Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Nigrospora oryzae* in Wild Rice in China

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In recent years, wild rice (*Oryza rufipogon* Griff) has been widely cultivated because of its health-promoting effects. In May 2019, leaf spot lesions on cultivar Haihong-12 were observed in Zhanjiang (20.93N, 109.79E), China. Leaf symptoms were yellow to brown, oval or circular lesions with a very distinctive large yellow halo. Black spores appeared on the leaves with advanced symptoms. The lesions coalesced, causing the entire leaf to become blighted and die. Disease incidence reached approximately 10% in the fields (8 ha) surveyed. Twenty leaves with symptoms were collected and cut into 2 × 2-cm pieces. They were surface disinfected with 75% ethanol for 30 s and 2% sodium hypochlorite (NaOCl) for 60 s, rinsed three times with sterile water, blotted dry on sterile paper, plated on potato dextrose agar (PDA) medium, and incubated at 28°C in the dark for 4 days. Ten pure cultures were obtained by transferring hyphal tips to new PDA plates, and monospore cultures were obtained from three isolates (Nos-1, Nos-2, and Nos-3). Those isolates exhibited very similar morphological characteristics on PDA. The colony of isolate Nos-1 was white at the early stage and became dark gray after 7 days. Conidia were produced from clusters of conidiophores, single celled, black, smooth, spherical, and 9.5 to 14.2 μm (average 10.6 ± 0.42 μm) in diameter. Morphological characteristics of the

isolates matched the description of *Nigrospora oryzae* Petch (Wang et al. 2017). The ITS region was amplified using primers ITS1 and ITS4 (White et al. 1990). Nucleotide sequences of isolates Nos-1, Nos-2, and Nos-3 deposited in GenBank under accession numbers MW042173, MW042174, and MW042175, respectively, were 100% identical to *N. oryzae* (KX985944, KX985962, and KX986007). A phylogenetic tree generated based on the ITS sequences and using a maximum likelihood method with 1,000 bootstraps showed that these three isolates from wild rice were grouped with other *N. oryzae* isolates downloaded from GenBank (bootstrap = 100%) but away from other *Nigrospora* spp. Pathogenicity testing was performed with these three isolates in a greenhouse at 24 to 30°C. Approximately 50 seedlings of wild rice cultivar Haihong-12 were grown in each pot. At the three-leaf stage, plants in three pots were inoculated with each isolate by spraying a spore suspension (10⁵ spores/ml) until runoff. Three pots sprayed with sterile water served as the controls. Each three-pot treatment was separately covered with a plastic bag. The test was conducted three times. Disease symptoms were observed on the inoculated leaves after 10 days, whereas no disease was observed in the control plants. Morphological characteristics and the ITS sequences of fungal isolates reisolated from the diseased leaves were identical to those of *N. oryzae*. *N. oryzae* has been reported to cause leaf spot on *O. sativa* (Wang et al. 2017) but not on *O. rufipogon*. Thus, this is the first report of *N. oryzae* causing leaf spot of *O. rufipogon* in China. The finding provides information important for further studies to develop management strategies for control of this disease.

References:

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