Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Corynespora cassiicola* Causing Target Spot on Soybean in Taiwan

Po-Kuan Lu,¹ Hsien-Hao Liu,² and Hao-Xun Chang^{2,†}

¹ Crop Environment Section, Hualien District Agricultural Research and Extension Station, Hualien County, 973044, Taiwan

² Department of Plant Pathology and Microbiology, National Taiwan University, Taipei City, 10617, Taiwan

Author contributions: PKL discovered the disease, isolated the pathogen, performed the morphological characterization, and executed Koch's postulates to confirm pathogenicity. HHL conducted the molecular and phylogenetic characterization of pathogen. PKL and HXC prepared the manuscript and supervised the project.

Data availability: The ITS, actin, tubulin, and elongation factor 1 alpha sequences of *Corynespora cassiicola* were deposited in NCBI GenBank under accession numbers MW548097, MW961420, MW961419, and MW961421.

Funding: This project is supported by the Yushan Young Scholar Program (Ministry of Education, Taiwan) to Hao-Xun Chang. Plant Dis. 105:3753, 2021; published online as <https://doi.org/10.1094/PDIS-02-21-0277-PDN>. Accepted for publication 4 May 2021.

Soybean (*Glycine max* [L.] Merr.) is an important crop in Taiwan. In October 2020, an unknown leaf spot disease was counted ($n = 100$) to occur over 70% of soybean cultivar 'Hualien No.1' in the Shoufeng Township of Hualien County, eastern Taiwan. Initial symptoms on leaves appeared as tiny lesions approximately 3 mm in diameter, which later enlarged and developed into round, irregular, and reddish-brown spots with concentric rings surrounded by a yellowish halo. The symptoms appeared on both young and old leaves but rarely on the stem or pods. The lesions at the margin of healthy and infected tissues were surface disinfested in 1% NaOCl for 30 s, washed twice in sterilized distilled water, dissected, and plated on potato dextrose agar (PDA) to isolate the potential pathogen. Colonies on PDA exhibited light to dark brown color at 24°C with 12-h light after 7 days of incubation. The average growth rate was 3 mm per day. Conidia were light brown in color and obclavate to cylindrical in shape. The size of a conidium was measured, with an average of 110.8 ± 28.2 µm in length and 15.2 ± 2.8 µm in width, typically with three to 18 septa ($n = 50$). To confirm the pathogenicity of this fungus, conidial suspension (10^4 conidia/ml) of two isolates, HL_GM-6 and HL_GM-7, was sprayed on the healthy leaves of 4-week-old soybean. Plants sprayed with sterile distilled water were used as a control. After inoculation, the plants were covered with plastic bags to maintain a

high humidity for 24 h before moving into a greenhouse with a condition of 20 to 25°C and relative humidity of 75 to 80%. After 7 days of inoculation, foliar symptoms began to appear, which were identical to the field observations. To complete Koch's postulates, pathogen isolation was attempted, and the identical fungus was retrieved from the foliar spots of the inoculated leaves. The foliar symptoms as well as the morphology of the conidiophores and conidia suggested the pathogen to be *Corynespora cassiicola* (Ellis et al. 1971). Molecular characterization was performed using the sequences of internal transcribed spacer (ITS) region of rDNA, actin (*act1*), tubulin, and translation elongation factor 1 alpha (*tef1*) genes after a PCR with ITS1/ITS4 (White et al. 1990), ACT-512F/ACT-783R (Carbone and Kohn 1999), BT2a/Bt2b (Udayanga et al. 2012), and EF1-728F/EF1-986R (Udayanga et al. 2012), respectively. BLASTN sequence analyses of the ITS, *act1*, tubulin, and *tef1* genomic regions of the isolate HL_GM-7 (GenBank accessions MW548097, MW961420, MW961419, and MW961421) showed high similarity with the isolates of *C. cassiicola* including 99.58% with sequence KF810854 (Déon et al. 2014), 99.11% with FJ853005, 99.34% with MH763700 (Duan et al. 2019), and 99.33% with KY112719 (Zhang et al. 2018), respectively. Based on the morphology, pathogenicity, and sequence results, this study becomes the first report of *C. cassiicola* causing target spot on soybean in Taiwan. *C. cassiicola* is known to infect a broad host range (Lopez et al. 2018), and it has been found to infect tomato, cucumber, papaya, and *Salvia miltiorrhiza* in Taiwan (Lu et al. 2019; Tsai et al. 2015). Therefore, the emergence of soybean target spot should be noted to avoid potential damage to soybean production in Taiwan.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Déon, M., et al. 2014. Fungal Biol. 118:32.
- Duan, Y., et al. 2019. Pestic. Biochem. Physiol. 153:95.
- Ellis, M. B., et al. 1971. CMI Description of Pathogenic Fungi and Bacteria No. 303. CMI, Kew, U.K.
- Lopez, D., et al. 2018. Front. Microbiol. 9:276.
- Lu, P. K., et al. 2019. Plant Dis. 103:769.
- Tsai, J. N., et al. 2015. Plant Dis. 99:1649.
- Udayanga, D., et al. 2012. Fungal Divers. 56:157-171.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Zhang, M., et al. 2018. Plant Dis. 102:2037.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: soybean (*Glycine max*), *Corynespora cassiicola*, target spot

[†]Indicates the corresponding author.
H.-X. Chang; hxchang@ntu.edu.tw