

RESEARCH NOTE

Fusarium oxysporum Causes Root Rot on *Gastrodia elata* in Korea: Morphological, Phylogenetic, and Pathogenicity Analyses

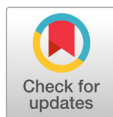
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ABSTRACT

Gastrodia elata infected with root rot disease was collected from cultivated *G. elata* fields in Gimcheon, Korea, in 2018. *G. elata* tuber surfaces exhibited root rot disease symptoms of dark-grey lesions and white fungal mycelial growth. The fungus was isolated from symptomatic tubers and cultured. Based on morphological characteristics and molecular analysis of the internal transcribed spacer region of ribosomal DNA and translation elongation factor 1-alpha, the isolated fungus was *Fusarium oxysporum*. This is the first report of root rot caused by *F. oxysporum* on *G. elata* tubers in Korea.

Keywords: *Fusarium oxysporum*, *Gastrodia elata*, Pathogenicity, Root rot



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Gastrodia elata is a saprophytic perennial plant [1] in the Achlorophyllous Orchidaceae family [2], which has a mycoheterotrophic lifestyle with reduced leaves and bracts in the scape [3]. The *G. elata* life cycle depends on two types of fungi: *Mycena* spp. support seed germination for vegetative propagation, and *Armillaria mellea* support vegetative growth to develop tubers [4]. *G. elata* rhizomes (tubers) accumulate valuable secondary metabolic compounds such as gastrodin (GA, p-(hydroxymethyl) phenyl-β-D-glucopyranoside), vanillyl alcohol, and p-hydroxybenzyl alcohol [5]. It is used as a traditional medicinal herb to treat many human diseases, such as convulsion, vertigo, ischemia, hemiplegia, epilepsy, and tremors [5,6]. *G. elata* is widely cultivated in northeast Asia, including Korea, China, Japan, and India [7].

Tuber rot disease of *G. elata* has reduced total yields in Korea by approximately 70% in recent years [8]. Multiple fungal pathogens were reported to infect *G. elata* in Korea, including *Clonostachys rosea*, *Fusarium solani*, and *Trichoderma hamatum* [9-11], and *Fusarium oxysporum* caused *G. elata* root rot during the growing and post-harvest seasons in China [12]. *Fusarium* pathogens cause various diseases in plants, produce a number of mycotoxins, and are recognized as human pathogens. *Fusarium* has a significant economic impact on global agricultural and horticultural industries [13]. *Fusarium* belongs to the Ascomycota phylum and contains a large number of species, which primarily exist in the soil and are related to various plants. These species cause rot diseases on plant bulbs, cobs, corms, roots, seedlings, stalks, and tubers [14]. *F. oxysporum* causes disease in many important plant species, including banana,

bean, cotton, chickpea, melon, tomato, and *Arabidopsis* [15].

In October 2018, we observed root rot disease (incidence 75% and severity 38%, $n=266$) on *G. elata* tubers from four commercial fields in Gimcheon, Korea (36°7'N 128°7'E), and obtained symptomatic tissues, which exhibited soft dark-grey lesions on the tuber surface (Fig. 1A). Pathogens were isolated as follows. The symptomatic tuber was surface-sterilized with 2% sodium hypochlorite (NaOCl) for 2 min, then rinsed twice with sterile distilled water for 1 min [16]. The tubers were dried with sterilized filter paper, and small pieces (5-7 mm diameter) of the lesions were incubated on potato dextrose agar (PDA, Difco, Detroit, MI, USA) in 90-mm Petri dishes for 7 days at 25°C in the dark. Pure cultures were generated by isolating single spores and culturing on PDA for 10 days. The early-stage colonies were white and became purplish-white with age. The pure culture was used for Koch's postulates, molecular analysis, and deposited in the Forest Bio Resources Bank, the National Institute of Forest Science (Accession no. NIFoS-FBRB181015-Fo01).

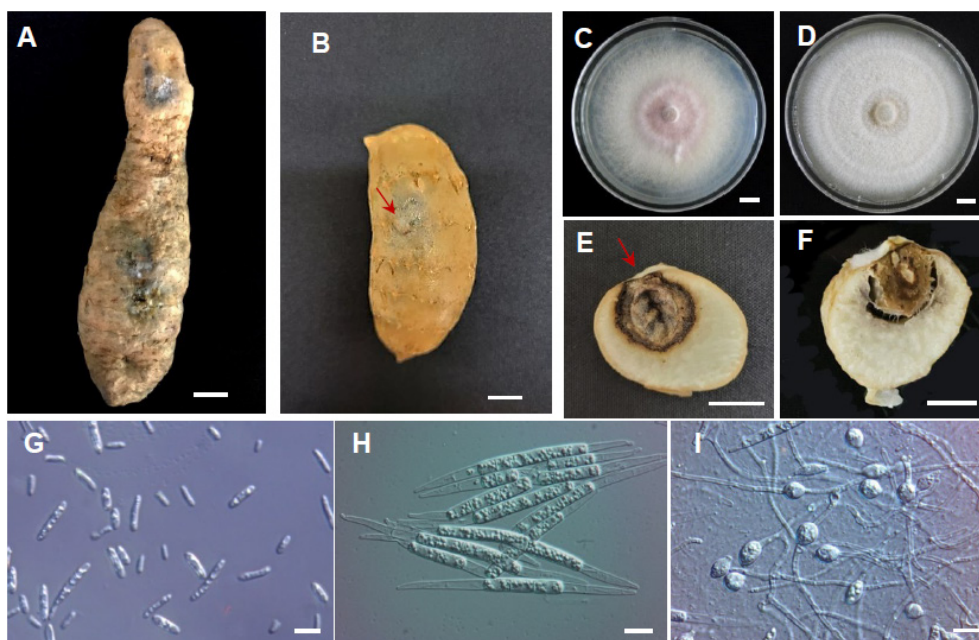


Fig. 1. Root rot caused by *Fusarium oxysporum* on *Gastrodia elata* tubers. (A) Symptoms of infected *G. elata* tubers collected from fields. (B) *G. elata* tubers inoculated with *F. oxysporum* developed rot symptoms after 10 days. (C) A 10-day-old colony of *F. oxysporum* growing on potato dextrose agar medium. (D) A 7-day-old colony of *F. solani* growing on potato dextrose agar medium. (E) Cross-section of the inoculated tuber in (B). (F) Cross-section of the inoculated tuber with *F. solani*. (G) microconidia and (H) Macroconidia. (I) Chlamydoconidia. Scale bar=1 cm (A-F) or 10 μ m (G-I). Inoculation point was indicated with red arrow.

We obtained 150-200 mg of mycelia from a 10-day-old culture that was grown on PDA at 25°C in the dark. The mycelia were ground using a pestle with liquid nitrogen (N_2), and genomic DNA was extracted using the AccuPrep Genomic DNA Extraction Kit (K-3032, Bioneer, Daejeon, Korea) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified

using ITS1/ITS4 primers [17], and translation elongation factor 1-alpha (TEF-1 α) was analyzed using EF-1/EF-2 primers [18]. The PCR reactions were performed in a 20 μ L mixture containing 5 μ L of template genomic DNA, 1 μ L each of ITS1/ITS4 or EF-1/EF-2 primer, 0.5 μ L of RealTaq DNA polymerase (RIT01, RBC, New Taipei, Taiwan), 0.5 μ L of each dNTP, 2 μ L of 10 \times PCR reaction buffer, and 15 μ L of distilled water. The PCR amplification was performed in a T100 Thermal Cycler (Bio-Rad, Foster, California, USA) under the following conditions: one cycle of pre-denaturation at 95 $^{\circ}$ C for 3 min, followed by 35 cycles of denaturation at 95 $^{\circ}$ C for 33 s, annealing at 58 $^{\circ}$ C for 1 min, and elongation at 72 $^{\circ}$ C for 1 min, with one final extension cycle at 72 $^{\circ}$ C for 5 min. The products were checked on a 1% agarose gel at 100 V for 35 min. The PCR product was sequenced using the ABI 3730xl system (Macrogen, Seoul, Korea). The ITS1/ITS4 primers generated a 472-bp amplicon (GenBank accession no. MW617294), while the EF-1/EF-2 primers generated a 712-bp amplicon (GenBank accession no. OM145982). NCBI BLASTn analysis identified a 100% match with *F. oxysporum* ITS (KY910846) and TEF-1 α (MH341211).

For Koch's postulates analyses, we incubated the culture on PDA for 10 days at 25 $^{\circ}$ C in the dark to obtain conidia. One-year-old healthy *G. elata* tubers harvested from fields were surface-sterilized. Experimentally inoculated tubers were injected with 10 μ L of conidial suspension (8×10^5 conidia/ml) in potato dextrose broth (PDB, Difco, Detroit, MI, USA) ($n=10$), whereas control disease-free tubers were injected with 10 μ L of sterile distilled water ($n=10$). All tubers were incubated at 25 $^{\circ}$ C with 80% relative humidity in the dark for 10 days. The rot symptoms on experimentally inoculated tubers were similar to that observed in the field (Fig. 1B), whereas control tubers did not exhibit symptoms. The dark-brown lesion on inoculated tubers developed with variable severity to cover an area of 28-60% of tuber diameter (Fig. 1E). The fungus was then isolated from symptomatic tissues, subjected to analysis, and determined to be 100% identical to *F. oxysporum*. These combined results satisfy Koch's postulates. In comparison, *F. solani* had abundant cottony mycelium with creamy white colored growth (Fig. 1D), and tissues infected with *F. solani* were softer than those infected with *F. oxysporum* (Fig. 1F) [10].

The fungal isolate FO2SA was obtained from an infected *G. elata* tuber. Morphological characterization and rDNA sequencing analysis identified the isolate as *F. oxysporum*. The fungal colonies were cultured on PDA at 25 $^{\circ}$ C for 10 days. The colonies were initially white and became bright violet with age (Fig. 1C). Conidiophores were shaped as branched monophialides. Microconidia lacked septa and were oval-ellipsoidal and straight to curved (Fig. 1G). Macroconidia were slightly curved and slender with 1-5 septa pointed at both ends (Fig. 1H). By contrast, other *F. oxysporum* had 3-5 septa pointed at both ends, and the macroconidia of *F. solani* isolated from *G. elata* had 2-5 septa [10]. In the FO2SA isolate, chlamydospores were single and globose-ellipsoidal, whereas other chlamydospores were pairs (Fig. 1I). The morphological characteristics of *F. oxysporum* infecting *G. elata* tubers are presented in Table 1.

Phylogenetic analysis of the combined multilocus (ITS and TEF-1 α) was performed using the maximum likelihood method and MEGA X (MEGA Software) [20]. The sequence distance was calculated based on the Tamura-Nei model, and bootstrap analysis was conducted with 1,000 replications. The FO2SA isolate

Table 1. Comparison of morphological characteristics of the *Gastrodia elata* tuber isolate with *Fusarium oxysporum*.

Characteristic	<i>G.elata</i> tuber isolate	<i>F. oxysporum</i> ^a
Conidiophore		
Shape	Branched monophialides	Unbranched and branched monophialides
Microconidia		
Shape	Oval-ellipsoidal, straight to curved	Oval-ellipsoidal, straight to curved
Septa	No septate	No septate
Length×width (µm)	3.1-18×2.0-8.1	5.0-12×2.2-3.5
Macroconidia		
Shape	Slightly curved, slender	Fusoid-subulate
Septa	1-5 septate pointed at both ends	Usually 3-5 septate pointed at both ends
Length×width (µm)	32-83×3.0-5.0	27-46×3.0-4.5
Chlamydo-spore		
Shape	Globose-ellipsoidal, single	Globose-ellipsoidal, single or in pairs
Length×width (µm)	5.9-11×4.7-9.1	4.0-16×4.0-12

^a Described by Booth [19].

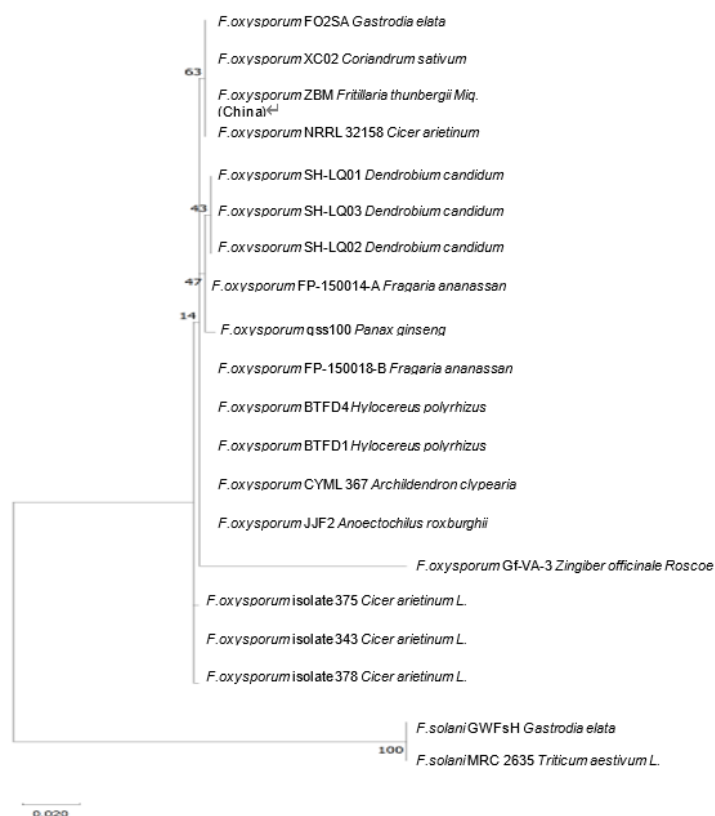


Fig. 2. Phylogenetic analysis using MUSCLE alignment based on a concatenated sequence (internal transcribed spacer [ITS] and translation elongation factor 1-alpha [TEF-1 α]) of *Fusarium oxysporum* isolated from *Gastrodia elata* with 12 members, and two strains of *F. solani* (GWFsH and MRC2635) are used as the outgroup. The evolutionary history was inferred using the maximum likelihood method and the Tamura-Nei model. GenBank accession numbers (ITS TEF-1 α) of the strains quoted are as follows: FO2SA (MW617294, OM145982), XC02 (MT579855, MT597425), ZBM (MH290452, MZ292517), NRRL32158 (MK074845, FJ538245), SH-LQ01 (JQ809654, JQ809657), SH-LQ03 (JQ809656, JQ809659), SH-LQ02 (JQ809655, JQ809658), FP-150014-A (MK085079, MK086961), gss100 (MH290452, MH341210), FP-150018-B (MK085070, MK086959), BTFD4 (MN727095, MN752124), BTFD1 (MN727096, MN752123), CYML367 (MN826824, MN839682), JF2 (MN626452, MN629915), Gf-VA-3 (MT337417, MW816632), isolate 375 (MK928423, MN788463), 343 (MK920204, MN788462), 378 (MK928424, MN788464), *F. solani* GWFsH (KX588131, KX951419), and MRC2635 (MH582403, MH582423), respectively. The scale bar indicates the number of nucleotide substitutions. The alignment and tree were constructed in MEGA X [20]. Values at nodes are based on 1,000 bootstrap replicates.

was closely related to three other strains (XC02, ZBM, NRRL 32158) (Fig. 2).

This study identified *F. oxysporum* as the causative element of root rot in *G. elata* tubers in Korea. Our result provides critical information for management strategies to control pathogen fungi in commercial fields of *G. elata* in Korea.

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