Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Bipolaris yamadae* and *Exserohilum rostratum* Associated with Leaf Spots of Maize (*Zea mays*) in Zambia

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In March 2023, leaf spot symptoms characterized by ovoid, oblong lesions with a pale brown center and small and narrow linear lesions were observed in seed maize production fields in Mkushi District, Zambia. The symptoms accounted for 70% of disease incidence and disease severity of 4 (disease scale of 0 to 9; 0 = resistant; 9 = susceptible). Six symptomatic leaf samples were collected, surface-sterilized in 70% ethanol and 1% NaClO for 30 s, rinsed three times in sterile distilled water, plated on potato dextrose agar (PDA; Becton, Dickinson, and Company, Franklin Lakes, NJ, U.S.A.) supplemented with 30 µg/ml of streptomycin (Chem Lab Suppliers), and incubated at 25°C in the dark for 5 days. Single spores were selected from each sample and cultured on fresh PDA medium. Colonies of the strains F3-1A, F3-1B, and F4-1 were pale gray after 4 days, turned dark gray after 7 days, and raised with an irregular margin. Conidia of F3-1A, F3-1B, and F4-1 were straight, curved, 7 to 9 distoseptate, and 65.0 to 90.0 × 13.0 to 15.0 µm (n = 20) and had a nonprotuberant hilum. The strain F3-2A was circular and taupe in color, with a light gray edge and cottony aerial mycelia. Conidia of F3-2A were curved, straight, ellipsoidal to fusiform, and 4 to 8 septate, had a truncated and protruding hilum, and were 50.0 to 85.0 × 10.0 to 20.0 µm (n = 20). Internal transcribed spacer (ITS) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene regions were amplified and sequenced using the primers ITS1/ITS4 (White et al. 1990) and Gpd1/Gpd2 (Berbee et al. 1999). Sequences for F3-1A, F3-1B, F4-1, and F3-2A were deposited in GenBank with accession numbers PP373829 to PP373831 and PP373833 for the ITS region and PP405110 to PP405113 for the *GAPDH* region. Nucleotide BLAST (BLASTn) analysis of F3-1A, F3-1B, and F4-1 sequences showed 100% pairwise identity (615 bp out of 615 bp for the ITS region) with the *Bipolaris yamadae* type strain CPC 28807 (GenBank accession no. MF490835). The F3-1A and F3-1B sequences showed 100% pairwise identity (527 bp out of 527 bp) while the F4-1 sequence showed 99% pairwise identity (526 bp out of 527 bp) with the *B. yamadae* strain KDI0124 (GenBank accession no. OQ538099). The F3-2A sequence showed 100% pairwise identity (571 bp out of 571 bp for the ITS region; 533 bp out of 533 bp for the GAPDH gene region) with the *Exserohilum rostratum* strain USJCC-0093 (GenBank accession no. OQ857389). The sequences of the four strains were aligned with sequences retrieved from GenBank for the ITS and *GAPDH* gene regions using MAFFT v7.505 (Katoh and Standley 2013). A concatenated maximum-likelihood phylogenetic tree (based on the ITS and *GAPDH* gene fragments) was constructed (Nguyen et al. 2015) and visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). The strains F3-1A, F3-1B, and F4-1 clustered with *B. yamadae* at 99.5% bootstrap support, whereas F3-2A clustered with *E. rostratum* at 96.7% bootstrap support. To complete Koch's postulates, B73 inbred maize plants at the V7 growth stage were whorl-inoculated with conidia at 1×10^5 conidia/ml in three replicate plants per strain. Three control plants per strain were inoculated with water. The plants were maintained in a phytotron at 20 to 25°C and at 16-h/8-h light/dark regimes. Three to five days postinoculation, the plants inoculated with fungal strains showed symptoms identical to those observed in the field, whereas no symptoms were observed on the control plants. The fungi were reisolated from the symptomatic leaves and confirmed as *B. yamadae* and *E. rostratum*, respectively. This research provides the phenotypic and molecular diagnostic tools to determine if these foliar maize pathogens in Zambia are isolated cases or part of a larger disease outbreak in the region.

References:

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