Disease Notes

First Report of *Alfalfa mosaic virus* Infecting *Lavandula × intermedia* in Croatia

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Abstract

Lavandin (*Lavandula* × *intermedia* Emeric ex Loiseleur) is cultivated on a large scale in some South European countries for the extraction of essential oils or as an ornamental plant for gardens and landscapes. In May of 2012, virus-like symptoms including bright yellow calico mosaic, leaf distortion, and growth reduction were observed on 15% of lavandin plants in a commercial nursery in Banovo Brdo locality, Baranja County, Republic of Croatia. Leaves from 15 symptomatic lavandin plants were collected and examined by double-antibody sandwich (DAS)-ELISA using commercial antisera (Bioreba AG, Reinach, Switzerland) against two viruses known to infect *Lavandula* spp.: *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV) (2,3). Commercial positive and negative controls and extracts from healthy lavandin leaves were included in each ELISA. Only AMV was detected serologically in all 15 tested samples. Five plants each of *Chenopodium quinoa*, *C. amaranticolor*, and *Nicotiana benthamiana* were mechanically inoculated with sap from an

ELISA-positive sample (70-12) using 0.01 M phosphate buffer (pH 7). Local chlorotic spots accompanied by systemic mosaic on both *Chenopodium* species and bright yellow mosaic on *N. benthamiana* were observed 6 and 12 days post-inoculation, respectively. Test plants were assayed by DAS-ELISA and all inoculated plants of each species tested positive for AMV. The presence of AMV in all symptomatic lavandin plants was further confirmed by reverse transcription (RT)-PCR assay. Total nucleic acid was extracted us RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RT-PCR was performed with the On Step RT-PCR Kit (Qiagen) using AMV specific primer pair CP AMV1 (5′-TCCATCATGAGTTCTTCAC-3′) and CP AMV2 (5′-AGGACTTCATACCTTGACC-3′) (1). Tot RNAs obtained from the Serbian AMV isolate from alfalfa (GenBank Accession No. FJ527748) and healthy *L.* × *intermedia* plant served as the positive and negative control, respectively. The 751-bp amplicons, covering the partial coat protein (CP) gene and 3′-UTR, were obtained from all 15 samples that were serologically positive to AMV as well as from positive control. No amplification product was observed when extract from healthy *L.* × *intermedia* plant was used as template in the RT-PCR assay. The RT-PCR product derived from isolate 70-12 was directly sequenced in both directions using the same primer pair as in RT-PCR and deposited in GenBank (JX996119). Multiple sequence alignment of the CP open reading frame was performed by MEGA5 software (4) and revealed that the isolate 70-12 showed the highest nucleotide identity of 99.4% (99.5% amino acid identity) with Serbian AMV isolate from tobacco (FJ527749). To our knowledge, this is the first report of AMV on *L.* × *intermedia* in Croatia. Because lavandin is an aromatic plant traditionally and widely grown in Croatia, the presence of AMV could be a limiting factor for its successful production.

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