Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Bipolaris oryzae* Causing Leaf Spot on Cultivated Wild Rice (*Oryza rufipogon*) in China

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Wild rice (*Oryza rufipogon*) has been widely studied and cultivated in China in recent years due to its antioxidant activities and health-promoting effects. In December 2018, leaf spot disease on wild rice (*O. rufipogon* cv. Haihong-12) was observed in Zhanjiang (20.93° N, 109.79° E), China. The early symptom was small purple-brown lesions on the leaves. Then, the once-localized lesions coalesced into a larger lesion with a tan to brown necrotic center surrounded by a chlorotic halo. The diseased leaves eventually died. Disease incidence was higher than 30%. Twenty diseased leaves were collected from the fields. The margin of diseased tissues was cut into 2 × 2-mm² pieces, surface disinfected with 75% ethanol for 30 s and 2% sodium hypochlorite for 60 s, and then rinsed three times with sterile water before isolation. The tissues were plated on potato dextrose agar (PDA) medium and incubated at 28°C in the dark for 4 days. Pure cultures were produced by transferring hyphal tips to new PDA plates. Fifteen isolates were obtained. Two isolates (OrL-1 and OrL-2) were subjected to further morphological and molecular studies. The colonies of the isolates on PDA were initially light gray but became dark gray with age. Conidiophores were single, straight to flexuous, multiseptate, and brown. Conidia were oblong, slightly curved, light brown with four to nine septa, and measured 35.2 to 120.3 × 10.3 to 22.5 μm (*n* = 30). The morphological characteristics of OrL-1 and OrL-2 were consistent with the description on *Bipolaris oryzae* (Breda de Haan) Shoemaker

(Manamgoda et al. 2014). The internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and translation elongation factor (*EF-1α*) were amplified using primers ITS1/ITS4, gpp1/gdp2 (Berbee et al. 1999), and EF-1/EF-2 (O'Donnell et al. 1998), respectively. Amplicons of OrL-1 and OrL-2 were sequenced and submitted to GenBank (accession nos. MN880261 and MN880262, MT027091 and MT027092, and MT027093 and MT027094). The sequences of the two isolates were 99.83 to 100% identical to that of *B. oryzae* (accession nos. MF490854, MF490831, and MF490810) in accordance with BLAST analysis. A phylogenetic tree was generated on the basis of concatenated data from the sequences of ITS, *GAPDH*, and *EF-1α* via the maximum likelihood method, which clustered OrL-1 and OrL-2 with *B. oryzae*. The two isolates were determined as *B. oryzae* by combining morphological and molecular characteristics. A pathogenicity test was performed on OrL-1 in a greenhouse at 24 to 30°C with 80% relative humidity. Rice (cv. Haihong-12) with three leaves was grown in 10 pots, with approximately 50 plants per pot. Five pots were inoculated by spraying a spore suspension (10⁵ spores/ml) onto leaves until runoff occurred, and five pots were sprayed with sterile water and used as controls. The test was conducted three times. Disease symptoms were observed on leaves after 10 days, but the controls remained healthy. The morphological characteristics and ITS sequences of the fungal isolates reisolated from the diseased leaves were identical to those of *B. oryzae*. *B. oryzae* has been confirmed to cause leaf spot on *Oryza sativa* (Barnwal et al. 2013) but as an endophyte has been reported in *O. rufipogon* (Wang et al. 2015). Thus, this study is the first report of *B. oryzae* causing leaf spot in *O. rufipogon* in China. This disease has become a risk for cultivated wild rice with the expansion of cultivation areas. Thus, vigilance is required.

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