Received: 15 December 2021 | Accepted: 10 January 2022

DOI: 10.1002/ndr2.12059

New Disease Reports Open Access BSPP WILEY

NEW DISEASE REPORT

First report of *Apium virus Y* infecting *Petroselinum crispum* in Turkey

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Funding information
TUBITAK-TOVAG, Grant/Award Number: 120O802

KEYWORDS
ApVY, parsley virus, infection

Apium virus Y (ApVY) is one of the most important potyviruses that infect apiaceous crops. In January-February 2021, parsley plants (*Petroselinum crispum*) with mild mosaic and vein clearing symptoms on their leaves were observed during field studies in the Balıkesir province of Turkey (Figure 1).

Leaf samples from ten different diseased plants from two commercial fields in the province of Balıkesir were sampled. The samples were brought to the laboratory in a cold box and stored on silica gel at 4°C. Total nucleic acid (TNA) isolation was performed using 100 mg of leaf tissue with the CTAB method (Li et al., 2008). The obtained TNA (adjusted to 1000 ng/μl) from each sample was tested by RT-PCR using universal potyvirus primers as described previously (Zheng et al., 2010). Fragments of the expected size (350 bp) belonging to the nuclear inclusion b (NIb) region of potyviruses were amplified from all ten samples. Amplicons from two randomly chosen samples, once from each field, were subjected to Sanger sequencing. BLASTN analysis of the nucleotide sequences (GenBank Accession nos. OL741312-OL741313) had the highest sequence identify (90%) with the corresponding region of an ApVY isolate (HM363516) from the USA. A new primer pair (ApVYCP-F 5′-CATCCAAAGACGTGTCAGAT-3′) and (ApVYCP-R 5′-GCTATGGTGCAATTTGAACG-3′) was designed to detect ApVY infection in all samples, producing a 1066 bp amplicon including the complete ApVY coat protein (CP) gene. Each PCR

FIGURE 1 Parsley plant with mild mosaic and vein clearing symptoms

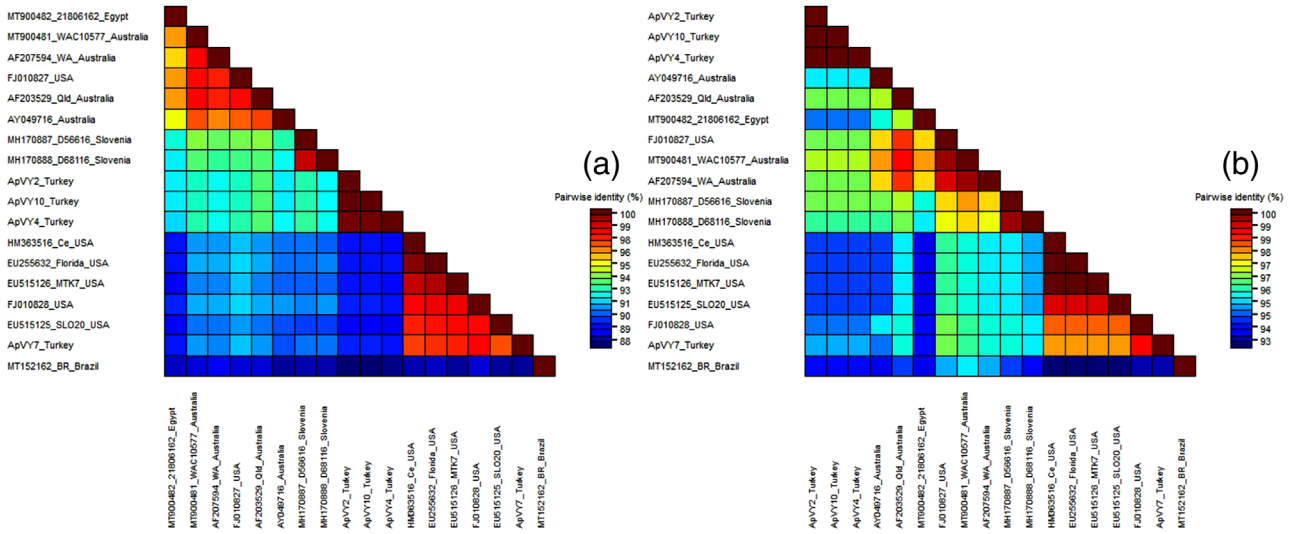

FIGURE 2 Pairwise identity scores of the (a) nucleotide and (b) amino acid sequences of the coat protein genes of *Apium virus Y* isolates as calculated by the Sequence Demarcation Tool software

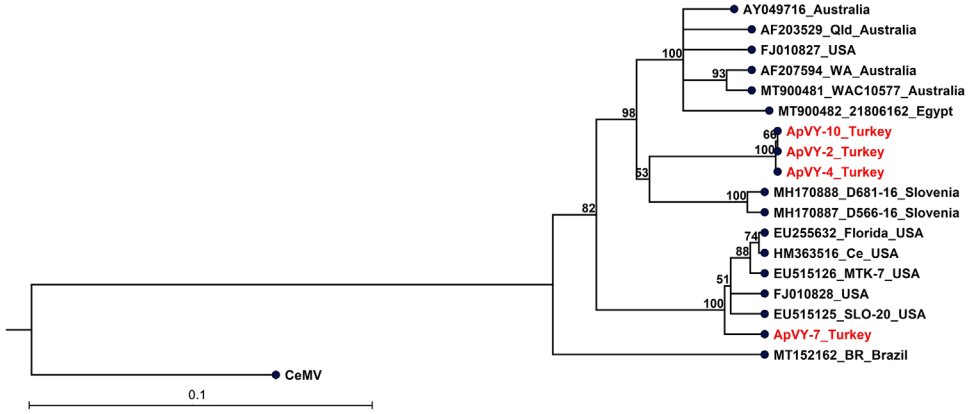

FIGURE 3 Phylogenetic tree of *Apium virus Y* (ApVY) isolates constructed by the maximum likelihood method using the nucleotide sequences of partial coat protein genes. *Celery mosaic virus* (CeMV, MK570304) was used as an outgroup for construction of the rooted phylogenetic tree. The scale bar represents a genetic distance of 0.1

reaction was prepared in a total volume of 25 μl including 2 μl cDNA (50 ng/μl), 12.5 μl 2x EmeraldAmp MAX PCR Mastermix (Takara, Japan), 0.5 μl primer mix (stock concentration 20 pmol/μl) and 10 μl nuclease-free water. The PCR conditions were 94°C for 5 min for initial denaturation, 40 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and a final extension for 5 min at 72°C. Fragments of the expected size were obtained from all samples. Amplicons from four samples, two from each field, were sequenced bi-directionally. The assembled sequences were submitted to GenBank (OL741314-OL741317). The identity of the isolates from this study with each other and the isolates from other countries were determined at the nucleotide and amino acid level by ClustalW alignment in the Sequence Demarcation Tool V.1.2 software (Muhire et al., 2014).

Sequence analyses using the obtained CP gene sequences revealed that Turkish ApVY isolates had 88–100% nucleotide and 95–100% amino acid identities between each other. Nucleotide and amino acid identities between the Turkish isolates and those from other countries were 88–100% and 93–100%, respectively (Figure 2). Phylogenetic analyses of the ApVY CP gene sequences of the four Turkish isolates in this study and isolates from other countries revealed that three Turkish isolates were closely related to Slovenian isolates and clustered together in the same branch of the phylogenetic tree, while one Turkish isolate grouped with the isolates from the USA (Figure 3). ApVY has been shown to be transmitted experimentally in 17 plant species in the family Apiaceae by mechanical inoculation, and by *Myzus persicae* with a transmission efficiency of 10–20 % (Xu et al. 2011). Seed transmission has not been shown (Xu et al. 2011; Koike et al. 2012) but we suspect it might be possible since aphid populations were not found in the ApVY-infected fields in this case. Alternatively, it is possible that some other insect vector is spreading this virus, thus further studies are needed to

understand the transmission of this virus under Turkish conditions. To the best of our knowledge, this is the first report of ApVY in Turkey.

ACKNOWLEDGEMENTS

This study was supported by a grant from TUBITAK-TOVAG (project # 120O802).

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How to cite this article: Karanfil A., Sarı, M., Randa-Zelyüt, F., Santosa, A.I. & Korkmaz, S. (2022) First report of *Apium virus Y* infecting *Petroselinum crispum* in Turkey. *New Dis Rep*, 45, e12059. https://doi.org/10.1002/ndr2.12059