Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Aspergillus niger* Causing Preharvest Ear Rot Infection of Maize in Pakistan

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Ear rots occur globally in maize causing reduction in yield and quality of grains. In recent years, Pakistan has been facing an increased mycotoxin contamination in maize caused mainly by *Aspergillus* spp. Information on the occurrence and precise identification of *Aspergillus* spp. in maize is an important step to mitigate the impact of mycotoxins and enhance food safety in Pakistan. During 2016, harvested maize cobs of 15 varieties were obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. To isolate the pathogen, grains from 30 cobs of each cultivar were collected, surface sterilized, placed onto potato dextrose agar in Petri plates, and the plates incubated at 26°C. Cultural and morphological characteristics studies showed that 10 to 15% of samples of each genotype were infected by *Aspergillus niger*. Fungal colonies were white first and then covered with a dense layer of conidiophores and conidia. The conidial heads were radiate, vesicles, and covered with irregular metulae and phialides. Conidia were globose or subglobose (3 to 4.8 µm in diameter), dark brown to black, with rough cell walls. The morpho-cultural characters were in compliance with the description of *A. niger* (Ahmed et al. 1993). For molecular identification,

genomic DNA of four representative isolates was extracted using DNAzol reagent (Thermo Fisher Scientific, U.S.A.). PCR amplification and sequencing of the internal transcribed spacer (ITS)-rDNA, β-tubulin (BenA), partial RNA polymerase II largest subunit (RPB2), and translation elongation factor-1 alpha (TEF-1α) gene regions were performed. Primers were ITS1/ITS4 (White et al. 1990), Bt2a/Bt2b (Glass et al. 1995), RPB2-5F/RPB2-7cR (Liu et al. 1999), and A-EF_F/A-EF_R (Perrone et al. 2011), respectively. The obtained sequences were deposited in GenBank with accession numbers MN922042.1, MN922043.1, MN922044.1, and MN922045.1 for ITS-rDNA; MT117920.1, MT117921.1, MT117922.1, and MT117923.1 for the BenA; MT318284.1, MT318285.1, MT318286.1, and MT318287.1 for RPB2; and MT318307.1, MT318308.1, MT318309.1, and MT318310.1 for TEF-1α. BLASTn analysis showed 99% identity with submitted sequence of *A. niger* for ITS rDNA, BenA, RPB2, and TEF-1α. To confirm Koch's postulates, maize cultivars were grown in the research field of the Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. For inoculation, a spore suspension was prepared by adding distilled water to Petri plate of *A. niger* culture to dissolve and harvest the spores. The concentration of the spore inoculum used was determined with a hemocytometer and adjusted to 1 × 10⁶ spores/ml in sterile 0.01% Triton X-100. Using a 10-ml syringe the top ears of maize were inoculated at 30 to 35°C with *A. niger* at milk growth stage by using 0.5 to 1 ml of inoculum per ear. After 14 days, disease symptoms similar to those observed in the field appeared in all inoculated but not in uninoculated ears of maize. *A. niger* had colonized the ears, as evidenced by visible sporulation on wounded kernels. These tests were repeated twice in the field, and Koch's postulates were satisfied after reisolating the *A. niger* from the inoculated maize. *A. niger* contamination in maize was previously reported in the United States (Wicklow et al. 1987); however, to the best of our knowledge this is the first report of *A. niger* causing preharvest ear rot of maize in Pakistan. This finding will help to plan effective disease management strategies against preharvest ear rot of maize in Pakistan.

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