Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spots on Oat Caused by *Alternaria alternata* in South Korea

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Oat, a gluten-free source of vitamins, minerals, fiber, and antioxidants required for human health (Rasane et al. 2015), is increasingly being cultivated in South Korea (Ju et al. 2011). In May 2020, ~30% of *Avena sativa* (cv. Samhan) leaves of a commercial oat field in Jeongeup, Korea (35.3859°N, 126.5607°E), displayed a leaf spot disease at the tillering growth stage. Small, brown spots appeared initially on the lower leaves but progressed to dark-brown oval or irregular, necrotic spots with a light-yellowish border. To isolate the pathogen, tissue excised from growing edges of about 50 lesions was surface sterilized (immersion in 70% ethanol and then 1% sodium hypochlorite), rinsed, and transferred to potato dextrose agar (PDA). Five isolates were purified using the single-spore culture method on water agar plates (Choi et al. 1999). Conidia from the PDA cultures ($n = 50$) were elongated, ellipsoidal, and obclavate, with one to five septa (primarily transverse), 18 to 35×7 to 12 μm . The morphological characteristics of the fungus from each culture were consistent with those observed for *Alternaria alternata* (Chen et al. 2020; Simmons 2007). To confirm the identity of the fungal pathogen, the internal transcribed spacer (ITS) region was amplified with universal primers ITS1/ITS4 and sequenced (White et al. 1990). BLAST analysis of the resulting nucleotide sequence of one representative isolate, Oaa-2 (GenBank MT786424), showed 100% identity with *A. alternata* type strains, such as CBS916.96. Further, nine nuclear protein-coding genes from the Oaa-2 isolate were amplified using previously described primers (Lawrence et al. 2013; Woudenberg et al. 2015). These sequences were deposited under the following GenBank accession numbers: MT795660 (*rpb2*), MT795656 (*His*), MT795657 (*CML*),

MT795658 (*ACT*), MT787343 (*CHSD*), MT795655 (*GAPDH*), MT795661 (*endoPG*), and MT795662 (*TEF1*). The nine sequences of the Oaa-2 isolate showed 99 to 100% identity to those of *A. alternata* type strain CBS916.96 in NCBI. The supplementary materials show the phylogenetic trees for the ITS and *GAPDH* sequences, indicating high homology. Pathogenicity of this isolate was confirmed by spraying leaves of surface-sterilized, glasshouse-grown Samhan oats with suspensions (5×10^6 spores/ml) of Oaa-2 conidia. Experimental seedlings were grown for 2 weeks in a chamber at $20 \pm 3^\circ\text{C}$ in 60% relative humidity with a 10-h light/14-h dark cycle ($200 \mu\text{mol/m}^2/\text{s}$). Conidial suspensions were prepared for inoculation by flooding with sterile water, followed by filtering the suspension through three layers of sterile cheesecloth to remove debris. Three pots each containing 20 oat seedlings were sprayed with the Oaa-2 isolate conidial suspension (5×10^6 conidia/ml) and incubated for 10 days at $20 \pm 3^\circ\text{C}$ in 100% humidity with a 10-h photoperiod. Three healthy seedlings were sprayed with sterile water as a control. After 10 days, dark brown spots developed in the inoculated plants; however, no spots were observed in the controls. Fungi with the same conidial characteristics as Oaa-2 were isolated from the infected plants, and the ITS sequence and the nine nuclear protein-encoding genes of these plant isolates was identical to those of Oaa-2. Pathogenicity tests also were conducted with isolate Oaa-2 with two oat cultivars, Sunyang and Joyang, with the same result. To our knowledge, *A. alternata* has not been previously reported to cause disease on oats in South Korea, although it does cause economic losses in China (Chen et al. 2020).

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