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Disease Notes

First Report of *Watermelon mosaic virus* in Zucchini Squash in Bosnia and Herzegovina

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Published Online: 5 Mar 2014 | <https://doi.org/10.1094/PDIS-08-13-0834-PDN>

Abstract

Aphid-borne *Watermelon mosaic virus* (WMV; genus *Potyvirus*, family *Potyviridae*) is widely distributed in the Mediterranean area and is one of the most prevalent cucurbit viruses in the region (4). In July 2012, approximately 20% of zucchini squash (*Cucurbita pepo* L.) plants showing virus-like symptoms were observed in one field in Kukulje locality (region of Banja Luka), Bosnia and Herzegovina. Infected plants exhibited mild to severe mosaic, chlorotic mottling, and dark green vein banding, as well as puckering and leaf deformation. Symptoms mostly developed on leaves, while fruits usually only failed to develop a normal coloration. Leaves from 15 symptomatic zucchini squash plants were sampled and analyzed utilizing double-antibody

sandwich (DAS)-ELISA kits (Bioreba, AG, Reinach, Switzerland) with commercial antisera specific for five commonly occurring cucurbit-infecting viruses: WMV, *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* (PRSV), *Cucumber mosaic virus* (CMV), and *Squash mosaic virus* (SqMV) (1,3,4). Commercial positive and negative controls were included in each test. WMV was detected serologically in all tested zucchini squash samples, while no presence of other tested viruses were found. Crude sap extracted from leaves of a serologically positive sample (307-12) using M phosphate buffer (pH 7) was mechanically inoculated onto five plants of *C. pepo* 'Ezra F1' and severe mosaic accompanied by bubbling and leaf malformation was observed 14 days post-inoculation. Viral identification in all naturally and mechanically infected plants was further confirmed by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and RT-PCR was performed using the One-Step RT-PCR Kit (Qiagen) with specific primers WMV 5' and WMV 3' (4), yielding a 402- to 408-bp fragment corresponding to the N-terminal part of the coat protein (CP) gene (2). Total RNAs obtained from the Serbian WMV isolate from oil pumpkin (GenBank Accession No. JF325890) and healthy zucchini squash leaves were used as positive and negative controls, respectively. A product of the correct predicted size was obtained in all naturally and mechanically infected plants as well as positive control. No amplicon was recorded in healthy control. After purification (QIAquick PCR Purification Kit, Qiagen) the amplicon obtained from one selected isolate 307-12 was sequenced directly in both direction, aligned and compared by MEGA5 software with WMV sequences available in GenBank. Sequence comparisons revealed that the zucchini squash isolate from Bosnia and Herzegovina (KF517099) showed the highest nucleotide identity of 100% with one isolate from Serbia (FJ325891) and two Slovakian WMV isolates (GQ241712 to 13), all belonging to the classical group of WMV isolates (4). To our knowledge, this is the first report of WMV infecting zucchini squash in Bosnia and Herzegovina. Since squash and other cucurbit species represent valuable crops in Bosnia and Herzegovina, with annual production close to US\$8.5 million (<http://faostat.fao.org>) and rising rapidly, the presence of a devastating pathogen like as WMV could be a serious constraint for their production.

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