Disease Notes

First Report of *Watermelon mosaic virus* Infectin Melon and Watermelon in Bosnia and Herzegovina

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Abstract

Hereby the expansion of host range of *Watermelon mosaic virus* (WMV, *Potyvirus, Potyviridae*), found previously on zucchini in Bosnia and Herzegovina (3), to two new hosts is reported. Also, this is the first finding of WMV "emerging" (EM) isolate causing more severe symptoms in some cucurbits than "classic" (CL) isolates (1). During a July 2013 survey to determine the presence of WMV on cucurbits in Bosnia and Herzegovina, in the Kosijerovo locality (Laktaši Municipality, Bosnia and Herzegovina), virus-like symptoms were observed on 10% of plants. Severe mosaic, puckering, and leaf deformation as well as necrosis and leaf distortion were observed in a melon (*Cucumis melo* L.) crop, while mosaic, green vein banding, and leaf curling with

reduced leaf size were observed in watermelon (*Citrullus lanatus* [Thunb.] Matsum and Nakai). Sampled melon and watermelon plants were tested for the presence of WMV with commercial double-antibody sandwich (DAS)-ELISA kit (Bioreba, AG, Reinach, Switzerland). Commercial positive and negative controls were included in each assay. Out of the 30 melon and 25 watermelon plants tested, 24 and 23 samples were positive for WMV, respectively, while no other cucurbit viruses were detected. The virus was mechanically transmitted from one of each of ELISA-positive melon (309-13) and watermelon (314-13) samples to five plants of each *Cucurbita pepo* 'Ezra F1', *C. melo* 'Ananas,' and *C. lanatus* 'Creamson sweet' using 0.01 M phosphate buffer (pH 7). Mild to severe mosaic and bubbling followed by leaf deformation were observed in all inoculated plants 10 to 14 days post-inoculation, regardless the isolate. Serological detection was verified with reverse transcription (RT)-PCR using the One-Step RT-PCR Kit (Qiagen, Hilden, Germany) with primers WMV 5' and WMV 3' (1), designed to amplify a 402- to 408-bp fragment overlapping the N-terminal part of the coat protein (CP) gene. Total RNAs were extracted with the RNeasy Plant Mini Kit (Qiagen). Total RNAs from the Serbian WMV oil pumpkin isolate (GenBank Accession No. JF325890) and RNA from healthy melon and watermelon plants were used as positive and negative controls, respectively. An amplicon of the expected size was produced from all serologically positive melon and watermelon plants, but not from healthy tissues. The RT-PCR products derived from isolates 309-13 and 314-13 were sequenced directly (KJ603311 and KM212956, respectively) and compared with WMV sequences available in GenBank. Sequence analysis revealed 91.5% nucleotide (nt) identity (94.6% amino acid [aa] identity) between the two WMV isolates. The melon WMV isolate shared the highest nt identity of 100% with four WMV isolates from Slovakia (GQ241712 to 13), Serbia (FJ325890), and Bosnia and Herzegovina (KF517099), while the sequence of isolate 314-13 had the highest nt identity with three Serbian isolates (JX262104 to 05 and JX262114) of 99.7% (99.2% aa identity). Phylogenetic analyses placed isolate 309-13 with CL isolates, while isolate 314-13 clustered with EM isolates (1,2). To our knowledge, this is the first report of WMV on melon and watermelon and the first report on EM isolates in Bosnia and Herzegovina. This could cause significant economic losses and become a limiting factor for cucurbit production with the potential of EM isolates to rapidly replace CL (2).

References: (1) C. Desbiez et al. Arch. Virol. 152:775, 2007. (2) C. Desbiez et al. Virus Res. 152:775, 2009. (3) V. Trkulja et al. Plant Dis. 98:573, 2014.

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