Diseases Caused by Fungi and Fungus-Like Organisms

Alternaria alternata Causing Leaf Spot of *Cucumis melo* (Muskmelon) in Pakistan

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In July 2019, leaf spot symptoms were observed on muskmelon (*Cucumis melo* L.) cultivar Jackball-1 plants in an experimental field of 2.02 ha with a disease incidence of 30% (31°26'05.4"N, 73°04'30.3"E) at the University of Agriculture, Faisalabad, Pakistan. Early symptoms consisted of small, circular, brown, necrotic spots 1 to 2 mm in size covering 10 to 30% of the leaf blade, which gradually enlarged and developed concentric rings. To identify the causal agent of the disease, a total of 20 symptomatic leaves were collected. Small pieces removed from the margin between healthy and diseased tissues were surface disinfected in 70% ethanol for 2 min, rinsed three times with sterile distilled water, plated on potato dextrose agar, and incubated at 25 ± 2°C with a 12-h photoperiod. Morphological observations were made on 7-day-old single-spore cultures. The colonies initially appeared white and then turned olive-green. All 20 fungal isolates were characterized by small, short-beaked, multicellular conidia. The conidia were ellipsoidal

or ovoid and measured 11.5 to 30 × 7.5 to 15 µm ($n = 50$) with longitudinal and transverse septa. Conidia were produced on short conidiophores in chains. The beaks were short (often less than one-third the body length) and conical or cylindrical. These morphological features concur with the description of *Alternaria alternata* (Fr.) Keissler (Woudenberg et al. 2013). For molecular identification, genomic DNA of four representative isolates (HMSMZA 07, 08, 09, and 10) was extracted, and PCR amplification of the internal transcribed spacer (ITS)-rDNA, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and translation elongation factor-1 alpha (TEF-1 α) gene regions was performed (Berbee et al. 1999; Carbone and Kohn 1999; White et al. 1990). The obtained sequences were deposited in GenBank with accession numbers MT253643.1 to MT253646.1 (ITS-rDNA), MT318260.1 to MT318263.1 (GAPDH), and MT318280.1 to MT318283.1 (TEF-1 α). BLASTn analysis of HMSMZA 07 sequences showed 100% identity with ITS rDNA (MN615420.1), GAPDH (MK637438.1), and TEF-1 α (MN807795.1) sequences of *A. alternata*. To confirm pathogenicity, 5- to 6-week-old muskmelon Jackball-1 plants (true leaf stage) were sprayed until runoff (1.5 to 2 ml per plant) with *A. alternata* conidial suspension (10⁶ conidia/ml; obtained from 1-week-old cultures) amended with 0.1% (vol/vol) of Tween 20 using an atomizer in the greenhouse. The experiment included four *A. alternata* isolates inoculated onto three muskmelon plants per each isolate, whereas control plants ($n = 3$) were sprayed with sterile distilled water amended with 0.1% Tween 20. The plants were incubated at 25 ± 2°C in a greenhouse, and the experiment was conducted twice. At 5 to 7 days postinoculation, necrotic leaf spots were observed on the inoculated plants, and *A. alternata* was reisolated and confirmed by morphological and molecular (ITS) features. No disease was observed on control plants. Previously, *A. alternata* on muskmelon has been reported in Pakistan (Ahmad et al. 1997); however, this study provides a detailed description of disease symptoms, as well as morphological and molecular identity of the causal agent, including completion of Koch's postulates. The disease could represent a threat for the muskmelon crop in Pakistan due to its increasing cultivation and therefore warrants the need to develop disease management strategies.

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