Disease Notes

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

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Abstract

Several potyvirus species cause severe economic losses in cucurbit crops in the Mediterranean region, but *Zucchini yellow mosaic virus* (ZYMV) is regarded as one of the most destructive (2,3). In June 2012, field-grown watermelon plants (*Citrullus lanatus* [Thunb.] Matsum and Nakai) showing mild to severe mosaic, mottling, and bubbling followed by leaf deformation with blistering were observed in the Kukulje locality (Region of Banja Luka) in Bosnia and Herzegovina. Incidence of virus infection in the field was visually estimated at 15%. Symptomatic watermelon plants were collected and tested for the presence of the most prevalent watermelon viruses including ZYMV, *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Papaya*

ringspot virus (PRSV), and *Squash mosaic virus* (SqMV) (1) using commercial double-antibody sandwich (DAS)-ELISA diagnostic kits (Bioreba AG, Reinach, Switzerland). Commercial positive and negative controls were included in each assay. Of the 14 watermelon plants tested, all were positive for ZYMV and negative for WMV, CMV PRSV, and SqMV. Sap prepared from an ELISA-positive sample (isolate 314-12) an healthy watermelon plants, using 0.01 M phosphate buffer (pH 7) was mechanic inoculated onto five carborundum-dusted plants of each *Chenopodium quinoa* a *Citrullus lanatus* 'Creamson sweet'. Mechanically inoculated *C. quinoa* plants exhibited chlorotic spots 5 days post-inoculation, while severe mosaic accompanied by crinkling and leaf deformation were observed on all inoculated watermelon plants 12 days post-inoculation. For further confirmation of the virus identity, total RNAs from all 14 naturally and 5 mechanically infected watermelon plants were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and subjected by reverse transcription (RT)-PCR. RT-PCR was carried out with One-Step RT-PCR Kit (Qiagen) using ZYMV-specific primer pair, CPfwd and CPrev (4), designed to amplify an 1,100-bp fragment covering the entire coat protein (CP) gene and part of the nuclear inclusion (NIb) and 3'-UTR. Total RNAs obtained from the Serbian ZYMV isolate from winter squash (GenBank Accession No. JN315861) and tissue sample from healthy watermelon leaves were used as positive and negative controls, respectively. The expected size of the RT-PCR product was amplified from each of the watermelon plants assayed confirming serological virus identification. One amplicon derived from isolate 314-12 was purified (QIAquick PCR Purification Kit, Qiagen) and sequenced directly (KF836440). Sequence analysis of the complete CP gene, conducted by MEGA5 software, revealed that watermelon isolate from Bosnia and Herzegovina showed the highest nucleotide identity of 99.8% (99.6% amino acid identity) with 14 ZYMV isolates originating from different hosts from Serbia (HM072431, JF308189 to 90, JN315856 to 57, JN315859 to 61) and Austria (AJ420012 to 17). To our knowledge, this is the first report of ZYMV in Bosnia and Herzegovina, which is an important discovery. It represents expansion of this virus to new geographical area. Considering that the ZYMV is among the most devastating pathogens of cucurbits (3), further survey is needed to determine its distribution in Bosnia and Herzegovina.

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