RESEARCH NOTE

Phytophthora Stem Rot of *Euphorbia hypogaea* Caused by *Phytophthora nicotianae*

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ABSTRACT

In August 2021, stem rot symptoms sporadically occurred in potted plants of *Euphorbia hypogaea* growing in a greenhouse in Pyeongtaek, Korea. The leaves of diseased plants wilted, and the epidermal layer of the stems blackened from the soil line portion and rotted. The disease incidence in plants in the greenhouse ranged from 1 to 6%. We obtained 12 oomycete isolates from the stems of diseased plants. All isolates were identified as *Phytophthora nicotianae* based on an analysis of their morphological and phylogenetic characteristics (NADH dehydrogenase subunit 1 and beta-tubulin genes). Three isolates of *P. nicotianae* were tested for pathogenicity in *E. hypogaea* plants using artificial inoculation. The isolates induced stem rot symptoms in the inoculated plants, similar to those observed in the greenhouse where the disease first occurred. This is the first report that *P. nicotianae* causes Phytophthora stem rot in *E. hypogaea*.

Keywords: *Euphorbia hypogaea*, *Phytophthora nicotianae*, Phytophthora stem rot

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Euphorbia hypogaea Marloth, which belongs to the family Euphorbiaceae, is native to Central Cape Province, South Africa, and grows primarily in desert or dry shrubland biomes [1]. This plant was introduced to Korea many years ago, where it is cultivated in greenhouses as an ornamental succulent.

In August 2021, stem rot symptoms sporadically occurred in potted plants of *E. hypogaea* growing in a greenhouse in Pyeongtaek, Korea. The leaves of diseased plants wilted, and the epidermal layer of the stems blackened from the soil line portion and rotted (Fig. 1A and 1B). Three cultivation pedestals were observed in the greenhouse and 200 plants grown in pots for each cultivation pedestal were investigated for disease incidence. The disease incidence in plants in the greenhouse ranged from 1 to 6%.

Fungi were isolated from the diseased stems of *E. hypogaea*. The 3−5 mm long samples of lesions cut from the stems were immersed in 1% sodium hypochlorite for 1 min and then plated on 2% water agar (WA; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Single hyphal tips growing from the lesion samples were transferred to potato dextrose agar (PDA; BD Difco, Sparks, MD, USA) slants after incubating the plates at 25°C for 2−3 days. Twelve oomycete isolates were obtained from the stems of diseased plants. Each isolate was cultured on PDA in 9-cm-diameter Petri dishes at 25°C in the dark for 10 days to investigate the cultural features. The colonies of the isolates displayed white mycelia with coralloid edges (Fig. 2A and 2B).

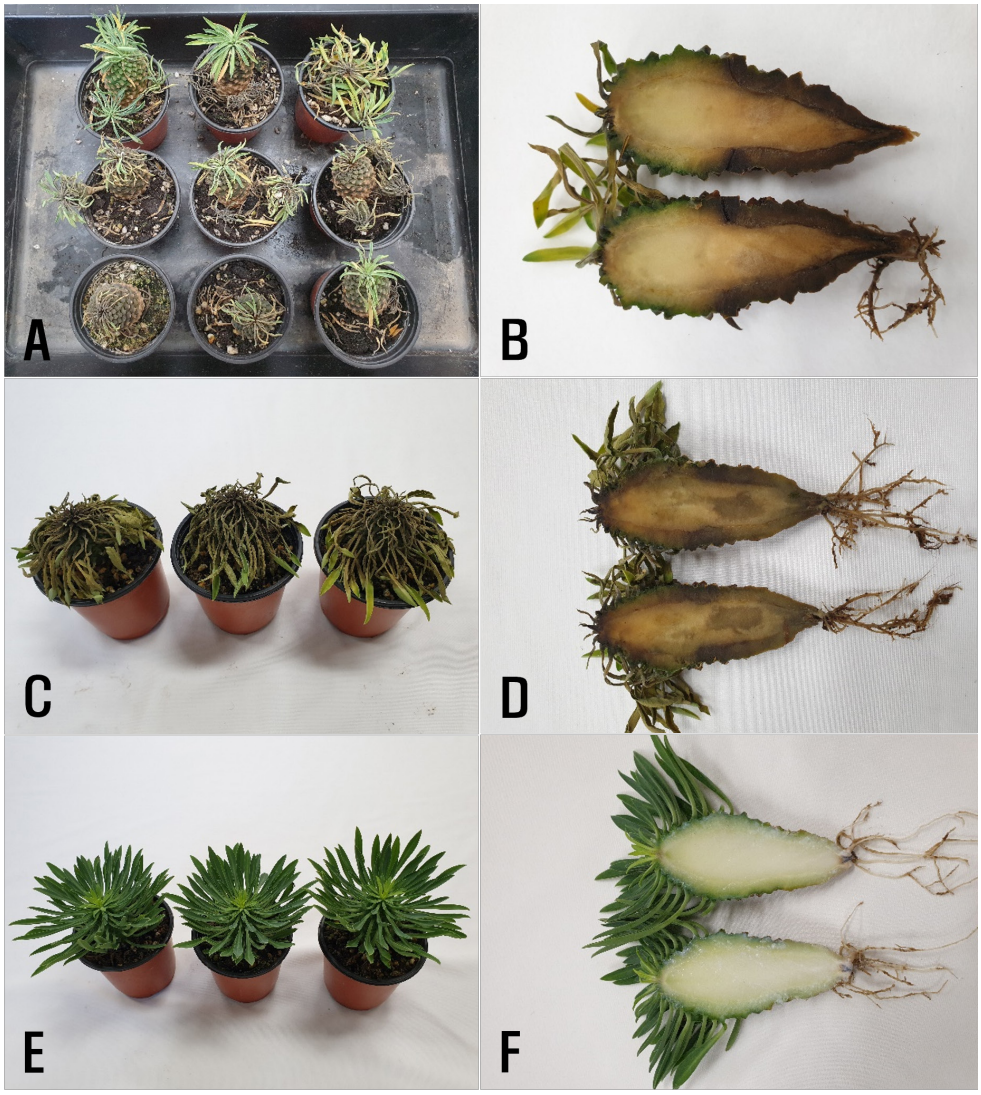

Fig. 1. Stem rot symptoms in *Euphorbia hypogaea* plants. A and B: Symptoms observed in the greenhouse. C and D: Symptoms induced by artificial inoculation tests with *Phytophthora nicotianae* isolates. E and F: Non-inoculated plants.

Each isolate was cultured on V8-juice agar (V8A) in 9-cm-diameter Petri dishes for 7 days. Twenty milliliters of sterile distilled water was then added to the cultures and they were incubated at 24–26°C under fluorescent light for 6 days to produce sporangia. The morphological characteristics of 10–20 sporangia per isolate were investigated under a light microscope (Eclipse Ci-L, Nikon, Tokyo, Japan). Hyphae were non-septate, and sporangia were globose or ovoid, papillate, persistent, and caducous with short pedicels (Fig. 2C–2E). Sporangia measured 37.6–58.1 × 28.9–36.2 μm (average 44.5 × 33.3 μm), and the papillae of sporangia measured 3.0–6.0 × 5.3–8.5 μm (average 4.0 × 7.1 μm). The morphological characteristics of the isolates were similar to those of *Phytophthora nicotianae* Breda de Haan described in a previous study [2].

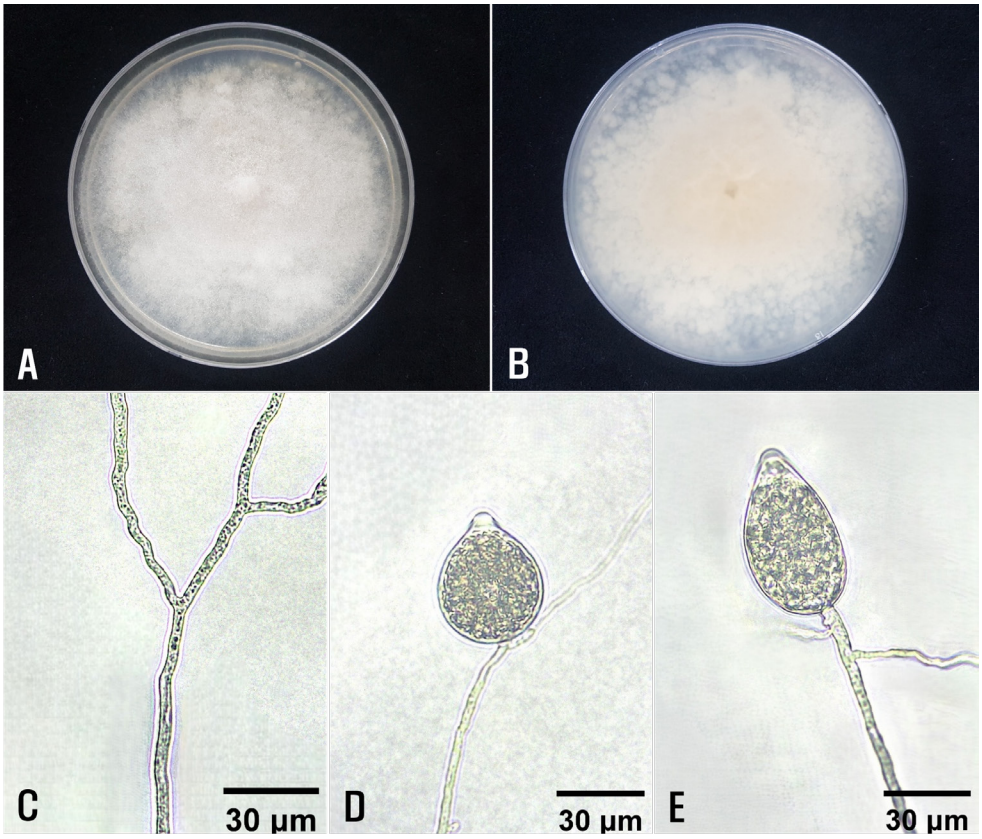

Fig. 2. Cultural and morphological features of *Phytophthora* sp. isolates from diseased *Euphorbia hypogaea* plants. Colony morphology on the surface (A) and reverse side (B) of potato dextrose agar. C: A non-septate hypha. D and E: Sporangiophores and sporangia.

Phylogenetic analysis was conducted to confirm the morphological identification of *P. nicotianae* isolates (EHPHY2101–EHPHY2112). Total genomic DNA was extracted from the isolates grown on PDA using a HiGene™ Genomic DNA Prep Kit (Biofact Co., Ltd., Daejeon, Korea) according to the manufacturer's protocol. For molecular identification, the NADH dehydrogenase subunit 1 (*NADH1*) and beta-tubulin (*TUB2*) genes of the isolates were amplified using the primer pairs NADH1F/NADH1R and TubuF2/TubuR1, respectively [3]. Amplified PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific Inc., Waltham, MA, USA) and sequenced by Solgent Co., Ltd. (Daejeon, Korea). All obtained sequences were registered with the National Center for Biotechnology Information (NCBI) under accession numbers OR161944–OR161955 and OR161956–OR161967 for *NADH1* and *TUB2*, respectively.

Phylogenetic analysis was performed using retrieved sequences registered in the NCBI database (Table 1). Partial sequences of *NADH1* and *TUB2* were analyzed for species identification, supported by the construction of phylogenetic trees using the neighbor-joining method, as described by the Kimura model [4,5]. The analysis was conducted using MEGA 11.0, with bootstrap values calculated from 1,000 replicates [6].

Table 1. List of species used in phylogenetic analyses along with their GenBank accession numbers

Species	Strain	GenBank accession number	
		NADH1	*TUB2*
Phytophthora cactorum	P6183	AY563994	AY564052
Phytophthora clandestina	IMI287317	AY563999	AY564057
	P3942	NC_067073	EU079867
Phytophthora cryptogea	SCRP206	KP288353	KP288386
	SCRP230	KP288354	KP288387
	SUC613	KP288355	KP288388
Phytophthora infestans	pic99186	AY563977	AY564035
	T30-4	MZ736507	MZ736454
Phytophthora iranica	CBS 374.72	AY564016	AY564074
Phytophthora meadii	CBS 219.88	AY564019	AY564077
Phytophthora multivesiculata	CBS 545.96	AY564022	AY564080
Phytophthora nicotianae	EHPHY2101	OR161949	OR161965
	EHPHY2102	OR161946	OR161966
	EHPHY2103	OR161955	OR161960
	EHPHY2104	OR161948	OR161961
	EHPHY2105	OR161951	OR161957
	EHPHY2106	OR161947	OR161956
	EHPHY2107	OR161944	OR161964
	EHPHY2108	OR161945	OR161967
	EHPHY2109	OR161954	OR161962
	EHPHY2110	OR161952	OR161963
	EHPHY2111	OR161953	OR161958
	EHPHY2112	OR161950	OR161959
	AR 238	DQ361212	DQ361149
	P582	AY564023	AY564081
Phytophthora phaseoli	CBS 556.88	AY563986	AY564044
Phytophthora tropicalis	PD97/11132	AY563988	AY564046
Nothophytophthora amphigynosa	BD268	KY788596	KY788515

NADH1: NADH dehydrogenase subunit 1, *TUB2*: beta-tubulin.
*The present isolates: EHPHY2101–EHPHY2112.

The *NADH1* (749 bp) and *TUB2* (857 bp) gene sequences were obtained from all 12 isolates, and the sequences of both genes were identical across all isolates. BLAST results of the partial *NADH1* gene sequences revealed high levels of similarity of 99.9% and 100% with *Phytophthora nicotianae* AR 238 and P582, respectively, compared to 97.5% similarity with *Phytophthora clandestina* IMI287317 and 97.3% with *Phytophthora iranica* CBS 374.72. High levels of similarity of 99.9% and 100% were observed with *TUB2* gene sequences from *P. nicotianae* P582 and AR 238, respectively, compared with similarities of 95.1% with *P. iranica* CBS 374.72 and 94.4% with *P. clandestina* IMI287317. Phylogenetic trees were constructed based on the *NADH1* and *TUB2* sequences using the neighbor-joining method (Fig. 3). The 12 isolates clustered with *P. nicotianae* AR 238 and P582. Accordingly, the isolates were identified as *P. nicotianae* based on their morphological characteristics and phylogenetic analysis.

Three isolates of *P. nicotianae* (EHPHY2101, EHPHY2105, and EHPHY2110) were tested for

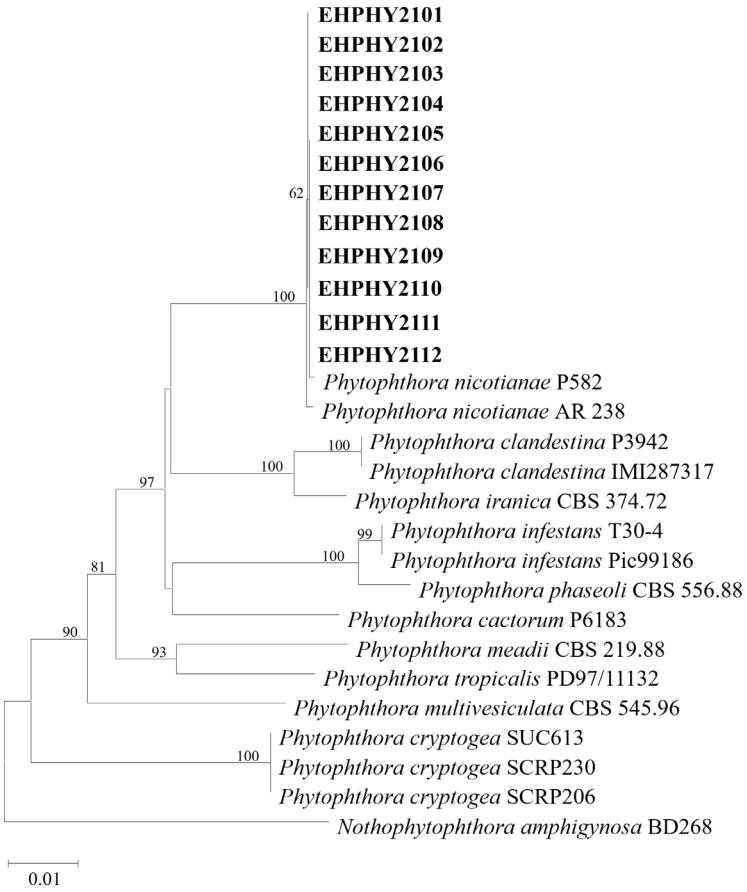

Fig. 3. Neighbor-joining phylogenetic tree based on a combined dataset of partial sequences of NADH dehydrogenase subunit 1 and beta-tubulin genes showing the phylogenetic position of the isolates (EHPHY2101–EHPHY2112) from *Euphorbia hypogaea* among *Phytophthora* species. Bootstrap values with more than 60% (percentage of 1,000 replicates) are shown at branching points. The tree was rooted using *Nothophytophthora amphigynosa* BD268 as an outgroup. The bar at 0.01 represents substitutions per nucleotide position.

pathogenicity in *E. hypogaea* plants using artificial inoculation. The 13-day-old V8A cultures in 20mL of sterile distilled water in Petri dishes were used to prepare the inoculum. Petri dishes containing the V8A cultures of each isolate were placed in a refrigerator at 4℃ for 2–4 hr to induce zoospore release from the sporangia. A zoospore suspension (10^5−10^6 zoospores/mL) was prepared by filtering through four layers of gauze. A 50 mL zoospore suspension of each isolate was poured into 8-month-old healthy plants of *E. hypogaea* grown in circular plastic pots (height: 9 cm; upper diameter: 10 cm; lower diameter: 7 cm) in a greenhouse. The same quantity of sterile distilled water was used for the control plants. The inoculated potted plants were placed in a greenhouse at 24−30℃. The inoculation test was repeated thrice. The pathogenicity of the isolates was investigated based on the degree of stem rot symptoms at 7 days after inoculation. All tested isolates of *P. nicotianae* caused stem rot symptoms in the inoculated plants (Fig. 1C and 1D), but no symptoms were observed in the control plants (Fig. 1E and 1F). The symptoms of the

plants used in the inoculation tests were similar to those observed in the greenhouse, where the disease first occurred. Morphologically identical pathogens were re-isolated from lesions of the inoculated stems.

P. nicotianae has a broad host range of more than 255 species and causes rot of roots, stems, leaves, and fruits [7]. This pathogen has been reported to attack many types of plants in Korea [8], and soft rot of *E. hypogaea* caused by a bacterial strain has been reported [9]. However, there are no reports of diseases in *E. hypogaea* caused by *P. nicotianae*. To the best of our knowledge, this is the first study to show that *P. nicotianae* causes Phytophthora stem rot in *E. hypogaea*.

CONFLICT OF INTERESTS

No conflict of interest was reported by the authors.

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