Diseases Caused by Fungi and Fungus-Like Organisms

Nigrospora sphaerica Causing Leaf Blight Disease on Sesame in Pakistan

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Sesame (*Sesamum indicum* L.) commonly known as “til”, is the most ancient and widely grown oilseed crop of Pakistan. During 2020, a field survey conducted in various research fields of Faisalabad (31°26′00.2″N, 73°04′25.4″E) revealed the occurrence of characteristic leaf blight disease with an incidence of 10 to 13%. The symptoms were characterized by yellow-brown and irregular lesions. At later stages, the lesions expanded and the affected leaves turned grayish to dark-brown and finally became wilted. Symptomatic leaves (both the diseased and healthy tissue) were cut into approximately 2 × 2-mm pieces, surface sterilized with 1% sodium hypochlorite for 30 s followed by 70% ethanol for 30 s, rinsed three times in sterile distilled water prior to culturing on potato dextrose agar (PDA), and incubated at 25°C under a 12-h photoperiod for 7 days. To obtain pure cultures, hyphal tips of growing mycelia from leaf tissues were carefully isolated and transferred onto fresh PDA plates. Fungal colonies of 11 isolates were initially white, becoming light to dark gray. The conidia were black, spherical to subspherical, single-celled, 12 to 14 × 18 to 20 µm in diameter, and borne on a hyaline vesicle at the tip of the conidiophore. Further, to identify the pathogen to the species level, genomic DNA was extracted using a modified CTAB protocol described by Guo et al. (2000). The internal transcribed spacer (ITS) region of the ribosomal DNA and translation elongation

factor 1-alpha (TEF1- α) gene were amplified using ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R primer sets (Carbone and Kohn 1999), respectively. The sequences were submitted to GenBank (accession nos. MW287214 for ITS and MW325222 for TEF1- α). Sequence comparison revealed 99 and 100% similarity to multiple sequences of *Nigrospora sphaerica* (GenBank accessions KX834822 and MN995332). On the basis of cultural features, conidial morphology, and molecular data, the fungus was identified as *N. sphaerica* (Sacc.) Mason (Chen et al. 2018; Wang et al. 2017). To test the pathogenicity, fresh and healthy leaves of ten 6-week-old growth stage sesame plants were spray inoculated with a conidial suspension (10⁵ conidia/ml) collected from a 7-day-old culture on PDA. In addition, 10 plants sprayed with sterile distilled water served as controls. Inoculated plants were covered with polyethylene bags to maintain high humidity and kept at 28°C, and observations were made at regular intervals. After 8 to 10 days of inoculation, leaves developed blight symptoms similar to those observed on naturally infected leaves, whereas control leaves remained asymptomatic. The pathogen was reisolated from the inoculated leaves, and its identity was confirmed by morphological and molecular (ITS and TEF1- α) means, thus fulfilling Koch's postulates. *N. sphaerica* is distributed on a wide range of hosts and has been reported from different host genera including monocotyledonous and dicotyledonous ones (Wang et al. 2017). Previously, *N. sphaerica* has been reported to cause leaf blight of *Cunninghamia lanceolata* in China (Xu and Liu 2016). To the best of our knowledge, this is the first report of *N. sphaerica* as the causal agent of leaf blight of sesame in Pakistan. Because sesame is an important oilseed crop of Pakistan, appropriate disease management practices should be developed and implemented.

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