

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

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15 Buckwheat (*Fagopyrum tataricum*) is recognized as a healthy food with
16 abundant nutrients and high levels of rutin. In April and May of 2020, an unknown
17 tartary buckwheat leaf spot distinct from *Nigrospora* leaf spot ([Shen et al. 2020](#)) was
18 observed in Xiangxiang, Hunan, China ([27°49'54"N](#), [112°18'48"E](#)). Disease
19 incidence was 60-70% within three fields (totally 7, 000 m²). The disease occurred
20 after plants emerged. Initial symptoms began as circular, or ellipsoid, chlorotic,
21 water-soaked spots, mostly on leaf apices or leaf margins. The small spots gradually

22 enlarged and often coalesced to form large circular or irregular, pale to light brown
 23 lesions, and the infected leaves eventually withered and fell off. Thirty 2×2 mm
 24 infected tissue pieces collected from five locations were sterilized in 70% ethanol for
 25 10 S, in 2% NaClO for 30 S, rinsed in sterile water for three times, dried, and placed
 26 on PDA with lactic acid (3 ml/L). After 3-5 days at 28°C in the dark, 17 fungal
 27 isolates were purified using single-spore isolation method. Almost all fungal isolates
 28 had similar morphology. Colonies were initially olive green with white margin and
 29 later turned dark olive or black with profuse sporulation. Conidia were borne in long
 30 chains, tawny to brownish green, with 1-3 longitudinal and 1-7 transverse septa,
 31 pyriform, and measured 9.5-39.6 μm long, and 5.1-12.6 μm wide (n=50). Based on
 32 morphological characteristics, the fungus was identified as *Alternaria alternata*
 33 (Simmons 2007). Partial internal transcribed spacer (ITS),
 34 glyceraldehyde-3-phosphate dehydrogenase (GAPDH), translation elongation factor
 35 1- α (TEF) and *Alternaria* major allergen (Alt a1) genes of isolate BLS-1 were
 36 amplified using ITS1/ITS4 (Mills et al. 1992), EF1-728F/EF1-986R (Carbone and
 37 Kohn 1999), Gpd1/Gpd2 and Alt-4for/Alt-4rev (Lawrence et al. 2013), respectively.
 38 Sequences were deposited into GenBank with acc. nos MW453091 (ITS), MW480219
 39 (GAPDH), MW480218 (TEF), and MW480220 (Alt a1). BLASTn analysis showed
 40 99.8% (ITS, MH854758.1), 100% (GAPDH, KP124155.1), 99.8% (TEF, KP125073.1)
 41 and 100% (Alt a1, KP123847.1) identity with reference strain CBS 106.24 of
 42 *A. alternata*, confirming isolate BSL-1 to be *A. alternata*. A neighbor-joining

43 phylogenetic tree constructed by MEGA7.0 based on concatenated sequences of the
44 four genes indicated that BSL-1 formed a distinct clade with *A. alternata* CBS 106.24
45 with 100% bootstrap values. Pathogenicity test was triplicately performed on healthy
46 leaves. Twenty leaves of five 20-day-old plants (cv. Pinku1) were sprayed with
47 conidial suspension (1×10^6 conidia/ml) collected from PDA cultures with 0.05%
48 Tween 20. An equal number of control leaves were sprayed with sterile water to serve
49 as the controls. Treated plants were kept in a greenhouse at 28 ± 3 °C with relative
50 humidity of $80 \pm 5\%$ for 24 h and transferred to natural conditions ($22-30^\circ\text{C}$, RH
51 50-60%). After 4 to 6 days, all inoculated leaves developed symptoms similar to those
52 observed in the fields, while the control leaves remained healthy. *A. alternata* was
53 re-isolated from all infected leaves. Occasionally-isolated *Diaporthe* isolates were not
54 pathogenic. *A. alternata* causes leaf spot of oat (Zhao et al. 2020) and leaf blight of *F.*
55 *esculentum* (Lu et al. 2019). To our knowledge, this is the first report of
56 *A. alternata* causing leaf spot on *F. tataricum* in China and the world. Effective
57 strategies should be developed to manage the disease.

58 *References:*

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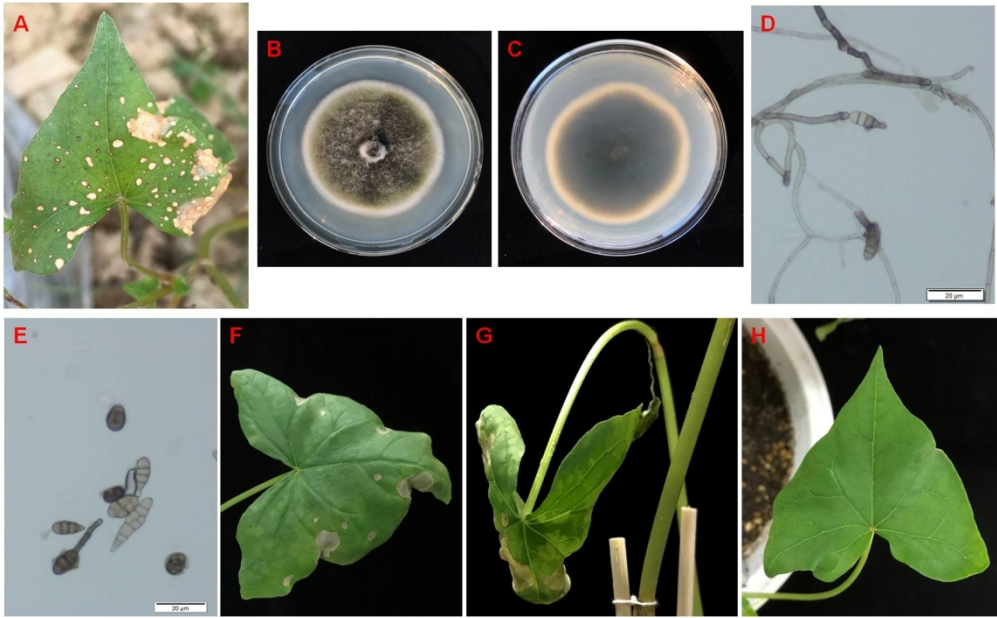


Fig. 1 Leaf spot symptoms caused by *A. alternata* on *Fagopyrum tataricum* in field (A); The front (B) and back (C) of the colony on the PDA; Conidiophores (D) and conidia (E), bar=20 µm; Symptoms of leaves inoculated with *A. alternata* conidial suspension after 5 d (F) and 8 d (G), or with sterile water (H) after 5 d.

288x177mm (150 x 150 DPI)

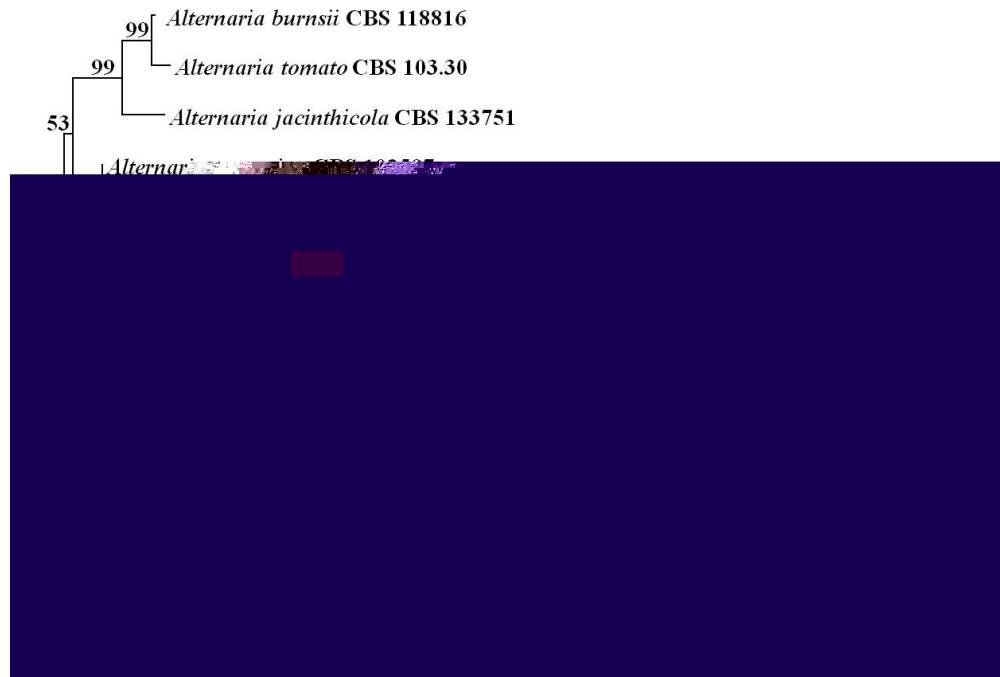


Fig. 2 Molecular phylogenetic tree based on ITS, TEF, GADPH and ALT gene sequence alignment generated from a neighbor joining analysis. Values above the branches represent bootstrap support values (>50%). *Alternaria alternantherae* was used as an outgroup. The isolate BSL-1 in this study is shaded in pink.

212x145mm (150 x 150 DPI)