Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Root Rot Caused by *Bipolaris zeicola* on Maize in Hebei Province

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Maize (*Zea mays* L.) is one of the most important cereal crops in China, and the planting area reached 41.3 million hectares in 2019. Root rot is a widespread disease that occurs at the seedling stage of maize, resulting in leaf wilting, root rot, and even plant death, and consequently causing yield and quality losses. During an investigation of spring maize in 2020, seedlings with wilted leaves and dark brown necrotic spots on roots were observed in the fields in Kuancheng Manchu Autonomous County, Hebei Province, China. Symptomatic plants were collected for pathogen isolation and identification. The soil on roots was washed off with running water. Then, 2- to 3-mm necrotic root segments were sampled and surface sterilized with 75% ethanol for 2 min, rinsed three times with sterile distilled water, air dried on sterile filter paper, and plated on potato dextrose agar (PDA). Plates were incubated at 28°C in darkness for 3 days. A nonsporulating, dematiaceous fungus growing from root segments was transferred to fresh PDA plates. The colonies were round or irregular round, black, and villiform with dense grayish white mycelia. Water agar amended with wheat straw was used for sporulation. Conidiophores were single, light brown, multiseptate, and geniculate. Conidia were 38.68×10.69 to 71.98×20.57 μm , brown, oval, slightly curved, with two to eight septa and an obviously flattened hilum on the basal cell. Conidia germinated from both poles. The causal agent was identified as *Bipolaris zeicola* (G.L. Stout) Shoemaker (teleomorph = *Cochliobolus carbonum* R.R. Nelson) based on its morphological features. For molecular identification, genomic

DNA was extracted from fresh mycelia cultured on PDA plates. Partial sequences of ITS-rDNA region and *Bml* reductase melanin biosynthesis gene were amplified using primers ITS1/ITS4 (TCCGTAGGTGAACCTGCGG/TCCTCCGCTTATTGATATGC) (White et al. 1990) and Brn01/Brn02 (GCCAACATCGAGCAAACATGG/GCAAGCAGCACCGTCAATACC AAT) (Shimizu et al. 1998), respectively. A DNA fragment of ~532 bp was obtained from the ITS-rDNA region, and the sequence (GenBank accession no. MW407046) was 100% identical to the sequence of *B. zeicola* (GenBank accession no. MH864760). The sequence of *Bml* gene was ~816 bp (GenBank accession no. MW415899) and was 99.75% identical to sequence of *C. carbonum* (GenBank accession no. AB011658). The morphological and molecular evidence proved that the causal agent isolated from maize roots in Hebei Province was *B. zeicola*. Pathogenicity assays were conducted with 1-week-old (V1 stage) maize seedlings grown from the surface-sterilized seed of cultivar Zhengdan 958. The mesocotyl and radicle of each plant ($n = 3$) were inoculated with a 5-mm fungal disk of *B. zeicola*. Mock-inoculated plants ($n = 3$) with a sterile PDA disk served as the negative control. After 7 days, plants inoculated with *B. zeicola* were wilted with dark brown necrotic spots on the mesocotyl and radicle. Meanwhile, the negative controls did not present any symptoms. Koch's postulates were proved with successful reisolation of the same fungus from the inoculated maize plants. These results confirmed the pathogenicity of *B. zeicola* on maize root. *B. zeicola* mainly causes an important foliar disease in many regions of the world, known as Northern corn leaf spot; in addition, it can also cause ear rot and stalk rot of maize (Liu et al. 2015). To our knowledge, this is the first report of root rot caused by *B. zeicola* on maize in China, which extends the known agents of maize root rot. Therefore, it is necessary to explore effective seed-applied fungicides for disease control. Also, more attention should be paid to develop hybrids with resistance to this disease.

References:

- Liu M., et al. 2015. PLoS ONE 10:e0119858.
- Shimizu, K., et al. 1998. J. Gen. Appl. Microbiol. 44:251.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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