Leaf Spot of Yam Caused by *Pseudophloeospora dioscoreae* in Korea

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Leaf spot symptoms were frequently observed on yam plants grown in the Yeosu area in Korea during a disease survey in 2008. A total of five isolates of *Pseudophloeospora* sp. were obtained from the infected leaves of yam plants. All of the isolates were identified as *Pseudophloeospora dioscoreae* based on their morphological and cultural characteristics. A phylogenetic tree derived from the internal transcribed spacer sequences of the fungal isolates showed that the fungus is distinctly separated from species in other related genera. *P. dioscoreae* isolates caused very tiny spots on leaves of yam plants two weeks after artificial inoculation which were similar to those observed in the field. This is the first report that *Pseudophloeospora dioscoreae* causes leaf spot in yams in Korea.

KEYWORDS : Leaf spot, *Pseudophloeospora dioscoreae*, Yam

Edible species of yam are a significantly important food and pharmaceutical crop used throughout the world [1]. Among them, the yam (*Dioscorea batatas* Decne.), a health food crop, is the most widely cultivated species in Korea [2]. Leaf spot is the most commonly encountered foliar disease in yam fields. The disease has been reported to be caused by *Cercospora dioscoreae* by Ellis and Martin, *Cylindrosporium dioscoreae* by Miyabe and Ito, and *Pseudocercospora contraria* (Syd. and Syd.) by Deighton [3].

This study was conducted to identify the fungus, based on morphological and cultural characteristics, causing leaf spot in yams in Korea. Additionally, to clarify the phylogenetic relatedness among taxa, rDNA-internal transcribed spacer (ITS) regions of the fungal isolates isolated from yam leaves were compared with those of related fungal species retrieved from the GenBank database. Finally, pathogenicity of the fungal isolates was tested.

Disease incidence and symptoms. White leaf spots were observed on yam leaves in the fields of the Yeosu area during a disease survey in 2008. The disease incidence was as high as 30% in some of the surveyed fields. The symptoms on the adaxial surface of leaves first appeared as white to pale gray mycelial masses, which are circular, discrete, or in aggregate and usually limited to less than 3 mm in diameter (Fig. 1A). As the disease progressed, the white masses disappeared, resulting in circular to somewhat irregular lesions with pale yellow edges and blackish brown acervuli in the center (Fig. 1B). The heavily diseased leaves eventually fell off the plant.

Pathogen identification. A total of five monoconidial isolates were obtained from symptomatic yam leaves. Morphological characteristics of all the isolates were similar to those described previously [4-6]. All of the isolates were identified as *Pseudophloeospora dioscoreae* (Miyabe and Ito) Braun. based on their morphological and cultural characteristics.

Colonies grown on potato dextrose agar reached 14~18 mm in diameter after 14 days of incubation at 25°C (Fig. 2A). The colonies consisted of compact, low aerial mycelium with irregular edges, a light olive gray to brown surface, and a dark reddish orange reverse (Fig. 2B). Conidiomata, with large, flat and subcuticular acervuli, on symptomatic yam leaves were well-developed. Conidiophores were densely arranged in acervuli (Fig. 2C). Conidiogenous cells were hyaline, smooth, straight, or slightly geniculate towards the apices, with inconspicuous conidial scars at apices and measured 5.5~8.6 µm in length and 1.8~2.4 µm in width. Conidia were holoblastic, solitary, hyaline, smooth, acicular, 1~2 septate, straight or gently curved, truncate in base, with an inconspicuous basal scar, and measured 36.5~73.3 × 2.2~3.2 µm (Fig. 2D).

Sequencing and phylogenetic analyses. ITS sequences of *P. dioscoreae* isolates OT0807 and OT0808 were used for the phylogenetic analysis. Sequences of 18 other related fungal species used for the analysis were obtained from the GenBank database. Sequences were edited using the computer program SEQMAN (DNASTAR Inc., Madison, WI, USA). A phylogenetic tree for ITS analysis was obtained with the sequence data by neighbor-joining methods using the software MEGA ver. 4.0 [7], and sequence distance was calculated with the Tamura-Nei

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Fig. 1. Leaf spots on yam leaves observed in the field. A, A leaf with white mycelial masses; B, Leaves with pale yellow to brown, circular or irregular shaped lesions.

Fig. 2. Morphological and cultural features of *Pseudophloeosporerlla dioscoreae* isolated from yam leaves. A (surface) and B (reverse), 14-day-old colonies grown on potato dextrose agar with conidial masses and brown pigments; C, Conidiophores bearing conidia (scale bar = 10 µm); D, Conidia (scale bar = 20 µm).

parameter model. Bootstrap analysis was performed with 1,000 replications to determine the support for each clade.

The size of the entire ITS region for *P. dioscoreae* isolates OT0807 and OT0708 was 452 bp, and they yielded 472 aligned nucleotide positions for all species included in the alignment. In all of the species, 108 (22.9%) variable nucleotide sites were observed, and 77 (16.3%) of these were parsimony-informative. A phylogenetic tree derived from parsimony analysis placed two *P. dioscoreae*

isolates under the clade that included *Mycosphaerella pyri* (Auersw.) Boerema and *Mycosphaerella ulmi* Kleb. (Fig. 3). Two *P. dioscoreae* isolates were separated from *M. ulmi* with 75% bootstrap support.

Although the teleomorph of *P. dioscoreae* was not found, this phylogenetic study revealed that the fungus is related to several species within the genus *Mycosphaerella* and most closely related to *M. ulmi* which form acervuli on host plants, of the anamorph genus *Phloeospora*.

Fig. 3. Phylogenetic tree of *Pseudophloeosporerlla dioscoreae* and related fungal species based on the entire internal transcribed spacer sequences of nuclear rDNA. The tree was constructed by the neighbor-joining method. The values of each clade are confidence levels from a 1,000 replicate bootstrap sampling. Numbers in parentheses are accession numbers found in the GenBank database.

Pathogenicity. The pathogenicity of *P. dioscoreae* to the host plants was tested using conidial suspensions (3×10^5 /mL). Yam leaves were wounded with a blunt pencil or left intact, and placed in plastic boxes ($30 \times 24 \times 6$ cm). Then, 15 μ L of the conidial suspension was placed on the surface of the leaves. The plastic boxes were kept in incubator at $25 \pm 1^\circ\text{C}$ for 14 days. The wounded leaves had symptoms that appeared as very tiny spots limited to less than 1 mm; there were no symptoms on unwounded leaves. The symptoms on wounded leaves developed very slowly.

P. dioscoreae was first described as *Cylindrosporium dioscoreae* by Miyabe and Ito. However, the fungus was transferred to the genus *Pseudocercospora* (= *P. miyabei* by Pons and Sutton) in 1988, and then placed in a new genus, *Pseudophloeosporerlla*, by Braun in 1993 that differentiated it from other related genera by the types and position of conidiomata and the arrangement of conidiphores [4]. This is the first report that *P. dioscoreae* causes leaf spot in yams found in Korea.

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