Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Alternaria brassicae* on *Avena sativa* in China

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Oat (*Avena sativa*) is an annual gramineous crop, which contains a source of soluble dietary fiber, β -glucan, unsaturated fatty acids, vitamins, minerals, phenolic acids, and avenanthramides. It widely cultivated in cool and semi-arid areas in northern China (Li et al. 2017). In July 2018, a severe leaf spot infection was observed in the Forage Germplasm Nursery (31°17'22"N, 103°40'15"E, 2,885 m elevation) in Tianzhu County, Wuwei City of Gansu Province in China. Disease incidence (total number of diseased leaves/total number of surveyed leaves $\times$ 100%) was 93% over 300 m² planting area. Symptoms initially appeared as small circular to irregular, gray-green, water-soaked spots on the leaves in the middle or along the margin of leaves, which enlarged and coalesced. The center of the leaf spots turned brown to reddish-brown. Infected tissues from symptomatic leaves were cut into small pieces (5 $\times$ 5 mm), surface sterilized with 70% ethanol for 60 s, soaked in 5% commercial bleach (~0.275% NaClO) for 5 min (Xue et al. 2018), rinsed five times with distilled water, plated on potato dextrose agar (PDA) medium, and incubated for 3 days in the dark at 25°C (Blagojević et al. 2020; Humpherson-Jones and Phelps 1989; Zhang 2003). Hyphae emerging from the tissue were subcultured on fresh PDA medium for purification. All colonies were light brown with intensive grayish white sporulation in rings that later became grayish brown. The back of the colony was dark brown. Conidiophores were light brown, unbranched, grew vertically on hyphae, and each conidiophore produced 3 to 7 conidia (mostly 6). Conidia were light brown, septate, straight to slightly curved, single or in chains, oval or obclavate, measured 17 to 32 μ m wide and 63 to 106 μ m long with the conical beak cell, 7 to 12 transverse septa, 0 to 5 longitudinal septa. These morphological characteristics were similar to the descriptions of *Alternaria* spp. (Simmons 2007). A single isolate, YMZZ1, was selected for molecular identification. The ITS region of rDNA,

partial GAPDH, and Tef1- α gene sequences were amplified by PCR with the primer pairs of ITS1/ITS4, gpd1/gpd2, and EF1-728F/EF1-986R, respectively (Woudenberg et al. 2013). Sequences were deposited in GenBank under accessions MN446739 (ITS), MN481462 (GAPDH), and MN464104 (Tef1- α). A nucleotide BLAST search revealed ITS, GAPDH, and Tef1- α sequences to be 99% similar to accessions numbers MN856410 (565/573 bp), MK026431 (575/575 bp), and MH754531 (211/211 bp), respectively, of *Alternaria brassicae*. Neighbor-joining (NJ) and maximum likelihood (ML) phylogenetic analysis were conducted based on ITS, GAPDH, and TEF-1 α sequences using MEGA7.0 under Kimura 2-parameter model. The isolate YMZZ1 clustered with a representative strain *Alternaria brassicae* LGBA22 with 100% bootstrap support. To test its pathogenicity, six healthy 3-week-old plants were spray-inoculated with a suspension of 3×10^5 conidia/ml of YMZZ1. The same number of plants were sprayed with sterilized water as control. All plants were covered with transparent plastic bags for 48 h to maintain high relative humidity and incubated in a 25°C growth chamber (16/8 h light/dark) for observation. Ten days after inoculation, leaf spot symptoms were observed on leaves similar to those previously observed in nursery; no symptoms were observed on the control. The pathogenicity test was repeated twice under the same conditions and *Alternaria brassicae* was reisolated from inoculated plants each time, fulfilling Koch's postulates. *Alternaria brassicae* has not been previously reported as a pathogen of *Avena sativa* in the world, but it has been mentioned as a pathogen of horse radish (*Armoracia rusticana*) in Serbia (Blagojević et al. 2015). To our knowledge, this is the first report of leaf spot caused by *Alternaria brassicae* on *Avena sativa* in China. This study stresses an urgent need to identify appropriate management strategies of *Alternaria brassicae* that help in preventing losses in quality and yield of oats in northern China.

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