DISEASE NOTES

First Report of *Cucumber mosaic virus* Causing Chlorotic Mottle on Pot Marigold (*Calendula officinalis*) in Serbia

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Calendula officinalis L. (family Asteraceae), commonly known as pot marigold, is a flowering perennial ornamental, rich in essential oils, that is commonly grown in gardens or as potted plant all over Europe. In July 2014, 10 samples of *C. officinalis* showing virus-like symptoms including chlorotic mottling and leaf deformation were collected in one field at Đurđevo locality (South Bačka District, Serbia). Disease incidence was estimated at 30%. These symptoms developed in naturally infected marigold plants were found to be identical to either *Cucumber mosaic virus* (CMV) as described by Naqvi et al. (1981) and Rahaman and Rao (1992). Collected samples were analyzed by double-antibody sandwich (DAS)-ELISA using commercial diagnostic kits (LOEWE Biochemica, Germany) against two of the viruses previously reported to infect pot marigold: *Cucumber mosaic virus* (CMV) and *Impatiens necrotic spot virus* (INSV). Commercial positive and negative controls and extract from healthy pot marigold tissue were included in each ELISA. CMV

was serologically detected in 7 out of 10 pot marigold samples, while all were negative for INSV. Five plants of each *Chenopodium quinoa* and *Nicotiana tabacum* 'Samsun' were mechanically inoculated with sap from one ELISA-positive sample (232Mrg) using 0.01 M phosphate buffer (pH 7). All inoculated plants produced symptoms typical of CMV (Iqbal et al. 2011) from 5 to 10 days postinoculation, chlorotic local lesions on *C. quinoa* a mosaic and leaf malformation on *N. tabacum* 'Samsun.' Also, the virus was success mechanically transmitted to *C. officinalis*, which reacted with symptoms identical t observed on the original host plants. The presence of CMV in all naturally and mechanically infected plants was further verified by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RT-PCR was performed with the OneStep RT-PCR Kit (Qiagen) using CMV-specific primer pair, CMVCPfwd and CMVCPrev (Milojević et al. 2012), which amplifies an 871-bp fragment of the entire coat protein (CP) gene. Total RNAs from Serbian pepper CMV isolate (GenBank Accession No. KC288146) and RNA extracted from a healthy pot marigold were used as positive and negative controls, respectively. A product of the expected size was obtained from all naturally and mechanically infected plants as well as positive control. No amplicon was recorded in negative control. The RT-PCR product obtained from one selected isolate 232Mrg was sequenced directly in both directions using the same primers and submitted to GenBank (KP034963). Sequence comparison of the complete CP gene, conducted with MEGA 5 software (Tamura et al. 2011), revealed that the Serbian isolate 232Mrg shared the highest nucleotide identity of 100% (100% amino acid identity) with four CMV isolates from Poland (EU191027, DQ018292), Italy (FN257306), and Serbia (KC414925). To our knowledge, this is the first report of natural infection of *C. officinalis* with CMV in Serbia. As a new CMV host in Serbia, pot marigold represents a potential virus reservoir and additional source of inoculum to recognize the known host range and prevalence of CMV in weed hosts.

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