Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Curvularia lunata* on Wild Rice in China

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Wild rice (*Oryza rufipogon*), a species only recently cultivated in China, is an invaluable resource for rice breeding and basic research. In June 2019, a leaf spot disease on wild rice (*O. rufipogon* 'Haihong-12') was observed in a 3.3-ha field in Zhanjiang (20.93° N, 109.79° E), China. The early symptoms were the presence of small, brown, and circular to oval spots that eventually turned reddish brown. The size of the spots was 1.0 to 5.0 × 1.0 to 3.0 mm. Disease incidence was higher than 20%. High-temperature and high-humidity climate were favorable for the disease occurrence. Twenty diseased leaves were collected from the field. The margin of the diseased tissues was cut into 2 × 2 mm 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 onto 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. Three isolates, namely, Cls-1, Cls-2, and Cls-3, were subjected to further morphological and molecular studies. The colonies of the three isolates on PDA were initially light gray, later becoming dark green. Conidiophores were erect, dark brown, geniculate, and unbranched. Conidia were fusiform, geniculate, or hook-shaped, smooth-walled, dark-brown, three-septate, with the second curved cell about 13.4 to 18.2 × 6.5 to 8.6 µm in size ($n = 30$). These morphological features agreed with previous descriptions of *Curvularia lunata* (Wakker) Boed (Macri and Lenna 1974). 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 and Cigelnik 1997), respectively. Amplicons of the three isolates were sequenced and submitted to GenBank (accession nos. MW042182, MW042183, and MW042184; MW091453, MW091454, and MW091455; and MW090049, MW090050, and MW090051). The sequences of the two isolates were 100% identical to those of *C. lunata* (accession nos. MG971304, MG979801, and MG979800) according to the results of BLAST analysis. A phylogenetic tree was built on the basis of concatenated data from the sequences of ITS, *GAPDH*, and *EF-1α* via the maximum likelihood method. The tree clustered Cls-1, Cls-2, and Cls-3 with *C. lunata*. The three isolates were determined as *C. lunata* by combining morphological and molecular characteristics. Pathogenicity tests were performed on Cls-1 in a greenhouse at 24 to 30°C with 80% relative humidity. Individual rice plants (Haihong-12) with three leaves were grown in 10 pots, with approximately 50 plants per pot. Five pots were inoculated by spraying a spore suspension (10^5 spores/ml) onto leaves until runoff occurred, and another five pots were sprayed with sterile water and used as controls. The test was conducted three times. Disease symptoms were observed on the leaves after 10 days, but the controls remained healthy. *C. lunata* occurs on *O. sativa* (rice) (Liu et al. 2014; Majeed et al. 2016), but it has not been reported on *O. rufipogon* until now. To the best of our knowledge, this study is the first to report that *C. lunata* causes leaf spots on *O. rufipogon* in China. Thus, vigilance is required for breeding *O. rufipogon*.

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