Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot of Tartary Buckwheat in China

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Buckwheat (*Fagopyrum tataricum*) is recognized as a healthy food with abundant nutrients and high levels of rutin. In April and May of 2020, an unknown tartary buckwheat leaf spot distinct from *Nigrospora* leaf spot (Shen et al. 2021) was observed in Xiangxiang, Hunan, China (27°49'54"N, 112°18'48"E). Disease incidence was 60 to 70% within three fields (7,000 m² in total). The disease occurred after plants emerged. Initial symptoms began as circular or ellipsoid, chlorotic, water-soaked spots, mostly on leaf apexes or leaf margins. The small spots gradually enlarged and often coalesced to form large, circular or irregular, pale to light brown lesions, and the infected leaves eventually withered and fell off. Thirty 2 × 2 mm infected tissue pieces collected from five locations were sterilized in 70% ethanol for 10 s, in 2% NaClO for 30 s, rinsed in sterile water three times, dried, and placed on potato dextrose agar (PDA) with lactic acid (3 ml/liter). After 3 to 5 days at 28°C in the dark, 17 fungal isolates were purified using the single-spore isolation method. Almost all fungal isolates had similar morphology. Colonies were initially olive green with white margins and later turned dark olive or black with profuse sporulation. Conidia were borne in long chains, tawny to brownish green, with 1 to 3 longitudinal and 1 to 7 transverse septa,

pyriform, and measured 9.5 to 39.6 µm long and 5.1 to 12.6 µm wide ($n = 50$). Based on morphological characteristics, the fungus was identified as *Alternaria alternata* (Simmons 2007). Partial internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), translation elongation factor 1- α (TEF), and *Alternaria* major allergen (*Alt a1*) genes of isolate BLS-1 were amplified using ITS1/ITS4 (Mills et al. 1992), EF1-728F/EF1-986R (Carbone and Kohn 1999), Gpd1/Gpd2, and Alt-4for/Alt-4rev (Lawrence et al. 2013), respectively. Sequences were deposited into GenBank with accession nos. MW453091 (*ITS*), MW480219 (*GAPDH*), MW480218 (*TEF*), and MW480220 (*Alt a1*). BLASTn analysis showed 99.8% (*ITS*, MH854758.1), 100% (*GAPDH*, KP124155.1), 99.8% (*TEF*, KP125073.1), and 100% (*Alt a1*, KP123847.1) identity with reference strain CBS 106.24 of *A. alternata*, confirming isolate BSL-1 to be *A. alternata*. A neighbor-joining phylogenetic tree constructed by MEGA7.0 based on concatenated sequences of the four genes indicated that BSL-1 formed a distinct clade with *A. alternata* CBS 106.24 with 100% bootstrap values. Pathogenicity test was performed three times on healthy leaves. Twenty leaves of five 20-day-old plants (cv. Pinku1) were sprayed with conidial suspension (1×10^6 conidia/ml) collected from PDA cultures with 0.05% Tween 20. An equal number of control leaves were sprayed with sterile water to serve as the controls. Treated plants were kept in a greenhouse at $28 \pm 3^\circ\text{C}$ with $80 \pm 5\%$ relative humidity (RH) for 24 h and transferred to natural conditions (22 to 30°C, 50 to 60% RH). After 4 to 6 days, all inoculated leaves developed symptoms similar to those observed in the fields, while control leaves remained healthy. *A. alternata* was reisolated from all infected leaves. Occasionally isolated *Diaporthe* isolates were not pathogenic. *A. alternata* causes leaf spot of oat (Zhao et al. 2021) and leaf blight of *Fagopyrum esculentum* (Lu et al. 2019). To our knowledge, this is the first report of *A. alternata* causing leaf spot on *F. tataricum* in China and the world. Effective strategies should be developed to manage the disease.

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