Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Leaf Spot of Rice Caused by *Bipolaris zeicola* in Pakistan

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Rice (*Oryza sativa* L.) is one of the highly consumed cereal grain crops in Pakistan. In September 2017, leaf samples of cultivar Basmati-385 showing brown to dark brown spots (5 to 9 mm in diameter) that were oval or cylindrical in shape with a chlorotic yellow halo and grayish tan centers were collected from fields near the University of Agriculture, Faisalabad (31.43633 N, 73.05981 E). Average disease incidence was 69% in six rice fields that were sampled for diseased plants with visible symptoms. To isolate the pathogen, from 20 diseased leaves, 5-mm² segments from the margins of lesions were cut, rinsed with sterile distilled water (SDW), surface disinfected by 70% ethanol, and again rinsed with SDW. The samples were dried on sterilized filter paper discs, plated on potato dextrose agar, and incubated at 27°C for 5 to 7 days. Twelve isolates were subcultured, and single-sporing was performed to obtain pure cultures. Fungal isolates with light to dark gray in color, thick or fluffy aerial mycelium, circular and smooth margins were obtained after 7 days of incubation. Conidia were 47 to 83 × 10 to 17 µm ($n = 100$), with four to 10 distosepta, dark or olivaceous brown, straight or moderately curved, and the cells at the ends occasionally looked paler than those in the middle. Conidiophores of the fungus were simple, smooth, cylindrical, septate, and straight to flexuous. These characteristics resembled those of *Bipolaris zeicola* (Stout) Shoemaker (Manamgoda et al. 2014). For molecular

identification, genomic DNA (isolate SU-11) was extracted, and the internal transcribed spacer (ITS) region, large subunit (LSU) of ribosomal DNA, translation elongation factor (*tef*), glyceraldehyde 3-phosphate dehydrogenase (*gpd*), and RNA polymerase II second largest subunit (*rpb2*) genes were amplified and sequenced by using the primers ITS1-F/ITS4-R (White et al. 1990), LROR-F/LR5-R (Schoch et al. 2012), EF1-983F/EF1-2218R (Rehner and Buckley 2005), GPD1F/GPD2R (Berbee et al. 1999), and 5F2/7CR (O'Donnell et al. 2007), respectively. BLASTn searches showed 100% homology with the LSU and *rpb2* sequences of *B. zeicola* (GenBank accession nos. MH876201 and HF934842) and 98 to 99% similarity with ITS, *tef*, and *gpd* sequences of *B. zeicola* (GenBank accession nos. KM230398, KM093752, and KM034815). The sequences of ITS, LSU, *tef*, *gpd*, and *rpb2* were deposited in GenBank with accession numbers MN871712, MN877767, MN867685, MN904511, and MT349837, respectively. To fulfill Koch's postulates, 25 greenhouse-grown rice plants (cv. Basmati-385) at two- to three-leaf stage were spray inoculated with a spore suspension (10⁵ spores/ml; isolate SU-11) prepared in SDW. Plants were covered with plastic wraps to maintain humid conditions for 24 h and incubated at 27°C for 1 week. Similarly, 10 noninoculated plants sprayed with SDW served as controls. After 1 week, observed symptoms were similar to those from natural infections, and no disease symptoms were observed on the noninoculated plants. The experiment was repeated twice, and the pathogen was reisolated from the infected leaves and characterized morphologically. Globally, *B. zeicola* has also been reported to cause the leaf spot of rice and maize plants (Kang et al. 2018; Sivanesan 1987). To our knowledge, this is the first report of *B. zeicola* causing brown leaf spot of rice in Pakistan. The increasing risk of this fungal pathogen in the rice-growing areas of Pakistan needs a rigorous exploration and outreach effort to develop effective management practices.

References:

- Berbee, M., et al. 1999. Mycologia 91:964.
- Kang, I. J., et al. 2018. Plant Pathol. J. 34:327.
- Manamgoda, D., et al. 2014. Stud. Mycol. 79:221.
- O'Donnell, K., et al. 2007. J. Clin. Microbiol. 45:2235.
- Rehner, S. A., and Buckley, E. 2005. Mycologia 97:84.
- Schoch, C. L., et al. 2012. Proc. Natl. Acad. Sci. U.S.A. 109:6241.
- Sivanesan, A. 1987. Mycol. 158:1.
- 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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