First Report of Watermelon Mosaic Virus Causing a Mosaic Disease on *Cucumis metuliferus* in China

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Cucumis metuliferus, also called horned cucumber or jelly melon, is considered as a wild species in the *Cucumis* genus and a potential material for nematode- or virus-resistant breeding (Chen et al. 2020; Provvidenti et al. 1977; Sigüenza et al. 2005). This species, originating from Africa, has been cultivated as a fruit in China in recent years. In July 2020, a mosaic disease was observed on *C. metuliferus* growing in five fields (approximately 0.7 ha) in Urumqi, Xijiang, China, where >85 to 100% of the field plants exhibited moderate to severe viral disease-like leaf mosaic and/or deformation symptoms. Delayed flowering and small and/or deformed fruits on the affected plants could result in yield loss of about 50%. To identify the causal pathogen, the symptomatic leaf samples were collected from the five fields (five plants/points for each field), and their total RNA was extracted using a commercial RNA extraction kit. The universal potyviral primers (Ha et al. 2008) and specific primers for a number of frequently occurring, cucurbit crop-infecting viruses, including papaya ringspot virus (Lin et al. 2013), cucumber mosaic virus, and watermelon mosaic virus (WMV), were designed and used for detection by reverse transcription polymerase

chain reaction (RT-PCR). The result showed that only the WMV primers (forward, 5'-AAGTGTGACCAAGCTTGGACTGCA-3'; reverse, 5'-CTCA-CCCATTTGCCCCAAGACGT-3') could amplify the corresponding target fragment from the total RNA templates, and direct sequencing of the RT-PCR products and GenBank BLAST confirmed the presence of WMV (genus *Potyvirus*) in the collected *C. metuliferus* samples. To complete Koch's postulates, the infected *C. metuliferus* leaves were ground in the sodium phosphate buffer (0.01 M, pH 7.0), and the sap was mechanically inoculated onto 30 four-leaf-stage *C. metuliferus* seedlings (two leaves for each seedling were inoculated) kept in an insect-proof, temperature-controlled greenhouse at approximately 25 to 28°C. Twenty-five of the inoculated plants were observed to have apparent leaf mosaic similar to the field symptoms 2 weeks after inoculation, and a positive result was obtained in RT-PCR detection for the symptomatic leaves of inoculated plants using the WMV primers aforementioned, confirming the virus as the pathogen of *C. metuliferus* in Urumqi. To our knowledge, this is the first report of WMV naturally infecting *C. metuliferus* in China. We obtained the full-length sequence of the WMV Urumqi isolation (WMV-Urumqi) by sequencing the RT-PCR amplicons from seven pairs of primers spanning the viral genome and the 5'RACE and 3'RACE products. The complete sequence of WMV-Urumqi (GenBank accession no. MW345911) is 10,046 nt long and contains an open reading frame that encodes a polyprotein of 3,220 amino acids. WMV-Urumqi shares the highest nucleotide identity (95.9%) and amino acid identity (98.0%) with the *Cucurbita pepo*-infecting isolation (KX664483) from Shanxi province, China. Our findings provide a better understanding of the host range and genetic diversity of WMV, and a useful reference for virus-resistant breeding involving *C. metuliferus*.

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