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Natural occurrence of *Malva vein clearing virus* in malva in Iran

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Malva plants (*Malva* spp.) are widespread in Iran and grown in different geographical regions in the country; virus-like symptoms, e.g. mosaic, are frequently observed on these plants. In the present study, eleven symptom-bearing malva leaf samples, showing severe and mild mosaic, vein clearing and leaf deformation, were collected from plants grown in natural ecosystems as well as in or around fields in the Markazi and Tehran provinces of Iran during the growing seasons of 2012-2014. Each leaf sample was tested for the presence of potyviruses by indirect ELISA using a "poty-group test" kit (Bioreba, Switzerland), according to the manufacturer's instructions. The ELISA results revealed a positive reaction for seven samples using the potyvirus antibodies. Mosaic, vein clearing and leaf deformation symptoms were associated with potyvirus infections.

Total RNA of three ELISA-positive samples was extracted using a LiCl protocol (Rosas-Cárdenas *et al*., 2011). The extracted RNAs were used as template in RT-PCR assays using universal potyvirus primers NIb2F and NIb3R (Zheng *et al*., 2010). The RT-PCR assays resulted in the amplification of fragments with the expected size of 350 bp, confirming the ELISA potyvirus results for all tested samples. The nucleotide sequences of the amplicons were determined using the amplification primers and deposited in GenBank (Accession Nos. KU962948, KU962952 and KU962957). BLAST analysis showed that the isolates had the highest nucleotide sequence identities (84-89%) with *Malva vein clearing virus* (MVCV). For further analysis, the 3′ end genome sequence of isolate Ma-W2243 from *Malva* sp. in Delijan (Markazi province) (Fig. 1), including the partial coat protein (CP) gene sequence, was amplified using NWCIEN and Tu3T9M primers, and sequenced using the primer Nseq (Tan *et al*., 2005; Valouzi *et al*., 2017). The gap between the NIb and CP regions of the isolate was subsequently amplified using a pair of newly designed primers (F: 5′- ATGGATTACCTGATGGTTGG-3′ and R: 5′-TGTCTGAACGTTGGTGCTGC-3′) and sequenced to obtain the complete sequence of the CP gene. The CP sequence of the isolate was then assembled (909 nts) and deposited in GenBank (KY653927). BLAST analysis showed that the sequence had 90% nucleotide and 95% amino acid identity with sequences of MVCV. Phylogenetic analysis of the sequence and representative sequences from the genus *Potyvirus* also revealed a clustering of the isolate Ma-W2243 with other isolates of MVCV, confirming the identity of the virus.

To our knowledge, this study reports the natural occurrence of MVCV on plants of *Malva* spp. in Iran for the first time. MVCV is a distinct species in the genus *Potyvirus* and has a narrow host range. The virus naturally infects weeds and garden plants belonging to the family Malvaceae (Brunt *et al*., 1996; Menzel *et al*., 2010). Work is ongoing to investigate the presence of MVCV in other species and to study other viruses occurring in malva in the country.

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Figure 1

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