RESEARCH NOTE

Occurrence of *Phytopythium vexans* Causing Stem Rot on *Anthurium andraeanum* in Korea

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ABSTRACT

In 2017, wilting symptom was observed on seedlings of *Anthurium andraeranum* in Youngin, Korea. Brownish lesions with water soaking were developed on the stems and roots of the infected plants. The stems and leaves wilted and finally died. One fungal isolate was obtained in pure culture. Morphological features and nucleotide sequences of internal transcribed spacer rDNA and cytochrome oxidase subunit II mt DNA were analyzed. The results of this study indicated that the fungus is identified as *Phytopythium vexans*. Pathogenicity tests showed the isolate was pathogenic to the seedlings of *A. andraeanum*. To our knowledge, this is the first report of *P. vexans* causing stem rot on *A. andraeanum* in Korea.

Keywords: *Anthurium andraeanum*, cytochrome oxidase subunit 2, *Phytopythium vexans*, Stem rot

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Anthurium andraeanum Linden (also called flamingo flower or flamingo lily) is grown for potted and cut-flower plant worldwide because of its attractive spathe and foliage. In September 2017, wilting symptoms appeared on seedlings of *A. andraeanum* in a farm located in Yongin, Korea (37°05′57.6″N, 127°08′26.3″E). The lower parts of the affected stems developed water-soaked appearance and brown discoloration which later extended upward into leaves. (Fig. 1A, 1B, 1C). Under moist conditions, white mycelium was found around the infected stems. The severely diseased leaves wilted, collapsed, dried, and finally died. These symptoms occurred on approximately 5% of seedlings.

A fungal isolate was obtained from the diseased plant tissues by single hyphal tip isolation. For isolation of the fungus, small size of plant tissue was surface-disinfected with 70% ethanol and 1% sodium hypochlorite, and then placed on potato dextrose agar (PDA) for cultivation. The colony on PDA grew slowly, producing white fluffy aerial mycelium, with an irregular margin, reaching 80 mm diam. after 4 weeks (Fig. 1E). The culture was deposited in the Korean Agricultural Culture Collection (accession number KACC48403). To observe morphological characteristics, the fungus was grown on water agar containing sterile grass blade for 7 days. Primary hyphae were up to 5.5 μm in width, and hyphal swellings were not observed. Sporangia

were papillate or non-papillate, globose to subglobose, terminal, 33-38 μm long × 20-28 μm wide (Fig. 1F, 1G). Encysted zoospores were 9-11 μm in diameter (Fig. 1H). The culture seems to be sterile as the sexual organs, oogonia and antheridia, were not produced on media. The morphological features are insufficient to identify the fungal species. Thus, the identity of the pathogen was confirmed by the data from the molecular sequence analysis.

Fig. 1. Characteristics of stem rot caused by *Phytopythium vexans* on *Anthurium andraeanum*. A, Diseased seedlings with wilting symptom; B, Lower parts of affected plants showing lesions of brown discoloration and rotting; C, Discolored vascular tissues; D, Inoculated plant(left) showing wilting symptom and uninoculated plant(right) showing no visible symptoms in pathogenicity test; E, Colony grown on PDA after incubation for 4 weeks; F, Subglobose sporangium with papilla; G, Globose sporangium; H, Zoospores. (scale bars: F-H = 10 μm).

Genomic DNA was extracted from fungal mycelia using DNeasy Plant Mini Kit (Qiagen, Valencia, USA) according to the manufacturer's protocol. The internal transcribed spacer (ITS) of ribosomal DNA region was amplified using the primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [1]. The cytochrome oxidase subunit II (*cox*2) mt DNA was amplified using the primers Cox2-F (5'-GGC AAA TGG GTT TTC AAG ATC C-3') and Cox2-RC4 (5'-TGA TTW AYN CCA CAA ATT TCR CTA CAT TG-3') [2]. The PCR products were sequenced by BIOFACT (Daejeon, Korea). The resulting sequences were edited using SeqMan program (DNASTAR Lasergene, Madison, WI, USA) and submitted to GenBank (accession number MH478301 for ITS, MH492367 for *cox*2). The NCBI BLASTn searches of the present sequences showed 100% identity with *cox*2 sequence (NBRC100105) and 99% with ITS sequence (CBS119.80) of *Phytopythium vexans*. The

phylogenetic relationship was inferred from the *cox*2 sequences for *P. vexans* and related species belonging to three allied genera, *Phytopythium*, *Pythium*, and *Phytophthora*. The reference sequences were retrieved from GenBank. *Saprolegnia parasitica* (CBS 127041) was used as an outgroup. A neighbor-joining tree was constructed by MEGA7 [3]. In the phylogenetic tree, the present isolate formed a well-supported clade with the reference isolates of *P. vexans* (bootstrap value 100%) (Fig. 2). *Phytopythium* species including *P. vexans*, formerly classified under the genus *Pythium*, were phylogenetically distinct from the current species of the genus *Pythium* (Fig. 2). The genus *Phytopythium* was newly established for *Pythium* species showing combined characteristics of both *Pythium* and *Phytophthora* [4, 5]. The result of molecular phylogeny indicates that the present isolate is identified as *P. vexans* [4].

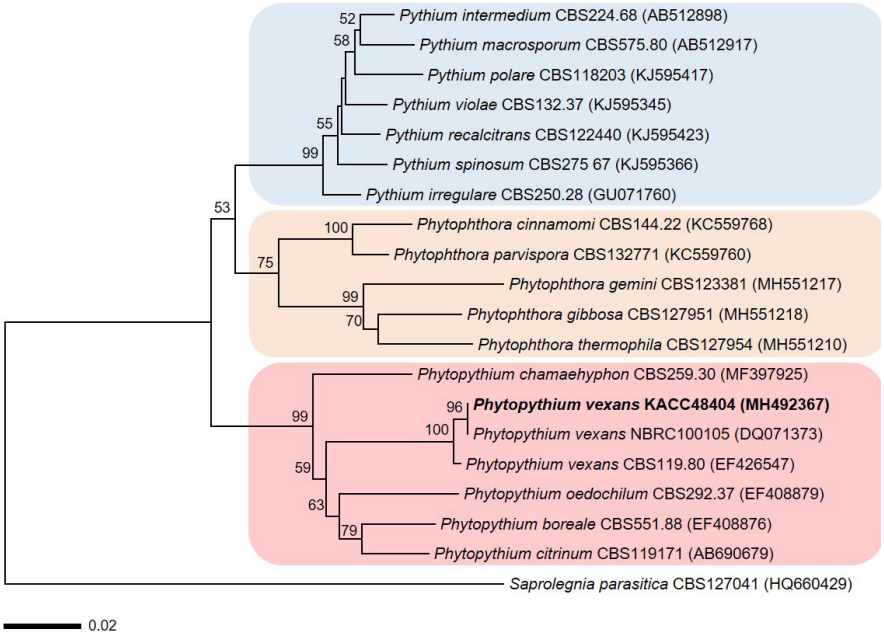

Fig. 2. Neighbor-joining tree based on the sequences of cytochrome oxidase subunit II mtDNA, showing a phylogenetic affinity of the Korean isolate (in boldface) with other *Phytopythium* species. *Saprolegnia parasitica* was designated as the outgroup. Bootstrap values above 50% were shown at the nodes. The scale bar represents 0.02 nucleotide substitutions per site.

Pathogenicity of the fungal isolate was tested on the seedlings of *Anthurium andraeanum*. For preparation of the fungal inoculum, mycelial mats were harvested from cultures grown for 2 weeks on PDA. The hyphal suspensions were poured into 5 pots (one seedling per pot). Five seedlings treated with sterile water served as control. All plants were kept in the glasshouse at 26°C. The soils in pot were kept in moist condition. After 2 weeks of inoculation, wilting symptom appeared on the leaves of the fungus-inoculated plants (Fig. 1D). No symptoms were observed on control plants. The fungus was consistently re-isolated from all inoculated plants. The pathogenicity testing showed that the isolate of *P. vexans* is pathogenic to *A. andraeanum*.

Phytopythium vexans known as saprobe and phytopathogen, displays a broad host range including economically important crops and trees worldwide [6]. The fungal species has been reported to cause root rot of *A. andraeanum* in Hawaii [7, 8]. However, there has been no previous record of *A. andraeanum* associated with *P. vexans* in Korea. To our knowledge, this is the first report of stem rot disease occurring on seedlings of *A. andraeanum* in Korea.

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