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Disease Notes

First Report of *Cucumber mosaic virus* on Melon in Bosnia and Herzegovina

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Abstract

During July 2012, field-grown melon plants (*Cucumis melo* L.) with symptoms of mosaic, chlorotic mottling, and vein banding as well as blistering and leaf malformation were observed in one field in the locality of Kladari (municipality of Doboj, Bosnia and Herzegovina). Disease incidence was estimated at 60%. A total of 20 symptomatic plants were collected and tested with double-antibody sandwich (DAS)-ELISA using commercial polyclonal antisera (Bioreba AG, Reinach, Switzerland) against four the most commonly reported melon viruses: *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and *Papaya ringspot virus* (PRSV) (1,3). Commercial positive and negative controls were included in each assay. Only CMV was detected serologically in all screened melon

samples. Sap from an ELISA-positive sample (162-12) was mechanically inoculated to test plants using 0.01 M phosphate buffer (pH 7.0). The virus caused necrotic local lesions on *Chenopodium amaranticolor* 5 days after inoculation, while mild to severe mosaic was observed on *Nicotiana rustica*, *N. glutinosa*, *N. tabacum* 'Samsun,' *Cucurbita pepo* 'Ezra F1,' and *Cucumis melo* 'Ananas' 10 to 14 days post-inoculation. Five inoculated plants of each experimental host were DAS-ELISA positive for CMV. The presence of CMV in all naturally and mechanically infected plants was further verified by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and used as template in RT-PCR. RT-PCR was carried out with the One-Step RT-PCR Kit (Qiagen) using primer pair CMVCPfwd and CMVCPREV (4), amplifying the entire coat protein (CP) gene and part of 3'- and 5'-UTRs of CMV RNA 3. Total RNAs obtained from the Serbian CMV isolate from *Cucurbita pepo* 'Olinka' (GenBank Accession No. HM065510) and healthy melon leaves were used as positive and negative controls, respectively. An amplicon of the correct predicted size (871 bp) was obtained from all naturally and mechanically infected plants as well as from positive control, but not from healthy tissues. The amplified product derived from isolate 162-12 was purified with QIAquick PCR Purification Kit (Qiagen) and sequenced directly using the same primer pair as in RT-PCR (KC559757). Multiple sequence alignment of the 162-12 isolate CP sequence with those available in GenBank, conducted with MEGA5 software, revealed that melon isolate from Bosnia and Herzegovina showed the highest nucleotide identity of 99.7% (100% amino acid identity) with eight CMV isolates originating from various hosts from Serbia (GQ340670), Spain (AJ829770 and 76, AM183119), the United States (U20668, D10538), Australia (U22821), and France (X16386). Despite the fact that CMV is well established in majority of Mediterranean countries and represents an important threat for many agriculture crops, including pepper in Bosnia and Herzegovina (2), to our knowledge, this is the first report of CMV infecting melon in Bosnia and Herzegovina. Melon popularity as well as production value has been rising rapidly and the presence of CMV may have a drastic economic impact on production of this crop in Bosnia and Herzegovina.

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