Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease Caused by *Stemphylium eturmiunum* on Garlic in Korea

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Garlic (*Allium sativum* L. cv. *namdo*) is one of the most popular vegetables grown in Korea due to its high demand from the food industry. However, garlic is susceptible to a wide range of pest infestations and diseases that cause a significant decrease in production (Schwartz and Mohan 2008). In early 2019, leaf blight disease was found spreading in garlic cultivation areas around Jeonnam Province (34.9671107°N, 126.4531825°E), Korea. Disease occurrence was estimated to affect 20% of the garlic plants and resulted in up to a 3 to 5% decrease in its total production. At the early stage of infection, disease symptoms were small, white-grayish spots with apical necrosis on leaves. This necrosis enlarged, producing a water-soaked lesion before turning black-violet due to the formation of conidia. As the disease progressed, the infected leaves wilted, and the whole plants eventually died. To identify the causal agent, symptomatic tissues (dry brown water-soaked lesions) were excised, surface sterilized with 1% NaOCl, and placed on potato dextrose agar (PDA) followed by incubation at 25°C in the dark for 5 days. Among 10 fungal isolates obtained, four were selected for further analyses. On PDA, fungal colonies were initially grayish white but gradually turned yellowish-brown after 15 days due to the formation of yellow pigments. Conidia were muriform, brown, oblong (almost round) with an average size of 18 to 22 × 16 to 20 μm ($n = 50$) and possessed 6 to 8 transverse septa. Fungal mycelia were branched, septate, and with smooth-walled hyphae. Morphological characteristics described above were consistent with the morphology of *Stemphylium eturmiunum* as reported by Simmons (2001). For molecular identification, the internal transcribed spacer (*ITS*) and calmodulin (*cmdA*) genes from the selected isolates

were amplified and sequenced (Carbone and Kohn 1999; White et al. 1990). Alignment analysis showed that *ITS* and *cmdA* sequences were 100% identical among the four selected isolates. Therefore, the representative isolate NIHHS 19-142 (KCTC56750) was selected for further analysis. BLASTN analysis showed that *ITS* (MW800165) and *cmdA* (LC601938) sequences of the representative isolates were 100% identical (523/523 bp and 410/410 bp) to the reference genes in *S. eturmiunum* isolated from *A. sativum* in India (KU850545 and KU850835, respectively) (Woudenberg et al. 2017). Phylogenetic analysis of the concatenated sequence of *ITS* and *cmdA* genes confirmed NIHHS 19-142 isolates were *S. eturmiunum*. Pathogenicity test was performed using fungal isolate representative, NIHHS 19-142. A conidial suspension (1×10^6 conidia/μl) of the fungal isolate was inoculated on intact garlic leaves (two leaves from 10 different individual plants) and bulbs (10 bulbs), respectively. Inoculation on intact leaves was performed at NIHHS trial farm whereas inoculated bulbs were kept in closed containers to maintain humidity above 90% and incubated in a chamber at 25°C. Water-soaked symptoms were observed at the inoculated site at 14 days post inoculation (dpi) on intact leaves and at 11 dpi on bulbs. As a control, the conidial suspension was replaced with sterile water and no symptoms were observed. Reidentification of fungal colonies from symptomatic leaves and bulbs showed that the morphological characteristics and molecular marker sequences of the three colonies selected were identical to the original isolates, thus fulfilling Koch's postulates. Early identification of *S. eturmiunum* as a causal agent of leaf spot disease is crucial information to employ effective disease management strategies or agrochemical applications to control disease outbreaks in the field. Although *S. eturmiunum* has been reported to cause leaf spot of garlic disease in China, France, and India (Woudenberg et al. 2017), to our knowledge, this is the first report of it causing leaf spot disease on garlic in Korea.

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