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First Report of *Cucumber mosaic virus* Infecting *Peperomia tuisana* in Serbia

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Abstract

Peperomia tuisana C.DC. ex Pittier (family Piperaceae) is an attractive succulent grown as an ornamental. Despite its tropical origins, it can be successfully grown indoors in any climate. In March 2012, three samples of *P. tuisana* showing virus-like symptoms were collected from a commercial greenhouse in Zemun (District of Belgrade, Serbia) in which estimated disease incidence was 80%. Infected plants showed symptoms including necrotic ringspots and line patterns that enlarged and caused necrosis of leaves. A serious leaf drop led to growth reduction and even death of the plant. Leaves from three symptomatic *P. tuisana* plants were sampled and analyzed by double-antibody sandwich (DAS)-ELISA using commercial diagnostic kits (Bioreba AG, Reinach, Switzerland) against the most common viral pathogens of ornamentals: *Cucumber*

mosaic virus (CMV), *Tomato spotted wilt virus* (TSWV), and *Impatiens necrotic spot virus* (INSV) (1,2). Commercial positive and negative controls were included in each ELISA. Serological analyses showed that all plants were positive for CMV and negative for TSWV and INSV. The ELISA-positive sample (isolate 1-12) was mechanically inoculated onto five plants each of three test species as well as of healthy young *P. tuisana* using 0.01 M phosphate buffer (pH 7). Chlorotic local lesions on *Chenopodium quinoa* and severe mosaic and leaf malformations were observed on all inoculated *Nicotiana tabacum* 'Samsun' and *N. glutinosa*. Also, the virus was successfully mechanically transmitted to *P. tuisana* that reacted with symptoms identical to those observed on the original host plants. All mechanically inoculated plants were positive for CMV in DAS-ELISA. For further confirmation of CMV infection, reverse transcription (RT)-PCR was performed on extracts made from symptomatic *P. tuisana*, *N. tabacum* 'Samsun,' and *N. glutinosa* leaf materials. Total RNAs were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and RT-PCR was carried out using One-Step RT-PCR Kit (Qiagen). A CMV-specific primer pair, CMVCPfwd and CMVCPrev (3), which amplifies an 871-bp fragment of the entire coat protein (CP) gene and part of 3′- and 5′-UTRs, were used for both amplification and sequencing. Total RNAs obtained from the Serbian CMV isolate (HM065510) and healthy *P. tuisana* were used as positive and negative controls, respectively. A product of the correct predicted size was obtained in all naturally and mechanically infected plants, as well as positive control. No amplicon was recorded in the healthy control. The amplified product derived from isolate 1-12 was purified (QIAquick PCR Purification Kit, Qiagen), directly sequenced in both directions, deposited in GenBank (KC505441), and analyzed by MEGA5 software (4). Sequence comparison of the complete CP gene (657 nt) revealed that the Serbian isolate 1-12 shared the highest nucleotide identity of 99.1% (99.5% amino acid identity) with the Japanese isolate (AB006813). To our knowledge, this is the first report on the occurrence of CMV in *P. tuisana* in Serbia. This is also an important discovery since *P. tuisana* is commonly grown together with other ornamental hosts of CMV, and thus could represent a serious threat for future expansion of CMV in the greenhouse floriculture industry in Serbia.

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