Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Leaf Spot of Rice Caused by *Curvularia hawaiiensis* in the United States

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Multiple diseases, including brown spot (*Cochliobolus miyabeanus*), leaf spot (*Epicoccum sorghinum*), and blast (*Magnaporthe oryzae*), can cause spot-like symptoms on the leaves of rice. In July 2021, a disease showing symptoms like brown spot was observed in an 8-ha field of rice, with disease incidence of >30%, in Beaumont, Texas. Lesions started as small pinhead-size blackish spots on leaf tips or from the edges of leaf blades. The spots enlarged to become irregular (most) or oval brown spots with a slight chlorotic halo. Diseased leaves were collected, washed in running tap water, and cut into small pieces. Pieces of the tissue were surface sterilized with 1% NaOCl for 2 min followed by 75% ethanol for 30 s and then washed in sterile distilled water for 1 min three times. The disinfected tissue pieces were air dried, placed on potato dextrose agar (PDA) medium, and incubated at 25°C. Initially, fungal colonies were hairy in texture with a light dark brown center and whitish edge and dark brown pigmentation on the reverse side. Mature colonies turned black in the center and dark brown toward the edge, with black on the reverse side after 2 or more weeks of incubation. Conidia were oval to narrowly oblong, rounded at the ends, two to six distoseptate, and 15 to 35 × 6 to 10 µm. These morphological characteristics were similar to those described for *Curvularia hawaiiensis* (Aslam et al. 2019; Ellis 1971; Kusai et al. 2015). For molecular identification, DNA was extracted and two

different rRNA regions, internal transcribed spacer (ITS) and large subunit (LSU), and two genes, RNA polymerase II (*RPB1*) and translation elongation factor 1 alpha (*EF1*), of the fungus were amplified using the primers of ITS1/ITS4 (Wang et al. 2014), D1/D2 domain region of LSU (Fell et al. 2000), and *RPB1* and *EF1* (Wang et al. 2014), respectively, and sequenced. The ITS sequence (OK397200) was 98.27% identical to *C. hawaiiensis* (KP131943); the *EF1* sequence (OK492159) was 99.78% identical to *C. hawaiiensis* (KC503942); the LSU sequence (OK397295) was 98.96% identical to multiple *C. hawaiiensis* (MN055715, MH160813, MH875853, etc.); and the *RPB1* sequence (OK492160) was 97.41% identical to *C. hawaiiensis* (JN992363). To evaluate pathogenicity, three rice plants (cv. Presidio) at the three-leaf stage were spray inoculated with a conidial suspension of 1×10^6 conidia/ml. Another set of three plants that were sprayed with sterilized distilled water served as the controls. Treated plants were maintained in a greenhouse with temperatures of 25 to 30°C. After 2 weeks, typical symptoms, like those observed in the field, developed on the inoculated plants while no symptoms developed on the control plants. The same fungus was consistently reisolated from the diseased plants. The pathogenicity test was conducted three times with similar results. To our knowledge, this is the first report of brown leaf spot caused by *C. hawaiiensis* in rice in the United States. *Curvularia* species are frequently associated with rice grain and cause blackish discoloration symptoms on grain kernels. Recently, however, *C. hawaiiensis* has also been reported to cause brown leaf spot in Malaysia (Kusai et al. 2015) and Pakistan (Aslam et al. 2019). This research will help identify this disease from other leaf spot-like diseases and develop effective management strategies.

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