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DISEASE NOTE

First report of Passiflora edulis symptomless virus in pomegranate in Spain

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Passiflora edulis symptomless virus (PeSV), family *Potyviridae*, genus *Roymovirus*, was first identified in Israel infecting *Passiflora edulis* plants (Jover-Gil et al. 2018). PeSV has also been reported in Turkey, China and India infecting pomegranate (Caglayan et al. 2020). Although it has not been formally associated with symptoms in pomegranate, there are indications that PeSV may induce symptoms in this crop. In June 2020 leaves from a pomegranate growing in Alicante (Spain) and showing chlorotic spots along the veins were analyzed by RNAseq high throughput sequencing (HTS, TrueSeq Illumina technology, 150 bp paired-end). Data was analyzed using CLC Genomics Workbench 10.1.1. After quality control and host genome subtraction, de novo assembly of 1,887,133 reads generated 10,946 contigs (> 200 nt), from which 128 were related to PeSV according to BLAST analysis (e-value < 10^{-4}). These contigs showed a high molecular variability indicating a mixed infection by several PeSV isolates. Contig extension performed by Geneious Prime 2020 allowed to recover a 9926 nt near full-length PeSV genomic sequence, (MZ361583, average coverage 528x). This sequence showed 78.1–80.8% nucleotide identity when compared to PeSV genomic sequences (MT680930-MT680935). No other virus was detected in the HTS analysis. RT-PCR from the original pomegranate plant using the newly designed primers SPeSV-6F (5'-GGCTAG AAACGGTGGGATGA-3') and SPeSV-6R (5'-ACCACC TGGCTCATGGCGA-3') generated the expected 165 nt amplicon, as confirmed by Sanger sequencing (100% nt identity with the HTS sequence). In further assays, the 165 nt fragment was also amplified from 17 symptomatic out of 65 tested trees from the same orchard. Although PeSV was not detected in asymptomatic plants, symptomatic trees that tested negative for PeSV were also found. These results could be explained by a limited inclusiveness of the primers used in this study and question the pathogenicity of PeSV in pomegranate. To our knowledge this is the first report of PeSV in Spain.

The authors have no conflicts of interest to declare that are relevant to the content of this article. HTS data supporting the results reported in the article are available from the corresponding author on reasonable request.

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