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Disease Notes

First Report of *Cucumber mosaic virus* in *Tulipa* s in Serbia

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Abstract

Tulips (*Tulipa* sp. L.), popular spring-blooming perennials in the Liliaceae family, are one of the most important ornamental bulbous plants, which have been cultivated for cut flower, potted plant, garden plant, and for landscaping. In May 2013, during a survey to determine the presence of *Cucumber mosaic virus* (CMV, *Cucumovirus*, *Bromoviridae*) on ornamentals in Serbia, virus-like symptoms, including the presence of bright streaks, stripe and distortion of leaves, and reduced growth and flower size, were observed in an open field tulip production in the Krnjaca locality (a district of Belgrade, Serbia). Disease incidence was estimated at 20%. Symptomatic tulip plants were collected and tested for the presence of CMV by double-antibody sandwich (DAS)-ELISA using commercial diagnostic kit (Bioreba, AG, Reinach, Switzerland).

Commercial positive and negative controls were included in each ELISA. Of the six tulip plants tested, all were positive for CMV. In bioassay, five plants of each *Chenopodium quinoa*, *Nicotiana tabacum* 'Samsun,' and *N. glutinosa* were mechanically inoculated with sap from selected ELISA-positive sample (79-13) using 0.01 M phosphate buffer (pH 7). Chlorotic local lesions on *C. quinoa*, and severe mosaic and leaf malformations on *N. tabacum* 'Samsun' and *N. glutinosa*, were observed 5 and 7 days post-inoculation, respectively. All mechanically inoculated plants were positive for CMV in DAS-ELISA testing. For further confirmation of CMV presence in tulip, total RNAs from all ELISA-positive symptomatic tulip plants were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Reverse transcription (RT)-PCR was performed with the One-Step RT-PCR Kit (Qiagen) using specific primer pair CMVCPfwd and CMVCPrev (1), which flank conserved fragment of the RNA3 including the entire coat protein (CP) gene and part of 3'- and 5'-UTRs. Total RNAs obtained from the Serbian watermelon CMV isolate (GenBank Accession No. JX280942) and healthy tulip leaves served as the positive and negative controls, respectively. The RT-PCR products of 871 bp were obtained from all six samples that were serologically positive to CMV, as well as from the positive control. No amplicon was recorded in the healthy control. The amplified product which derived from isolate 79-13 was purified (QIAquick PCR Purification Kit, Qiagen), directly sequenced in both directions using the same primer pair as in RT-PCR, deposited in GenBank (KJ854451), and analyzed by MEGA5 software (4). Sequence comparison of the complete CP gene (657 nt) revealed that the Serbian isolate 79-13 shared the highest nucleotide identity of 99.2% (99% amino acid identity) with CMV isolates from Japan (AB006813) and the United States (S70105). To our knowledge, this is the first report on the occurrence of CMV causing mosaic on *Tulipa* sp. in Serbia. Taking into account vegetative reproduction of tulips and the large scale of international trade with tulip seeding material, as well as wide host range of CMV including a variety of ornamentals (2,3), this is a very important discovery representing a serious threat for the floriculture industry in Serbia.

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