DISEASE NOTES

First Report of *Alfalfa mosaic virus* on Safflower in Serbia

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Safflower (*Carthamus tinctorius* L.) is a thistle-like annual plant that is grown commercially for the production of oil and birdseed and recently as a host for the production of transgenic pharmaceutical proteins (Mayerhofer et al. 2011). In July 2014, symptoms resembling those caused by *Alfalfa mosaic virus* (AMV; genus *Alfamovirus*, family *Bromoviridae*) including bright yellow leaf mosaic, leaf distortion, and growth reduction were observed in field-grown safflower plants in Rimski Šančevi locality (South Bačka District) in Serbia. Symptomatic plants were found throughout the field and disease incidence was estimated at 50%. Symptomatic leaves from 15 plants were collected and tested for the presence of AMV using commercial double-antibody sandwich (DAS)-ELISA diagnostic kits (Loewe Biochemica, Sauerlach, Germany). Commercial positive and negative controls were included in ELISA. AMV was detected serologically in 12 of 15 safflower samples. Five plants each of *Chenopodium amaranticolor*, *Ocimum basilicum*, and *Nicotiana benthamiana* were mechanically inoculated with sap from an ELISA-positive

sample (292Saff) using 0.01 M phosphate buffer (pH 7). Local chlorotic lesions accompanied by systemic mosaic on all inoculated *C. amaranticolor*, and bright yellow mosaic on all inoculated *O. basilicum* and *N. benthamiana* were observed. The virus was transmitted mechanically to safflower and induced symptoms resembling those observed on the source plants. All five inoculated plants of each experimental host DAS-ELISA positive for AMV. Presence of AMV in safflower plants was further confi by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and RT-PCR was carried out with the OneStep RT PCR Kit (Qiagen) following the manufacturer's instructions. An AMV-specific primer pair, CP AMV1 and CP AMV2 (Finetti-Sialer et al. 1997), designed to amplify 751 bp covering the part of coat protein (CP) gene and 3'-UTR, was used for both amplification and sequencing. A Serbian isolate of AMV from pepper (GenBank Accession No. KC182569) and RNA extracted from a healthy safflower plant were used as positive and negative controls, respectively. Amplicons were obtained from all naturally infected plants as well as from positive control; all yielded an amplicon of the predicted size (751 bp), while no amplification products were recorded in negative control. After purification with QIAquick PCR Purification Kit (Qiagen), the RT-PCR product derived from 292Saff was directly sequenced in both directions and deposited in GenBank (KP034961). Sequence analysis of the part of CP gene, conducted with MEGA5 software (Tamura et al. 2011), revealed that the Serbian safflower isolate showed the highest nucleotide identity of 100% (100% amino acid identity) with pepper isolate (KC182567) from Serbia and alfalfa isolate (JQ691185) from Spain. To our knowledge, this is the first report of natural occurrence of AMV on safflower in Serbia. Considering the importance of safflower in pharmaceutical industry and increase of its production, the presence of new and potentially harmful disease may have impact on production of this crop in Serbia.

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