Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Didymella glomerata* Causing Didymella Leaf Blight on Maize

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Maize (*Zea mays* L.) is a staple food crop worldwide. In July 2021, gray leaf blight was observed on maize leaves in a field in Panjin (41°7'11.98" N, 122°4'14.57" E), Liaoning Province, China. Nearly 5% of the maize plants were affected in the field. The leaves of the affected plants showed oval to oblong, gray, sunken lesions with yellow or tan margins. The lesions were scattered all over the leaf surface; however, they were absent on the stalks and other parts of the affected plants. To isolate the pathogen, leaf discs (1.25 mm²) excised from the blight lesions were surface-sterilized with 70% ethanol for 30 s followed by 20% NaOCl for 2 min and rinsed three times with sterilized water. The discs were cultured on potato dextrose agar (PDA) plates supplemented with streptomycin (100 mg/liter) and incubated at 25°C under a 12-h photoperiod for 7 days. Six single spore isolates (two per sampled infected leaf) were purified from the PDA culture plates. The fungal colonies of three selected isolates (one per sampled infected leaf; Pj-1, Pj-2, and Pj-3) were dark brown on the PDA plates and devoid of aerial hyphae; all three isolates grew 11 mm/day on the PDA plates. The number of conidia

produced by the isolates on the 6-cm PDA plates 7 days after incubation ranged from 160×10^8 to 208×10^8 ($n = 36$). Conidia were hyaline, single-celled, and ellipsoidal (3.35 to 3.56×6.47 to 6.70 μm ; $n = 36$). To identify the pathogen, four loci, the 28S subunit (large subunit [LSU]) of the nuclear ribosomal (nr) DNA, internal transcribed spacer (ITS) region (ITS1, 5.8S subunit of nrDNA, and ITS2), the second-largest subunit of RNA polymerase II (*rpb2*), and β -tubulin (*tub2*), were amplified using the primer sets described by Chen et al. (2015). A BLASTn search against GenBank revealed that the four amplicon sequences from Pj-1, Pj-2, and Pj-3 showed 99 to 100% homology to the type strain CBS 528.66 of *D. glomerata*. A phylogenetic tree deduced from a maximum likelihood analysis of a concatenated MUSCLE-based alignment of LSU, ITS region, *rpb2*, and *tub2* sequences of 12 isolates/strains showed that the Pj isolates clustered together with CBS 528.66, along with other *D. glomerata* isolates/strains, with a high bootstrap support value (i.e., 99%). Based on both morphological characteristics and molecular phylogeny, Pj-1, Pj-2, and Pj-3 were identified as the *D. glomerata* isolates. Since the amplicon sequences of the three isolates were identical, only Pj-2 sequences were deposited in GenBank with accession numbers OM372474 (LSU), OK485138 (ITS), OM406188 (*rpb2*), and OK485135 (*tub2*). To confirm pathogenicity, 14-day-old plants (V3 growth stage) of maize cultivar P178 were spray-inoculated with Pj-2 conidia (1×10^7 conidia/ml) in a growth chamber. The inoculated leaves exhibited typical gray leaf blight lesions (similar to those detected in the maize field) 7 days post-inoculation at 25°C and 95 to 100% humidity under a 12-h photoperiod, whereas the leaves spray-inoculated with sterilized water remained healthy. The pathogenicity assay was repeated three times; the pathogen was reisolated from the inoculated leaves each time and confirmed by morphological characteristics and molecular phylogeny based on the four loci to be *D. glomerata*, fulfilling Koch's postulates. This first report of *D. glomerata* causing Didymella leaf blight on maize will help develop robust disease management strategies against this emerging fungal pathogen.

Reference:

Chen, Q., et al. 2015. Stud. Mycol. 82:137.

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