First report of cherry virus Turkey in sweet cherry in Greece

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The number of viruses identified in sweet cherry has been constantly increasing over the last few years, following the broad application of high throughput sequencing (HTS). Some of these were reported to cause leaf symptoms and yield reduction. In 2009-2013, surveys were performed on sweet cherry orchards located in Northern Greece for the presence of *Betaflexiviridae* viruses using generic and specific molecular assays (Foissac et al., 2005). Sanger sequencing of a generic reverse transcription polymerase chain reaction (RT-PCR) product originated from a sample collected from a symptomless sweet cherry cv. Ferrovia revealed a virus sequence sharing 75% nucleotide (nt) similarity with a highly conserved portion of the betaflexivirus polymerase gene. Total RNA, extracted with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from leaves of this sample, was subjected to HTS analysis on an Illumina Hi-seq 4000 platform (Novogene Co., Tiangen,China). The run, after quality control, yielded 26,002,319 of 150 nt long paired-end reads. *De novo* assembly of these reads using Geneious Prime 2019.2.3 and Velvet and subsequent blast analysis (BLASTn/x) of 82 contigs revealed, apart from several known sweet cherry viruses (cherry virus A, prunus virus F, little cherry virus 1), the presence of a newly described *Robigovirus* species. More specifically, the BLAST results revealed that 8 *de novo* contigs ranging from 277 to 2287 nt long were similar to the sequence of the recently reported cherry virus Turkey (CVTR) (Caglayan et al., 2019). The near complete genome sequence consisting of 8362 nucleotides (99.5%) of the Greek CVTR isolate (CVTR-C18GR, accession no. MT043307) was reconstructed using iterative mapping of the reads to the *de novo* contigs, with 4690 reads mapped in total, and Sanger sequencing of RT-PCR amplicons to fill the sequence gaps. Similarity analysis of the CVTR-C18GR sequence with the full virus sequences from GenBank CVTR-BUR12 (MK600387) and CVTRAZQ (MH177869) showed 90.47% and 90.60% nucleotide identities, respectively. Phylogenetic analyses confirmed the clustering of C18GR with other CVTR isolates.To investigate for the virus presence in Greece, primers F2Rob (5′-GTCAGAGAGAGGTATCTATGTC-3′) and RT1Rob (5′-CAGGCTGTTCATAACCT-3′), which amplify 577 nts of CVTR replicase, were designed. Sixty six samples from sweet cherry trees showing no virus-like symptoms were collected from various orchards and screened for the presence of CVTR. Eight of them were found to be infected with the new virus. Sanger sequencing of sample C11GR amplicon confirmed the presence of CVTR in Greek fields (96% nts similarity with C18GR, accession no. MT043308). Sequence similarity between C11GR and C18GR isolates was also high (97% nts) in partial TGB2, TGB3 and CP genomic regions amplified with primer pair 7006 F (5′-GAAAAGTGATTATTCAGCRCCAGT-3′) and 7708 R (5′-CTTTCACCCACTCATCACCTATCTCC-3′) (accession no. MT461407). This is the

first report of this virus in Greece, thus extending the information on its geographical distribution. Robigoviruses cause variable symptoms and diseases in sweet and sour cherries and for that reason they are included in the European Commission Directive concerning official inspections (2019/2072/EU). Additional screenings are necessary to evaluate the presence and impact of CVTR in sweet cherry orchards in Greece.

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