Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Brown Leaf Spot on Wild Rice (*Oryza rufipogon*) in China

F. T. Zhong, Y. L. Liu,[†] D. F. Zheng, and S. L. Lu

Guangdong Ocean University, Zhanjiang, China 524088

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Oryza rufipogon Griff is a wild rice germplasm that might contain genes valuable for rice breeding. In May to June 2019, a leaf disease on wild rice (*O. rufipogon* ‘Haihong-12’) was observed in a 3.3-ha field in Zhanjiang (20.93° N, 109.79° E), Guangdong, China. Early symptoms were yellow spots on the tips of leaves. Later, the spots gradually expanded down the entire leaf to turn brown in turn. Symptoms were found in the tillering to the grain-filling stages. The disease incidence on plants was between 10 and 40%. 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 and 2% sodium hypochlorite for 30 and 60 s, respectively, and rinsed three times with sterile water before isolation. The tissues were plated onto potato dextrose agar (PDA) medium and incubated at 28°C. After a 5-day incubation, grayish fungal colonies appeared on PDA. The single-spore isolation method was used to recover pure cultures for three isolates (Aas-1, Aas-2, and Aas-3). The colonies first produced light-grayish aerial mycelia, which turned dark grayish upon maturity. Conidiophores were branched. Conidia were two to four in chains, dark brown, ovoid or ellipsoid, and mostly beakless; had one to four transverse and zero to three longitudinal septa; and measured 7.0 to 18.5 (average = 12.5) × 3.0 to 8.8 (average = 4.5) μm ($n = 30$). Morphological characteristics of the isolates were consistent with the description of *Alternaria alternata* (Fr.) Keissler

(Simmons 2007). The internal transcribed spacer (ITS) region, partial RNA polymerase II largest subunit (*RPB2*) gene, translation elongation factor (*TEF*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were amplified with primers ITS1/ITS4, RPB2-6F/RPB2-7R, EF-1α-F/EF-1α-R, and GDF1/GDR1, respectively (Woudenberg et al. 2015). Amplicons were sequenced and submitted to GenBank (accession nos. MW042179 to MW042181, MW090034 to MW090036, MW090046 to MW090048, and MW091450 to MW091452, respectively). The sequences of the three isolates were 100% identical (ITS, 570/570 bp; *RPB2*, 1,006/1,006 bp; *TEF*, 254/254 bp; and *GAPDH*, 587/587 bp) with those of CBS 479.90 (accession nos. KP124319, KP124787, KP125095, and KP124174) through BLAST analysis. The sequences were also concatenated for phylogenetic analysis by maximum likelihood. The isolates clustered with *A. alternata* CBS 479.90. The fungus associated with brown leaf spot on wild rice was thus identified as *A. alternata*. Pathogenicity tests were done in a greenhouse at 24 to 30°C with 80% relative humidity. Individual rice plants (cv. Haihong-12) with three leaves were grown in 10 pots, with around 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 to serve as controls. The test was done three times. Disease symptoms were found on the leaves after 7 days. The tips of the leaves turned yellow and spread downward. Then, the whole leaf turned brown and dried out, but the controls stayed healthy. The pathogen was reisolated from infected leaves and phenotypically identical to the original isolate Aas-1 to fulfill Koch’s postulates. To our knowledge, this report is the first one on *A. alternata* causing brown leaf spot on wild rice (*O. rufipogon*). The pathogen has the potential to reduce wild rice yields, and future breeding should consider resistance to this pathogen.

References:

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[†]Indicates the corresponding author.
Y. L. Liu; mushwoman@126.com