First report of tomato spotted wilt virus on lisianthus (*Eustoma grandiflorum*) in Bulgaria

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Tomato spotted wilt virus (TSWV) is one of the most destructive pathogens worldwide. In Bulgaria the virus was found to severely damage a number of solanaceous vegetables and *ca.* 25 ornamentals (Hristova et al. 2001). In spring 2014, about 30% of lisianthus plants (*ca.* 1000 plants) grown in a private farm nearby Plovdiv, Bulgaria showed yellowing and/or light brown necrosis on leaves and petals followed by subsequent wilting. To identify the causal agent of this infection, *Chenopodium quinoa*, *Nicotiana glutinosa*, and *Petunia hybrida* plants were sap inoculated with material from 10 symptomatic individuals. *C. quinoa* displayed large necrotic local lesions. Primary and systemic symptoms in *N. glutinosa* appeared as translucent necrotic lesions accompanied with yellow areas, while *P. hybrida* showed dark necrotic ring spots. Observations of lisianthus leaf sap by electron microscopy revealed the presence of isometric particles *ca.* 85 nm in size resembling those of tospoviruses. A TAS-ELISA test was conducted with antisera specific for impatiens necrotic spot virus (INSV, RT-0115-0117/1) and TSWV (RT-0105-0106/3) purchased from DSMZ, Germany. The samples from lisianthus and those from the inoculated indicators were positive only for TSWV. RNA was isolated from symptomatic leaves of lisianthus by TRI reagent® (Sigma-Aldrich) and

reverse transcription (RT) was performed using random hexamer primers. The PCR amplification conducted with specific primers targeting N gene, described by Roberts et al. (2000), revealed an amplicon of about 600 bp corresponding to the expected size. Further, the amplicon was sent for sequencing. A high-confidence consensus nucleotide sequence of 567 bp (accession No. MK388405) was selected for analysis, which encoded a protein of 188 amino acids. This protein was queried against a database of all NCBI viral protein sequences by blastp and showed 99% sequence identity to TSWV nucleocapsid protein, including several accessions from Bulgaria (e.g. CAC01282.1, CAC01285.1) (Heinze et al. 2001). The results from TAS-ELISA and RT-PCR tests clearly show that the virus-specific antibodies do not have interspecies cross-reactivity. Based on our knowledge and viruses reported in literature, this appears to be a first report of TSWV on lisianthus in Bulgaria.

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