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Disease Notes

# First Report of *Cucumber mosaic virus* Infecting Watermelon in Serbia

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## Abstract

In June 2012, field-grown watermelon plants (*Citrullus lanatus* L.) with virus-like symptoms were observed in Silbaš locality, South Backa District of Serbia. Plants infected early in the growing season showed severe symptoms including stunting, mosaic, mottling, blistering, and leaf curling with reduced leaf size, while those infected at later stages exhibited only a mild mosaic. Affected plants were spread across the field and disease incidence was estimated at 40%. Thirteen symptomatic watermelon plants were sampled and analyzed by double-antibody sandwich (DAS)-ELISA using a commercial diagnostic kit (Bioreba AG, Reinach, Switzerland) against the most important watermelon viruses: *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* (PRSV), and *Squash mosaic virus* (SqMV) (1). Commercial positive and negative controls and an

extract from healthy watermelon tissue were included in each ELISA. Serological analyses showed that all plants were positive for CMV and negative for ZYMV, WMV, PRSV, and SqMV. The virus was mechanically transmitted from an ELISA-positive sample (449-12) to five plants of each *Citrullus lanatus* 'Creamson sweet' and *Chenopodium amaranticolor* using 0.01 M phosphate buffer (pH 7) with Serbian CMV isolate from *Cucurbita pepo* 'Olinka' (GenBank Accession No. HM065510) and healthy watermelon plants as positive and negative controls, respectively. Small necrotic lesions on *C. amaranticolor* and mild mosaic with dark green vein banding on watermelon leaves were observed on all inoculated plants 5 and 14 days post-inoculation, respectively. For further confirmation of CMV infection, reverse transcription (RT)-PCR was performed with the One-Step RT-PCR Kit (Qiagen, Hilden, Germany) using specific primers CMVCPfwd (5'-TGCTTCTCCRCGARWTTGCGT-3') and CMVCPREV (5'-CGTAGCTGGATGGACAACCCG-3'), designed to amplify an 871-bp fragment of the RNA3 including the whole CP gene. Total RNA from 12 naturally infected and five mechanically infected watermelon plants was extracted with the RNease Plant Mini Kit (Qiagen). Total RNA obtained from the Serbian CMV isolate (HM065510) and healthy watermelon plants were used as positive and negative controls, respectively. The expected size of RT-PCR products were amplified from all naturally and mechanically infected watermelon plants but not from healthy tissues. The PCR product derived from isolate 449-12 was purified and directly sequenced using the same primer pair as in RT-PCR (JX280942) and analyzed by MEGA5 software (3). Sequence comparison of the complete CP gene (657 nt) revealed that the Serbian isolate 449-12 shared the highest nucleotide identity of 98.9% (99.1% amino acid identity) with the Spanish melon isolate (AJ829777) and Syrian tomato isolate (AB448696). To our knowledge, this is the first report of CMV on watermelon in Serbia. CMV is widely distributed within the Mediterranean basin where it has a substantial impact on many agricultural crops (2) and is often found to be prevalent during pumpkin and squash surveys in Serbia (4). The presence of CMV on watermelon could therefore represent a serious threat to this valuable crop in Serbia.

*References:* (1) L. M. da Silveira et al. Trop. Plant Pathol. 34:123, 2009. (2) M. Jacquemond. Adv. Virus Res. 84:439, 2012. (3) K. Tamura et al. Mol. Biol. Evol. 28:2731, 2011. (4) A. Yucurovic et al. Eur. J. Plant Pathol. 133:935, 2012.



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