Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria tenuissima* Causing Leaf Spot of Celery (*Apium graveolens*) in China

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Celery (*Apium graveolens*) is one of the most widely grown vegetables in the world. A survey in Anding District of Gansu Province in 2019 showed that the incidence of celery leaf spot was 25 to 45%. The disease mainly occurs in late June and July. A high-temperature and high-humidity environment is conducive to the onset of the leaf spot. The initial symptoms were many small, light brown, irregular-shaped spots on the leaves. The lesions gradually enlarged in the later stage of the disease, and multiple lesions coalesced to form large irregular brown spots, and eventually the whole leaves died. A 3- to 4-mm leaf tissue was cut from the junction of the diseased leaf and the healthy area, and the leaf tissue was surface sterilized in 1.5% NaClO for 1 min and washed with sterile water. Then it was incubated on potato dextrose agar and a pure culture (Q1) was obtained. After 5 days of cultivation at 25°C, the fungal colonies were olivaceous to dark olive with white margins and abundant aerial mycelia. The conidia were obclavate or ellipsoid, pale brown, with three to four longitudinal septa and two to seven transverse septa, and they measured 20.0 to 50.0 × 3.5 to 14.0 μm ($n = 50$). Conidiophores were septate, arising singly, and measured 3.5 to 40.0 × 2.5 to 4.5 μm ($n = 50$). Based on morphological characteristics, the fungus was preliminarily identified as *Alternaria tenuissima* (Simmons 2007). To further confirm the identification, the internal transcribed spacer region (ITS), translation elongation factor 1-α gene (TEF), RNA polymerase II second largest subunit (RPB2), major

allergen Alt a 1 gene (Alt a 1), endopolygalacturonase gene (endoPG), anonymous gene region (OPA10-2), and glyceraldehyde 3-phosphatedehydrogenase (GAPDH) were amplified and sequenced using primers ITS1/ITS4 (Peever et al. 2004), EF1-728F/EF1-986R (Carbone and Kohn 1999), RPB2-5F2/RPB2-5R (Sung et al. 2007), Alt-for/Alt-rev (Hong et al. 2005), EPG-specific/EPG-3b (Peever et al. 2004), OPA10-2R/OPA10-2L (Peever et al. 2004), and Gpd1/Gpd2 (Berbee et al. 1999) (GenBank accession nos. MN046364, MW016001, MW016002, MW016003, MW016004, MW016005, and MW016006). DNA sequences of TEF, RPB2, endoPG, OPA10-2, and GAPDH were 100% identical to those of *A. tenuissima* (MN256108, MK605866, KP789503, JQ859829, and MK683802), but ITS and Alt a 1 were 100% similarity with *A. tenuissima* (MN615420, JQ282277) and *A. alternata* (MT626589, KP123847). The ITS and Alt a 1 sequences did not distinguish *A. tenuissima* from the *A. alternata* complex. Maximum likelihood phylogenetic analyses were performed for the combined data set with TEF, RPB2, and endoPG using MEGA6 under the Tamura–Nei model (Kumar et al. 2016). The isolate Q1 clustered with *A. tenuissima* type strain CBS 918.96. Twenty celery plants of four- to seven-leaf age were used to test the pathogenicity of Q1. Ten plants were sprayed with 20 ml of spore suspension (1×10^5 spores/ml), and the control was sprayed with 20 ml of sterile water; they were placed in a growth chamber (25°C, a 14-h light and 10-h dark period, relative humidity > 80%). Eight days after inoculation, 40% of the leaves formed lesions, which were consistent with the field observation, and the control group was asymptomatic. The pathogen was reisolated from infected leaves to fulfill Koch's postulates. To our knowledge, this is the first report of *A. tenuissima* causing leaf spot on celery in China.

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