Diseases Caused by Fungi and Fungus-Like Organisms

Stemphylium lycopersici Causing Leaf Spot of Watermelon (*Citrullus lanatus*) in China

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Watermelon (*Citrullus lanatus*) is an important cucurbit crop in China. During September 2020, an unknown leaf spot disease was observed on watermelon in two greenhouses (640 m² per greenhouse) of Sangzi town, Jizhou district, Tianjin, China (117°10'E, 39°55'N), where approximately 10% of plants were infected. Disease symptoms began as small, circular, brown spots on leaves. As these spots increased in size, they developed confluent, irregular lesions surrounded by dark brown edges. Severely affected plants had many wilted leaves followed by defoliation. Ten symptomatic leaves were collected for pathogen isolation. Diseased tissues (3 × 3 mm) were cut from the margins of lesions and surface disinfected with 1% NaClO for 1 min, rinsed three times with sterile distilled water (SDW), and then placed on PDA at 25 ± 2°C with a 12-h photoperiod for 7 to 10 days. Seven morphologically similar isolates were obtained from the 10 infected leaves and purified by single-spore culturing for further study. The initial growth of the isolates on PDA appeared grayish white in obverse and bright yellow pigmentation in reverse. Colony color gradually deepened to grayish brown in obverse and brownish red in reverse. Conidia (*n* = 50) were solitary, light brown, oblong to long elliptic, pointed or obtusely rounded at the top, constricted at the transverse septum, with verrucous processes on the surface, 36.3 to 64.2 × 16.6 to 25.1 μm, and the length-to-width ratio of conidia was 1.5 to 2.5. All characteristics were consistent with *Stemphylium lycopersici* (Ellis 1971; Woudenberg et al. 2017). Total genomic DNA was extracted from a representative isolate (XG2-2) using a Fungal DNA Kit (GBCBIO, Guangzhou, China). The ITS and EF1-α genes

(Sun et al. 2016) were amplified and sequenced with the primer pairs ITS1/ITS4 (5'-TCCGTAGGTGAACCTGCGG-3'/5'-TCCTCCGCTTATTGATA TGC-3') and EF-1α-F/EF-1α-R (5'-TCACTTGATCTACAAGTGC GG-3'/5'-CGATCTTTAGACATCTGGAGG-3'), respectively. The two sequences of strain XG2-2 (GenBank MW362344 and MW664941) showed 100 and 99% identity to *S. lycopersici* strain 01 and strain KuNBY1 (KR911814 and AB828256), respectively. Phylogenetic analysis using MEGA7 based on the sequences of ITS and EF1-α regions showed that XG2-2 clustered with *S. lycopersici* isolates (strain 01 and strain KuNBY1). For the pathogenicity test, a spore suspension (1 × 10⁶ spores/ml) in SDW from a 7-day-old culture of the fungus grown on PDA and counted with a hemacytometer was sprayed on leaves and stems of five healthy watermelon plants, grown for 2 months in a greenhouse at 25 to 30°C, with 85% RH. Conditions remained the same for inoculation experiments. Negative controls were healthy plants inoculated with SDW. The experiment was repeated twice. Six days after inoculation, typical leaf spot symptoms were observed on inoculated leaves, whereas control leaves remained symptomless. To satisfy Koch's postulates, the causal fungus was reisolated from the lesions of inoculated plants, with morphological and cultural characteristics identical to the original isolate. *S. lycopersici* is a common fungus with a relatively extensive host range (Kee et al. 2018). In recent years, new host plants infected by *S. lycopersici* have been reported in Asia including physali (Yang et al. 2020), common bean (Li et al. 2020), and potato (Kee et al. 2018). To our knowledge, this is a new host recorded for *S. lycopersici* causing leaf spot on watermelon in China. Sangzi watermelon is a special local product in the Jizhou district of Tianjin. At present the cultivated area is 1,000 ha including 667 ha in controlled conditions and 333 ha of field-grown plants with a total annual output of 45,000 Mg. In this survey, we found the disease caused by *S. lycopersici* on watermelon only in these two greenhouses, but we cannot rule out the possibility of large-scale spread in the future. Therefore, integrated management strategies for this fungus need to be developed to reduce economic losses in commercial cultivation.

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