



First report of *Tomato apical stunt viroid* in *Solanum jasminoides* in Slovenia

M. Viršček Marn* and I. Mavrič Pleško

Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia

*E-mail: mojcavm@kis.si

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A survey of *Potato spindle tuber viroid* (PSTVd) in ornamental host plants was initiated in Slovenia in December 2006 after a PSTVd infection was confirmed in an ornamental plant *Brugmansia cordata* imported from the Netherlands. By the end of 2010, PSTVd had also been detected in *Solanum jasminoides*, *S. rantonnetii*, *S. muricatum* and *Petunia* spp. Furthermore, after the detection of *Tomato chlorotic dwarf viroid* (TCDVd) in *Petunia* sp. (Viršček Marn & Mavrič Pleško, 2010) and *Citrus exocortis viroid* (CEVd) in *S. jasminoides* (Viršček Marn & Mavrič Pleško, 2011) in 2010, an assessment of the risk of pospiviroids for Slovenia was performed at the beginning of 2011. The results of pest risk analysis showed that pospiviroids pose a serious threat to important agricultural plants. Symptomless pospiviroid-infected ornamental plants were proposed to be a probable source of infection for agricultural crops. Sampling of all pospiviroid hosts and destruction of all pospiviroid-infected lots was therefore implemented in Slovenia in 2011.

In 2011, 14 samples were taken from symptomless *S. jasminoides* plants in greenhouses and retail trade and were tested by one-step RT-PCR using semi-universal pospiviroid primers Posp1-RE/FW and Vid-RE/FW (cited in Verhoeven *et al.*, 2008). With three samples, PCR products were obtained with both primer pairs. They were all directly sequenced (Macrogen, The Netherlands). Sequence analysis of PCR products obtained with both primer pairs from two samples confirmed infection with PSTVd. Sequences of amplification products obtained with the Vid-RE/FW primer pair from the third sample showed the highest similarity with PSTVd whereas the sequences of the amplicon obtained with Posp1-RE/FW primers suggested the presence of *Tomato apical stunt viroid* (TASVd). A second total RNA extraction was made; both extracted RNAs were further used in RT-PCRs using primer pairs of Shamloul *et al.* (1997) and of Di Serio (2007) for detection of PSTVd, and primer pairs of Elleuch *et al.* (2003) and Verhoeven *et al.* (2008) for detection of TASVd and CEVd. Sequence analysis confirmed the presence of PSTVd and TASVd. PSTVd and TASVd sequences of this doubly infected sample were deposited in the GenBank (Accession Nos. JN559763 and JN543957, respectively).

Successful amplification with only Posp1-RE/FW was obtained with 10 samples. These amplification products were also directly sequenced and sequence analysis revealed the presence of CEVd in three samples and the presence of TASVd in seven samples. To obtain full viroid sequences

further analyses were made using primer pairs of Elleuch *et al.* (2003) and Verhoeven *et al.* (2008). These sequences have also been deposited in GenBank (JQ083645 to JQ083647 for CEVd and JN872141 to JN872144 and JQ904911 for TASVd). This is the first record of TASVd in Slovenia. All Slovene TASVd sequences had 370 nt and were identical to each other at 366 positions. They shared 98.9 to 100% identity among themselves and 97.5 to 100% identity with other TASVd sequences from *S. jasminoides*. The variability of sequences detected in *S. jasminoides* in Slovenia and worldwide is rather small. This data suggest a possible common origin of TASVd infection of *S. jasminoides*. Higher variability was observed between TASVd sequences from *S. jasminoides* and from tomato. The most distant sequences shared only 92.0% identity.

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