Disease Notes

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Nigrospora sphaerica* Causing Leaf Spot on Watermelon (*Citrullus lanatus*) in Malaysia

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Watermelon (*Citrullus lanatus* L.) is widely cultivated and consumed in Malaysia for its nutritional value. In June 2018, nearly 40% of the 'Red Rocky' watermelon plants in experimental plots of the research farm at the Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, had leaf spot symptoms. Leaf spots were small, ranging 5 to 30 mm, yellow to brown, and circular to irregular in shape. With age, the leaf spots gradually enlarged and coalesced. To investigate the disease, 10 symptomatic leaves were collected from the experimental plots. Diseased tissues (5 × 5 mm) were excised and surface sterilized with 0.5% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water, plated on potato dextrose agar, and incubated at 25°C for 5 days. A total of 10 isolates with similar colony morphologies were obtained from tissue samples. A single representative isolate (F) was further characterized by molecular analysis. All colonies were initially white in color but later turned gray to black upon sporulation after 7 days. Conidia were produced in culture and were single-celled, black, smooth-walled, spherical in shape, measuring 11.4 to 14 × 13.8 to 19 µm in diameter (n = 40). These were borne on hyaline vesicles at the tip of a conidiophore. For molecular identification, genomic DNA was extracted from fresh mycelium of isolate F using a DNeasy Plant Mini kit (Qiagen, Germantown, MD). The internal transcribed spacer (ITS) region of rDNA and the translation elongation factor 1-alpha (TEF1-α) gene were amplified using the ITS5/ITS4 (White et al. 1990) and EF1-728F/EF1-986R primer sets (Carbone and Kohn 1999), respectively. BLASTn analysis of the ITS sequence revealed 100% identity (526 of 526 bp) to *Nigrospora sphaerica* (GenBank HQ608063). TEF1-α sequence had 100% identity (494 of 494 bp) with *N. sphaerica* (GenBank MN995332). The resulting sequences were deposited in GenBank (ITS, MK544066; TEF1-α, MT708197). Based on morphological and molecular characteristics, isolate F was identified as *N. sphaerica* (Sacc.) Mason (Chen et al. 2018). A pathogenicity test was conducted on five healthy leaves of five 1-month-old Red Rocky watermelon plants grown in a greenhouse. Leaves were wounded using a 34-mm-diameter florist pin frog and spray-inoculated until runoff with a conidial suspension (1×10^6 conidia/ml) of a 7-day-old culture. Five leaves from an additional two plants were sprayed with sterile distilled water to serve as controls. Inoculated plants were covered with polyethylene bags for 48 h to maintain high humidity. Ten days postinoculation, symptoms on inoculated leaves developed brown-to-black lesions similar to those observed in the field, whereas control leaves remained asymptomatic. *N. sphaerica* was reisolated from all symptomatic tissues, confirming Koch's postulates. *N. sphaerica* is distributed on a wide range of hosts and has been reported from 40 different host genera including monocotyledonous and dicotyledonous hosts (Wang et al. 2017). *N. sphaerica* has been reported to cause leaf spot of date palm in Pakistan (Alam et al. 2020) and kiwifruit in China (Chen et al. 2016). To our knowledge, this is the first report of *N. sphaerica* causing leaf spot of watermelon in Malaysia. This new disease could reduce fruit quality, because sweetness and ripening are dependent on healthy foliage. Additionally, this disease can cause premature defoliation, which would also reduce watermelon productivity.

References:

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