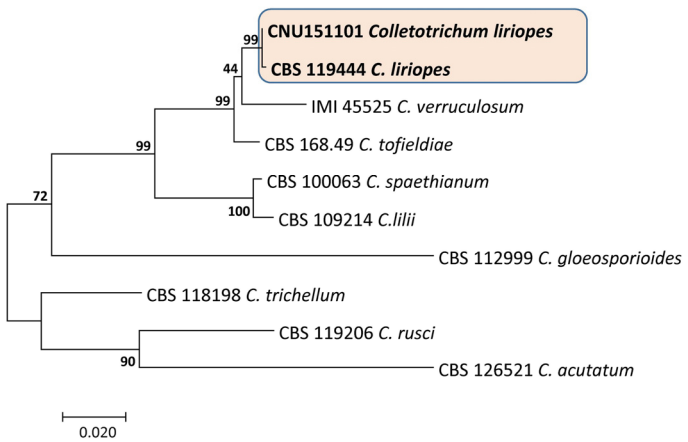

Fig. 2. Phylogenetic tree generated by a maximum parsimony analysis of a combined dataset of internal transcribed spacer, glyceraldehyde-3-phosphate dehydrogenase, and actin gene sequences of *Colletotrichum liriopes* and other *Colletotrichum* spp. obtained from the GenBank database. The numbers beside each branch represent the bootstrap values obtained after a bootstrap test with 1,000 replications. The fungal strain identified in this study is shown in boldface. Bar indicates the number of nucleotide substitution.

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