Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Verticillium Wilt of Watermelon Caused by *Verticillium dahliae* in China

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Watermelon (*Citrullus lanatus* T.) is one of the most important economic crops in China. Soilborne diseases are becoming more and more serious with longer growing seasons and continuous cropping of watermelon in greenhouses. In May 2020, symptoms were observed on plants in greenhouses located at Xingtai, Hebei province of China and included wilted leaves, chlorosis, and plant death. Among the 26 greenhouses examined, symptomatic plants were observed in 17 greenhouses. The incidences of infected plants ranged from 1 to 35% and caused an average 10% yield loss. Symptoms began on the lower part of the plants and progressed upward to the vines and leaves. At the early stage of infection, the edge of watermelon leaves changed from green to yellow and became soft. As the disease progressed, infected leaves wilted and desiccated. The vascular tissue of the stem exhibited a uniform brown discoloration that often extended throughout the vine. To identify the causal agent, small pieces approximately 3.0 × 3.0 mm in size of infected stem tissues were collected and sterilized with 0.5% sodium hypochlorite solution for 1 min, rinsed three times with sterile water, and transferred onto potato dextrose agar (PDA) medium amended with 100 µg/ml of chloramphenicol. The plates were incubated at 25°C for 3 days in the dark, and fungal isolates were purified using the single-spore isolation method. A total of 22 fungal isolates with identical colony morphology were collected from diseased plants. The color of the fungal colonies on PDA medium was creamy white with an abundance of mycelia that darkened after 5 days of growth due

to the formation of microsclerotia. Fungal colonies consisted of fine, hyaline hyphae with verticillate conidiophores producing hyaline, ellipsoidal to oval conidia with an average size of $5.12 \times 3.41 \mu\text{m}$ ($n = 50$). The morphological characters of the fungal isolates were identical to those of *Verticillium dahliae* Kleb. described by Hawksworth and Talboys (1970). Pathogenicity tests were performed by soaking 30 watermelon seedlings with wounded root tips in the fungal conidial suspension (1×10^7 conidium/ml) for 30 min (Ma et al. 2004). The same number of noninoculated watermelon seedlings was used as a control. All plants were kept in a greenhouse at 25°C and 90 to 95% relative humidity. Seven days postinoculation (dpi), leaves of treated plants began to show symptoms of wilt. At 10 dpi, lower leaves wilted and dried, and by 15 dpi, whole plants were dead. Pathogenicity tests were repeated three times with consistent results. The pathogen was reisolated from the diseased plants and displayed identical morphological characteristics to the original isolates. To further identify the pathogens, the ribosomal DNA internal transcribed spacer (rDNA-ITS) region was amplified by PCR (Bellemain et al. 2010; Liu et al. 1999; White et al. 1990). The amplicon was sequenced and showed 99 to 100% identity to the ITS region of the *V. dahliae* reference strains deposited in the NCBI database (MK093977.1, MK287620.1, MT348570.1, and LC549667.1). Based on morphological and ITS sequence information, the fungal pathogen was identified as *V. dahliae*. *V. dahliae* is an economically important pathogen with a wide host range worldwide. The discovery of Verticillium wilt on watermelons indicates that there might be a risk of Verticillium wilt when watermelons are planted in subsequent crops of the host plants of the disease, such as cotton or eggplant. To our knowledge, this is the first report of *V. dahliae* causing Verticillium wilt of watermelon in China.

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