

RESEARCH NOTE

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Leaf Spot and Stem Rot on Wilford Swallowwort Caused by *Stemphylium lycopersici* in Korea

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In June 2012, leaf spot and stem rot were observed on Wilford Swallowwort plants grown in Cheonan, Korea. Three fungal isolates obtained from the diseased leaves and stems were identified as *Stemphylium lycopersici*, based on morphological, cultural, and molecular characteristics and pathogenicity. This is the first report of leaf spot and stem rot on Wilford Swallowwort caused by *S. lycopersici*.

KEYWORDS : Leaf spot, *Stemphylium lycopersici*, Stem rot, Wilford Swallowwort

Wilford Swallowwort [*Cynanchum wilfordii* (Maxim.) Hemsl.], which is native to Korea, is a perennial herb plant belonging to the family Asclepiadaceae. The plant, named Baekhasuo or Keujorong in Korean, is an important medicinal crop that has long been cultivated in Korea. In June 2012, leaf spot and stem rot were observed on Wilford Swallowwort seedlings planted in a greenhouse in Cheonan, Korea. More than 80% of the young plants in the greenhouse were infected with the disease, which affected both the leaves and stems (Fig. 1A). During the early stage, the disease appeared as one or more small spots with purple discoloration on leaves of affected plants (Fig. 1B). The spots showed gradual enlargement with age and became large lesions with a gray to brown center surrounded by a dark purple to red area (Fig. 1C). Several lesions sometimes coalesced, forming large, irregular, and spreading lesions. Stem rot appeared as somewhat sunken black spots at the base of the diseased stems (Fig. 1D). As the disease progressed, leaves of plants with stem rot turned yellow, resulting in premature defoliation. All of the plants affected by the disease eventually withered and died.

Three monoconidial fungal isolates were obtained from leaves and stems of symptomatic Wilford Swallowwort. Morphological characteristics of the isolates were investigated on lesions and V-8 juice agar (V-8A) cultures under 12 : 12 hr near-ultraviolet light : darkness. Conidiophores were solitary or in fascicles, straight,

smooth, pale to mid brown, septate, cylindrical, slightly swelling in apical conidiogenous cells, measuring 91~237 × 4~5 μm, with an average of 183 × 4.7 μm on lesions, and 118~283 × 4~6 μm, with an average of 208 × 5 μm on V-8A (Fig. 2A). Conidia were solitary, oblong, rounded, or sometimes pointed at the apex, bluntly rounded at the base, pale to mid brown, smooth or minutely verruculose, usually with 3~4 transversal septa and several longitudinal septa, which were constricted at the transversal septa, measuring 23~46 × 11~22 μm, with an average of 37 × 17 μm on naturally infected leaves, and 24~54 × 13~23 μm, with an average of 41 × 18 μm (length to width ratio of 2.3 : 1) on V-8A (Fig. 2B). Colonies of isolates on potato dextrose agar (PDA) reached 68~70 mm in diameter after seven days at 25°C in the dark. The colonies were gray, brown to dull yellow in color, with regular margins, secreting yellow to dark red pigment into the medium (Fig. 2C and 2D). Compared to the *Stemphylium* species described previously, the current isolates, NAAS12164, NAAS12167, and NAAS12168, were similar in length, width, and length to width ratio of conidia to *Stemphylium solani* G. F. Weber [1, 2], and were similar to *Stemphylium lycopersici* (Enjoji) W. Yamam. in cultural features, particularly secretion of a yellow to dark red pigment on PDA [2, 3].

To overcome the limitations of morphological and cultural approaches for determination of *Stemphylium* species, multi-locus sequence analysis was employed. The

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Fig. 1. Symptoms of leaf spot and stem rot on Wilford Swallowwort. A, Diseased plants in the green house; B, Small spots on leaves at the early stage; C, Enlarged lesions with a gray to brown center and a dark purple border at the late stage; D, Sunken black spots at the base of the diseased stems.

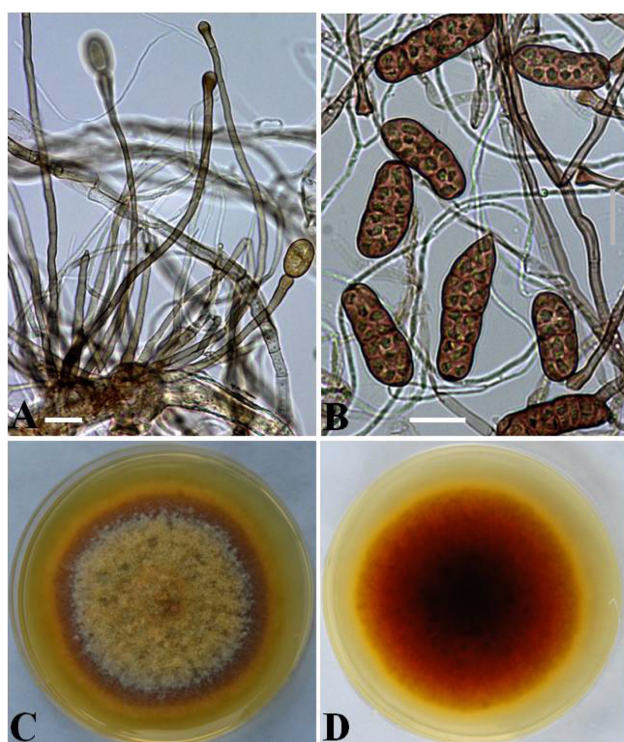


Fig. 2. Morphological and cultural features of *Stemphylium lycopersici*. A, Conidiophores arising in fascicles; B, Conidia with transversal and longitudinal septa produced on V-8 juice agar (V-8A); C, D, Seven-day-old colonies grown on potato dextrose agar at 25°C (scale bars: A, B = 20 µm).

ribosomal internal transcribed spacer (ITS) region, including the 5.8S gene, elongation factor 1 α (*EF-1 α*), partial glyceraldehyde-3-phosphate dehydrogenase (*GPD*), and the noncoding region between the vacuolar membrane ATPase catalytic subunit A gene (*vmaA*) and a gene involved in vacuolar biogenesis (*vpsA*) were amplified by PCR using the ITS1/ITS4 [4], EF1-688F/EF1-1251R [5], *gpd f/gpd r* [6], and ATPF2/GTPr primers [7], respectively. The PCR products were purified, sequenced, and deposited in GenBank (JX845138 to JX845140 and KC160506 to KC160514). Nucleotide sequences generated in this study were aligned with those of *Stemphylium* species deposited in GenBank by Inderbitzin *et al.* [7]. DNASTAR Seqman software (DNASTAR Inc., Madison, WI, USA) was used in editing of sequences. Sequence alignment of ITS, *EF-1 α* , *GPD*, and *vmaA-vpsA* indicated that sequences of the current isolates differ significantly from those of *S. solani*, but are nearly identical to those of *S. lycopersici* and *S. xanthosomatis* B. Huguenin, except for a few nucleotide differences (data not shown). A phylogenetic tree based on combined sequences of the four loci (ITS+ *EF-1 α* + *GPD*+ *vmaA-vpsA*) was constructed using the maximum likelihood method using MEGA version 4.0 [8]. The tree showed that current isolates are distinguished clearly from *S. solani* and other *Stemphylium* species, placed in a clade with *S. lycopersici* and *S. xanthosomatis* with 100% bootstrap support and are more closely related to *S. lycopersici* than *S. xanthosomatis* (Fig. 3).

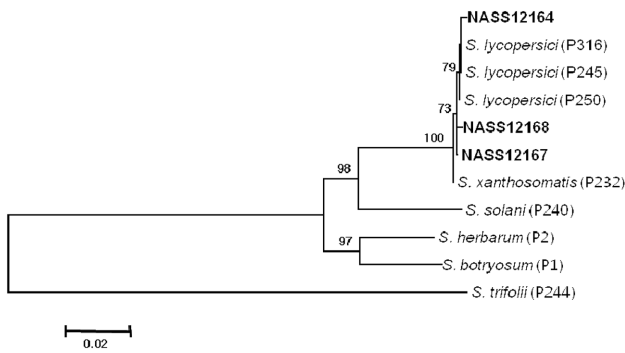


Fig. 3. Maximum likelihood tree based on combined internal transcribed spacer, glyceraldehyde-3-phosphate dehydrogenase, elongation factor 1 α , and vacuolar membrane ATPase catalytic subunit A gene-gene involved in vacuolar biogenesis sequence data for *Stemphylium lycopersici* and its allied species. The numbers above each branch indicate bootstrap values of distance. The bootstrap values were obtained after a bootstrap test with 1,000 replications. The bar represents 0.02 substitutions per site.

Pathogenicity test was performed by inoculation on detached leaves of Wilford Swallowwort, chili pepper, tomato, and tobacco with 30 μ L of conidial suspension of the three *Stemphylium* isolates (1×10^5 conidia/mL). The inoculated leaves were placed in plastic containers (30 \times 24 \times 6 cm) layered with wet paper towels and kept at 25°C for seven days. All of the tested isolates induced symptoms on leaves of Wilford Swallowwort, chili pepper, and tomato, but not on leaves of tobacco (Table 1). Results of the inoculation tests indicated that pathogenicity of the tested isolates is highly virulent on leaves of Wilford Swallowwort and chili pepper, but weakly virulent on leaves of tomato. No difference in pathogenicity was observed among the tested isolates. No development of symptoms was observed on control leaves inoculated with sterilized distilled water. The pathogen was re-isolated from artificially inoculated leaves. *S. lycopersici* has been recorded on different plants, including *Allium*, *Carthamus*, *Capsicum*, *Lycopersicon*, and others in Asia, Africa, America, Australasia, and India [3, 9], whereas, *S. xanthosomatis* was recorded only on Tania [*Xanthosoma sagittifolium* (L.) Schott] and Kalanchoe, in New Caledonia

and Korea [9, 10]. Based on the morphological, cultural, and molecular characteristics, and pathogenicity described above, the *Stemphylium* isolates from Wilford Swallowwort were confirmed to be *S. lycopersici*. *S. lycopersici* and *S. xanthosomatis* have been reported to share such characteristics as long conidiophores and conidia with pointed apices, and 1~3 major transverse septa, and are nearly identical for ITS and *gpd* sequences [3, 6, 10]. Therefore, it is not outside the realm of possibility that *S. xanthosomatis* may be a synonym of *S. lycopersici* with intra-specific variation; however, additional information is needed in order to clarify whether the two species are actually identical. Findings of this study suggest that *S. lycopersici* causing leaf spot and stem rot on Wilford Swallowwort seedling served as a source for spread of gray leaf spot to economically important crops such as tomato and chili pepper. To the best of our knowledge, this is the first report of leaf spot and stem rot caused by *S. lycopersici* on Wilford Swallowwort seedlings.

Acknowledgements

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Table 1. Pathogenicity of *Stemphylium lycopersici* isolates on leaves of different plants by artificial inoculation

Isolates	Pathogenicity ^a of tested isolates on leaves of different plants			
	Wilford swallowwort	Chili pepper	Tomato	Tobacco
NAAS12164	++	++	+	-
NAAS12167	++	++	+	-
NAAS12168	++	++	+	-
Control	-	-	-	-

^aPathogenicity was rated based on the lesion diameter seven days after inoculation. ++, above 15 mm lesion diameter; +, 5~8 mm lesion diameter; -, no symptom.

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