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NEW DISEASE REPORT

First report of *Apium virus Y* in wild carrot (*Daucus carota* ssp. *carota*) in Spain

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Apium virus Y (ApVY), a member of the genus *Potyvirus*, infects cultivated, weedy, and wild *Apiaceae* species, in which it may be asymptomatic (Eastwell *et al.*, 2008) or cause symptoms such as leaf mosaic, interveinal chlorosis, vein clearing, deformation and/or stunting. Since its first identification in Australia where it is considered endemic (Moran *et al.*, 2002), ApVY has also been reported in Germany, Slovenia, New Zealand and the USA (EFSA, 2022). Natural infection of ApVY has been reported in celery, coriander, dill, parsley, *Ammi majus* and *Conium maculatum*, while other *Apiaceae* species as well as some *Chenopodiaceae*, and *Nicotiana benthamiana* have been reported as experimental hosts (EFSA Panel on Plant Health, 2022).

In June 2021, six carrot populations (five cultivated carrot fields and a wild carrot population) were sampled near Segovia, Central Spain. From each population, leaf samples were randomly collected from fifty plants, including asymptomatic and symptomatic (reddening or chlorosis), and pooled into one sample. Double-stranded RNAs were extracted from each pool and converted to complementary DNA (Marais *et al.*, 2018) before being sequenced (2 × 125 nt paired reads; Illumina HiSeq2500), yielding between 2 and 6.5 million reads per sample. Analysis of the obtained sequence data by mapping reads on viral reference genomes using CLC Genomics Workbench v22.0 revealed a high number of reads from the wild carrot population (331,258 reads or c. 13% of the total) mapping at high stringency

(>90% identity over >95% of read length) on the genomic RNA sequence of ApVY (GenBank Accession No. HM363516). *De novo* assembly of reads from the wild carrot population and contig BlastX annotation allowed the identification of 15 contigs of between 360 and 2,530 nt which could be scaffolded and extended by successive rounds of mapping of remaining reads into two complete, 9,916 nt-long genomic sequences of ApVY. These two sequences are 95.1% identical and share 84.8–86.2% identity with other full-length ApVY genomic sequences available in GenBank. To confirm the presence of ApVY in the sampled wild carrot population, a two-step RT-PCR assay with detection primers (ApVY_458_F: 5'ACGCCATGCATTCAAGTGCC3' and ApVY_458_R: 5'ATGTGTTTCGTGATCAGGGTGC3') designed to generate a 458 nt amplicon was used on the dsRNAs extracted from the pooled sample. Sanger sequencing of the amplicon (OM801196) showed it to be identical to one of the sequences obtained by Illumina sequencing.

To our knowledge, this is the first reported case of natural ApVY infection in wild carrot and the first report of the virus in Spain, enabling us to increase the information on ApVY's distribution throughout Europe and suggesting this pathogenic virus might pose a problem at some point in carrot crops. Given the mixed infection status of the pool of plants sampled in the wild carrot population as evidenced by the HTS results, it is not possible to evaluate the pathogenicity of

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ApVY in this host. Efforts are clearly needed to evaluate its presence and potential impact in cultivated carrot crops.

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