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Occurrence of Leaf Spot on Mulberry Caused by *Phloeospora maculans* in Korea

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Mulberry (*Morus alba* L.) is native to China, and has long been widely cultivated to feed the silkworms. Recently, it is being used as a material for medical applications and health food in Korea. In July 2009, leaf spot disease severely occurred in some local varieties of mulberry grown in Suwon, Korea. Among different varieties surveyed, a variety, Hasusang, was found to be highly susceptible to the disease where disease incidence reached nearly 90 percent. The disease usually occurred on leaves close to the ground and gradually progressed to upper leaves. The symptoms appeared initially in the form of small, circular, dark-brown spots less than 1 mm and with age these spots enlarged to large lesions with diffuse chlorotic halo (Fig. 1A and 1B).

A total of eight fungal isolates were obtained from lesions on the leaves of mulberry plants. Acervular conidiomata produced on necrotic lesions in concentric circles were white to pinkish white, subepidermal, separate or confluent, mostly 150–200 μm wide (Fig. 1C). Conidiophores were hyaline, cylindrical, holoblastic and measured 7.3–21.5 × 2.3–4.5 μm (Fig. 1D). Conidia were hyaline, cylindrical, straight or curved, tapered towards the apex, 1–6 septate and measured 21.0–68.8 × 2.5–5.2 μm (Fig. 1E). Colonies on potato dextrose agar were slow growing, compact, producing pale red diffuse pigment, white on top surface and dark green with pinkish white edge on reverse side (Fig. 1F). All fungal isolates were identified as *Phloeospora maculans* (Sandri) Allesch. [teleomorph *Mycosphaerella mori* (Fuckel) Wolf] based on their morphological and cultural characteristics. The morphological characteristics of present isolates were also similar to those described previously (Punithalingam, 1990; Sutton, 1980).

To confirm the results of conventional identification, the ribosomal DNA-internal transcribed spacer (rDNA-ITS) regions from MP0930, MP0938 and MP0940 were amplified using the primers ITS1/ITS4, and sequenced. The sequences obtained were deposited in GenBank with accession numbers of HQ874466, 874467 and 874468. An ITS-based phylogenetic tree was constructed by the neighbor-joining method with Kimura's two-parameter distance model using MEGA version 4.0. The phylogenetic tree showed that present isolates could be distinguished from the other *Mycosphaerella* species and form an independent clade together with *P. maculans* in GenBank (Fig. 2).

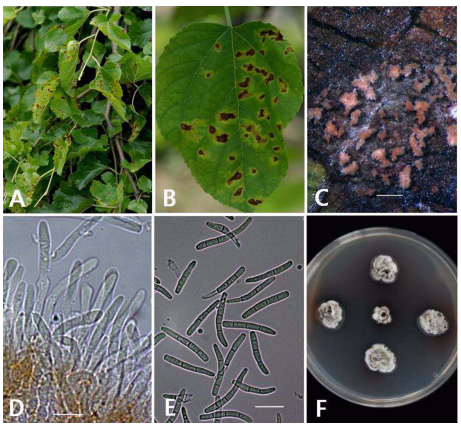

Fig. 1. Natural symptoms of leaf spots on mulberry (A, B), acervular conidiomata on necrotic lesion (C, bar=200 μm), conidiophores (D, bar=10 μm), conidia (E, bar=10 μm) and colonies on potato dextrose agar (F) of *Phloeospora maculans*.

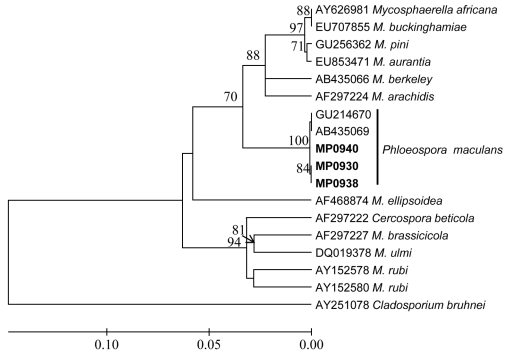

Fig. 2. Neighbor-joining tree based on sequences of rDNA-ITS regions of *Phloeospora maculans* isolates and related species. The numbers above the nodes represent bootstrap values of >60% out of 1,000 bootstrap replication. Bar represents the number of nucleotide substitutions per site.

Pathogenicity tests were made on mulberry leaves spray-inoculated with conidial suspension (5×10^5 conidia mL^{-1}). Inoculated plants were kept in a moist chamber at 23°C for three days, and then moved to a growth chamber at 23°C with a 12 h photoperiod. Many small, necrotic lesions were produced on the leaves 15 days after inoculation. However, no symptoms developed on control plants inoculated with sterilized distilled water. The pathogen was reisolated from inoculated leaves.

P. maculans has been recorded on the genus *Morus* including *M. alba* in Africa, Asia, Australia, Europe, North America and South America (Farr and Rossman, 2010; Punithalingam, 1990). In Korea, *P. maculans* was first recorded on mulberry as its a synonym *Septogloeum mori* (Park, 1967) but no description for the fungus was given there or elsewhere. It has been reported that the fungus overwinters in young parts of branches and in debris lying on the ground and then is dispersed by rain splash (Punithalingam, 1990). Frequent rain and high humidity coupled with rainy season in Suwon, in July 2009 might be a major cause for high incidence of the disease.

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