Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Cercospora* Leaf Spot Caused by *Cercospora* cf. *flagellaris* on Okra in China

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Okra (*Abelmoschus esculentus* [L.] Moench), which belongs to the family Malvaceae, is widely grown in the tropics, subtropics, and warmer areas of the temperate zones for its immature seed pods, which are consumed as a vegetable. In China, okra pods are consumed not only as vegetables but also as a traditional medicine to cure dental diseases and gastric ulcers. During September 2018 to June 2019, extensive spots on okra leaves were observed in several commercial fields (approximately 2.0 ha), with disease incidence of approximately 25 to 50% in the Yanqing District (116.63°E, 40.77°N) of Beijing, China. Symptoms of the disease initially appeared as small pale brown spots with yellow haloes. As the disease progressed, some spots gradually coalesced, forming larger irregular dark brown lesions. The centers of the lesions became grayish white. A total of 13 small fragments (3 to 5 mm) excised from the lesion margins were sterilized in 1% NaClO for 1 min, followed by three washes with sterile distilled water, and then placed on potato dextrose agar (PDA) and incubated at 25°C in the dark for 5 days. In total, 21 cultures were obtained and purified by single-spore subcultures on PDA for morphological identification. The colonies on PDA were whitish to gray, with cottony aerial mycelium. Conidiophores were fasciculate, olivaceous brown, straight or geniculate, uniform in width, multiseptate, and ranged from 286 to 711 µm (average = 578 µm, $n = 50$). Conidia were hyaline, slightly curved or straight, needle shaped, truncate at the base, and terminal at the tip, three- to 17-septate, and measuring 52 to 231 µm (average = 182 µm, $n = 50$). The morphological features were consistent with *Cercospora* cf. *flagellaris* Ellis & G. Martin (Groenewald et al. 2013). Pathogenicity tests were conducted on potted okra plants (cv. 'Jiayuan'). Twenty-four healthy

okra plants at the true leaf stage were sprayed with conidial suspensions (1×10^6 conidia/ml) and incubated at a glass cabinet maintained at 25°C and 90% relative humidity. To each leaf approximately 10 ml of conidial suspension was applied. Plants sprayed with water were used as controls. Seven days later, dark brown spots, which were identical to those observed in the fields, were observed on inoculated leaves, whereas the control plants remained healthy. *C. cf. flagellaris* was reisolated from symptomatic leaves, confirming Koch's postulates. Genomic DNA was extracted from fungal mycelium using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China). The nuclear ribosomal internal transcribed spacer region (ITS) and portions of the actin (ACT), histone H3 (HIS3), and translation elongation factor 1- α (TEF1) genes were amplified using primers ITS1/ITS4 (Groenewald et al. 2013), ACT-512F/ACT-783R (Carbone and Kohn 1999), CYLH3F/CYLH3R (Crous et al. 2006), and EF1-728F/EF1-986R (Carbone and Kohn 1999). The resulting 542-bp ITS, 226-bp ACT, 410-bp HIS3, and 306-bp TEF1 sequences of isolate QK14091813 were deposited in GenBank (accession nos. MT949700, MT949701, MT949702, and MT949703, respectively). The ITS, ACT, HIS3, and TEF1 sequences shared 99.42 to 100% identities to previously published sequences of *C. cf. flagellaris* (accession nos. MN633275 for ITS, MF680960 for ACT, MK991295 for HIS3, and MK991292.1 for TEF1, respectively). Multilocus phylogenetic analyses (ITS, ACT, HIS3, and TEF1) were performed by the neighbor-joining method using MEGA 7.0. The resulting trees showed that *C. cf. flagellaris* isolate QK14091813 (this study) nested within the clade that includes other isolates of *C. cf. flagellaris* with a 99% confidence level. To our knowledge, this is the first report of *C. cf. flagellaris* causing leaf spot on okra (Farr and Rossman 2020). The pathogen has a worldwide distribution and an unusually broad host range, which can be of great significance, and the plant protection policy of priority to prevention and synthetic prevention should be followed.

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