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Research Note

First Report of *Fusarium subglutinans* Causing Leaf Spot Disease on *Cymbidium* Orchids in Korea

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Abstract In 2006~2010, leaf spot symptoms, that is, small, yellow spots that turned into dark brown-to-black lesions surrounded by a yellow halo, were observed on *Cymbidium* spp. in Gongju, Taean, and Gapyeong in Korea. A *Fusarium* species was continuously isolated from symptomatic leaves; in pathogenicity testing, isolates caused leaf spot symptoms consisting of sunken, dark brown lesions similar to the original ones. The causal pathogen was identified as *Fusarium subglutinans* based on morphological and translation elongation factor 1-alpha sequence analyses. This is the first report of *F. subglutinans* as the cause of leaf spot disease in *Cymbidium* spp. in Korea.

Keywords *Cymbidium*, *Fusarium subglutinans*, Leaf spot

Members of the genus *Cymbidium*, belonging to the family Orchidaceae, are popularly grown as potted plants and used as cut flowers throughout Asia due to their esthetic value. Many cultivars of *Cymbidium* developed by breeding systems have been released in the commercial market [1]. *Cymbidium* currently occupies the largest cultivation area of any orchid in Korea, accounting for 47% (ca. 101 ha) of the total cultivated land [2]. During 2006~2010, some leaf spots were observed on *Cymbidium* species cultivated in greenhouses located in Gongju, Taean, and Gapyeong in Korea. Tiny yellow spots were initially observed on the upper side of the leaves, which later turned dark brown to black with a surrounding yellow halo (Fig. 1A and 1B). The lesions became larger and sunken with dark brown, raised edges. In the advanced stage, the centers of leaf spots that were more than 50 mm in size fell out, leaving holes in these older lesions (Fig. 1C and 1D). The disease finally resulted in leaf deformation, thereby reducing the market value of the affected plants.

Small sections cut from the diseased tissues were surface-disinfected in 70% ethanol and 1% sodium hypochlorite, washed with sterile distilled water, and subsequently placed on potato dextrose agar (PDA) plates supplemented with streptomycin sulfate (200 ppm). The hyphal tips of emerging colonies were transferred onto new PDA plates and subcultured to obtain pure isolates. A total of 5 fungal isolates were derived from different collections and deposited at the Korean Agricultural Culture Collection (Table 1). Fungal structures from fresh materials were examined under a light microscope and their dimensions were measured with the aid of a micrometer eyepiece. Microscopic images were obtained using a Zeiss AXIO microscope equipped with AxioCam ICc3 (Carl Zeiss, Jena, Germany). Colonies on the PDA plates were white to pale pinkish, with sparse, fluffy, aerial mycelium in the outer region; were submerged in the middle region; and had an average diameter of 55 mm after 4 days (Fig. 1G). Morphological characteristics of the present isolates were observed after 7 days of incubation at 25°C. Conidiophores were unbranched or branched, bearing monophialides and polyphialides (Fig. 1H). Microconidia were aseptate, ellipsoid to allantoid, and 8~18 × 2.5~3 µm (Fig. 1I). Macroconidia were straight to slightly curved, 3-5 septate, and 32.5~75 × 2.5~4 µm (Fig. 1I). The morphological and cultural characteristics of the causal fungus were consistent with the description of *Fusarium subglutinans* (Wollenw. & Reinking) PE Nelson, Toussoun & Marasas [3].

Total genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Mycelial mats and conidia were harvested by scraping the surface of colonies on PDA plates after for 1 mon. The genomic DNA was used as a template for PCR. A portion of the translation

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Table 1. Information on *Fusarium subglutinans* isolates obtained from *Cymbidium* orchids

Isolate	Year of isolation	Locality of collection	KACC accession No.	GenBank accession No.
				TEF1
06-115	2006	Gongju	47732	KM213990
09-104	2009	Taean	47733	KM213991
10-173	2010	Gapyeong	47735	KM213993
10-181	2010	Gongju	47736	KM213994

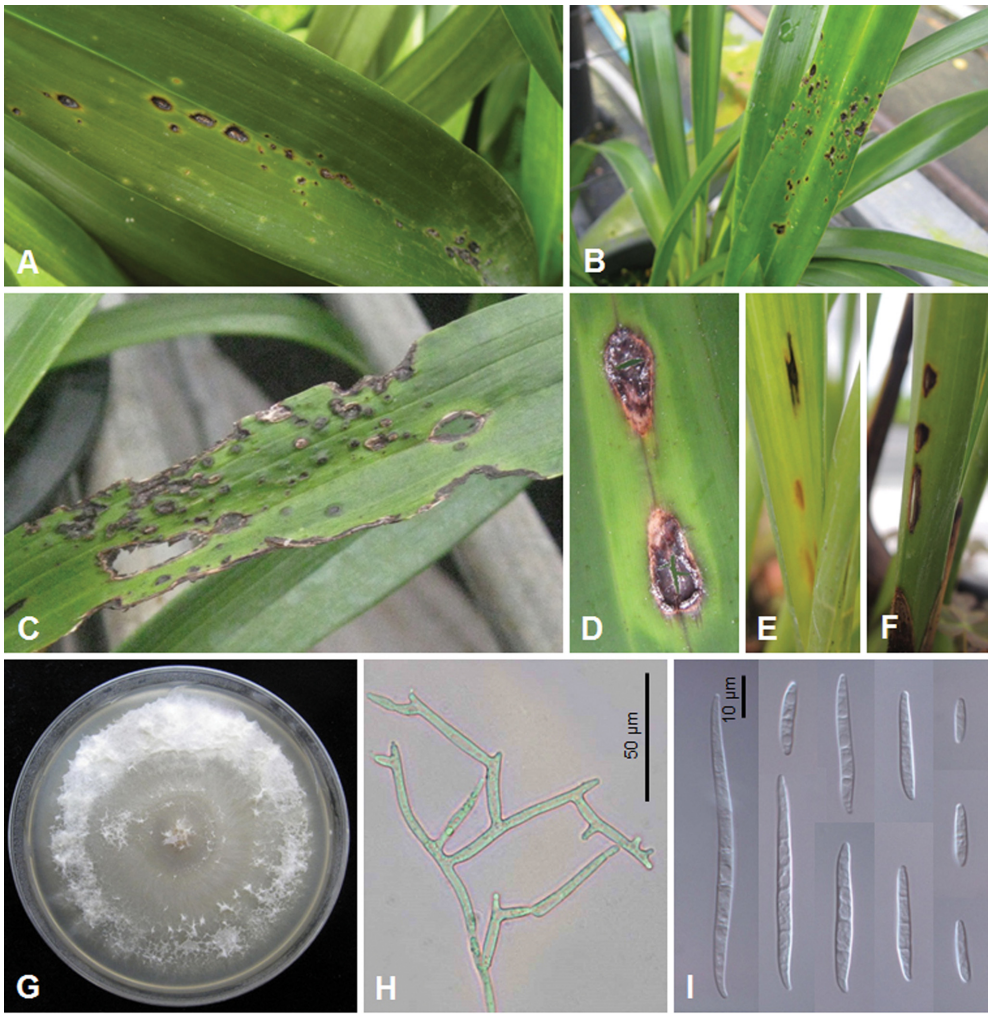

Fig. 1. Leaf spot symptoms occurring on *Cymbidium* sp. and morphological characteristics of *Fusarium subglutinans*. A, B, Early symptoms on leaves appearing as tiny, dark brown lesions surrounded by a yellow halo; C, D, Advanced foliar lesions producing holes; E, F, Leaf spot symptoms on inoculated plants; G, Colony on potato dextrose agar after 1 wk of incubation; H, Conidiophores with polyphialides; I, Macroconidia and microconidia.

elongation factor 1-alpha gene was amplified with the primers EF1 (5′-ATG-GGT-AAG-GAR-GAC-AAG-AC-3′) and EF2 (5′-GGA-RGT-ACC-AGT-SAT-CAT-GTT-3′) [4]. The PCR cycling conditions were as follows: initial denaturation for 2 min at 96°C; 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 56°C, and extension for 30 sec at 72°C; and a final extension for 7 min at 72°C. The PCR products were purified using a PCR purification kit (Inclonebiotech Co., Seoul, Korea), and both strands were directly sequenced using the ABI Prism 3730xl automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). NCBI BLAST analysis showed high similarity (97~98%) to the sequences of *F. subglutinans*. The resulting sequences of the present isolates were submitted to GenBank (accession Nos. KM213990, KM213991, KM213993, and KM213994). To infer the phylogenetic relationships, 19 reference sequences of *F. subglutinans* were retrieved from the GenBank database. *F. solani* (AB674290) was used as an outgroup. A neighbor-

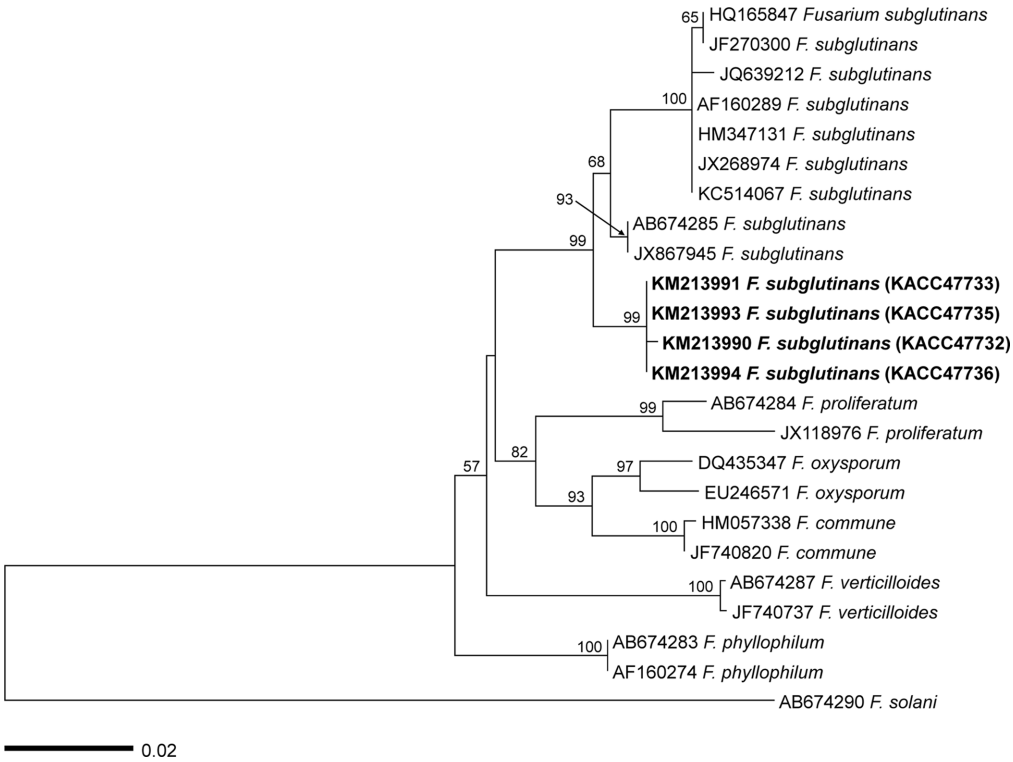

Fig. 2. Phylogenetic tree inferred from translation elongation factor 1-alpha sequences of *Fusarium* spp. using neighbor-joining analysis. Bootstrap values ≥ 50% obtained from 10,000 replicates are indicated above the branches. The scale bar represents 0.02 nucleotide substitutions per site.

joining tree was generated in MEGA5 [5]. In the phylogenetic tree (Fig. 2), the present isolates were grouped into a clade accommodating *F. subglutinans* isolates with a bootstrap value of 99%. The molecular data verified the identity of the present isolates.

A pathogenicity test was carried out using 6-mon-old *Cymbidium* plants in a glasshouse. Two isolates (06-115 and 10-173) were used as inocula that consisted of conidial masses and mycelium harvested from the 30-day-old cultures. Each conidial suspension, adjusted to 10^6 conidia per mL, was sprayed on 3 young plants whose leaves had been injured with needles. Three control plants were sprayed with sterilized water. All plants were covered with polyethylene bags and kept in a growth chamber at 28°C for 48 hr. Leaf necrosis symptoms appeared on the inoculated leaves within 7 days (Fig. 1E and 1F). The fungal pathogen was successfully re-isolated from the leaves of all inoculated plants, confirming Koch's postulate. In contrast, no symptoms were observed in the control plants.

Until date, 3 *Fusarium* species, viz., *F. oxysporum*, *F. proliferatum*, and *F. solani*, which cause dry rot, have been recorded on *Cymbidium* spp. in Korea [6]. There have been no previous records of *F. subglutinans* associated with *Cymbidium* orchids in Korea, whereas the *Fusarium* species has been known to occur on these plants in New Zealand [7] and Japan [8]. As leaf spot disease may pose a serious threat to commercial *Cymbidium* growers, adequate disease control is required to reduce economic losses.

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