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First Report of Leaf Spot Disease Caused by *Cladosporium pseudocladosporioides* on *Morus alba* in South Korea

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Abstract

Morus alba, known as White Mulberry, is one of the most common species of mulberry found in South Korea, along with *M. australis*, known as Korean Mulberry. During a routine survey to investigate fungal diseases on deciduous broad-leaved trees in 2020, leaf spots were consistently observed on the White Mulberry in Sejong-si (36°30'12.8"N 127°17'34.5"E) and Wonju-si (37°15'29.6"N 128°11'37.9"E), South Korea, with a disease incidence of approximately 70 to 80%. Symptoms included circular, tan or necrotic lesions surrounded by a dark margin on leaves, which, in some cases, the lesions coalesced to form relatively large necrotic regions. The pathogen was successfully isolated from *M. alba*, and was identified as *Cladosporium pseudocladosporioides* based on the phylogenetic analysis and morphology. To the best of our knowledge, this is the first report of leaf spot disease on *M. alba* caused by *C. pseudocladosporioides* in South Korea.

Key Words: mulberry, phylogenetics, morphology, fungal pathogens

Morus alba, known as White Mulberry, is one of the most common species of mulberry found in South Korea, along with *M. australis*, known as Korean Mulberry. During a routine survey to investigate fungal diseases on deciduous broad-leaved trees in 2020, leaf spots were consistently observed on the White Mulberry in Sejong-si (36°30'12.8"N 127°17'34.5"E) and Wonju-si (37°15'29.6"N 128°11'37.9"E), South Korea, with a disease incidence of approximately 70 to 80%. Symptoms included circular, tan or necrotic lesions surrounded by a dark margin on leaves, which, in some cases, the lesions coalesced to form relatively large necrotic regions. Conidiophores on the lesion surface, usually solitary and occasionally in loose fascicles, were observed under a dissecting microscope. To isolate the causa-

tive fungal pathogen, conidia directly taken from randomly collected symptomatic leaves were suspended and plated on PDA to make monosporic isolates. Each isolate was then transferred and cultured on PDA media at 25°C for two weeks. All the cultures obtained in this study were deposited in the culture collection (CDH) of the National Institute of Forest Science, Korea (Accession No. CDH 2020-13 and CDH2020-15). The colonies developed into olivaceous-brown, smooth or verrucose, and velvet-like colonies. All isolates recovered in this study contained dense masses of dematiaceous, septate and branched hyphae. Conidiophores were macronematous or micronematous, apically or laterally branched, and septate, with profuse conidial chains branching in all directions. Conidia

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were ovate or elongate, sometimes septate, and 5.8 (4.4 to 7.6) $\mu\text{m} \times 2.9$ (2.3 to 3.7) μm ($n=50$) in diameter, with a distinct distal hilum. Secondary ramoconidia were sub-cylindrical to cylindrical or oblong, and 10.4 (7.4 to 15.7) $\mu\text{m} \times 3.4$ (2.6 to 4.3) μm ($n=25$) in diameter, with a distal hilum, compared to a broadly truncate base of ramoconidia. To assure the identity of the pathogen retained in this study, genomic DNAs of the isolates, CDH2020-13 and CDH2020-15, were extracted and subjected to molecular identification based on the internal transcribed spacer (ITS) regions, actin and translation elongation factor 1-alpha (TEF) gene. The resulting sequences of the isolates were deposited in GenBank with the accession numbers (MW412557–558 for ITS, MW419293–294 for actin and MW419295–296 for TEF). To further understand the phylogenetic relationship between the isolates and other *Cladosporium* spp., maximum likelihood (ML) analysis was performed using the concatenated sequences of the isolate with six closely related *Cladosporium* spp. This was achieved using RAxML HPC BlackBox ver.8.1.11 (Stamatakis 2006; Stamatakis et al. 2008) to which the default option with the GTR substitution model implemented in the CIPRES cluster server (<https://www.phylo.org/>) at the San Diego Supercomputing Center. *Cercospora beticola* was used as the outgroup taxon for the ML analyses. Based on the phylogenetic analysis and morphology, the identity of the isolates from *M. alba* was confirmed as *C. pseudocladosporioides* (Bensch et al. 2010). To confirm the pathogenicity, inoculation trials were conducted by spraying the leaves of five *M. alba* seedlings (two-year-old) with 5 μL of conidial suspensions prepared from the isolate, CDH2020-13, and diluted to contain 1×10^5 conidia per ml until runoff, with two control seedlings sprayed with sterile distilled water. After seven weeks in the greenhouse at 23 to 28°C and in 80 to 85% relative humidity, symptoms identical to those observed in the field were observed on inoculated leaves, while the control remained asymptomatic. Then, the pathogen was successfully re-isolated from all inoculated leaves and found to be identical to the inoculated isolate based on morphology and sequence comparisons, fulfilling Koch's postulates. It has been reported that several fungal species are associated with *Morus* spp. in South Korea, including *Pseudocercospora snelliana* causing leaf spot on *M. bombycis* and *Phyllactinia moricola* causing

powdery mildew on *M. alba* (Farr and Rossman 2021). In cases of *C. pseudocladosporioides*, it was reported as a causal agent of the passionfruit scab and some postharvest rots on kiwi and taro in Brazil (Rosado et al. 2019) and as an endophytic fungus from *Quercus* sp. in Korea (Lee et al. 2019). However, this is the first report of *C. pseudocladosporioides* causing leaf spot on *M. alba* in South Korea.

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Ethical Statements

All the authors have sufficiently contributed to the work, have agreed to this submission and are responsible for its contents. This manuscript including the data that are supporting the aim and the conclusion of this research is new and is not being considered elsewhere. No data have been fabricated or manipulated for being published to the journal.

Conflict of Interest

The authors have no conflicts of interest to declare.

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