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GENOME SEQUENCES

Genome Characterization of Two *Carrot Virus Y* Isolates from Australia

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ABSTRACT The near-complete genome sequence of the original *Carrot virus Y* (CarVY) type isolate (CarVY-Vic) collected in 1999 in Victoria, Australia, and a near-complete genome sequence from an isolate collected in 2019 from the same region (CarVY-2-22) were determined following deep sequencing. The two CarVY genome sequences shared 98% nucleotide identity.

Carrot virus Y (CarVY) (family *Potyviridae*, genus *Potyvirus*) is an economically important pathogen of carrots (*Daucus carota* subsp. *sativus*) that causes chlorotic mottle, marginal leaf necrosis or reddening, mild stunting, and increased subdivision of leaflets, giving a "feathery" appearance (1). It has a narrow natural host range and is known to infect only commercially grown and wild *Apiaceae* species (1, 2). CarVY is nonpersistently transmitted by aphids and also is transmitted in seeds (1). CarVY was first reported in Australia in 2002 (2). The type isolate CarVY-Vic (NCBI RefSeq accession number NC_043142.1) is represented only by a 3′-terminal sequence of 1,754 nucleotides (nt) that covers part of the NIb polymerase and the complete coat protein genes and was detected by reverse transcription (RT)-PCR (2).

Tissue from the original carrot sample containing the CarVY-Vic type isolate was preserved in the Agriculture Victoria reference specimen collection, and high-throughput sequencing (HTS) was used to obtain a near-complete CarVY-Vic genome sequence. HTS was also used to obtain a near-complete genome sequence of a second carrot isolate, CarVY-2-22, which was collected in May 2019 from a symptomatic plant from the same region in Victoria, Australia. Total nucleic acids were extracted from the two samples using the QIAxtractor (Qiagen) as described previously (3). A transcriptome sequencing (RNA-Seq) library was prepared using the TruSeq stranded total RNA sample preparation kit with Ribo-Zero Plant (Illumina, San Diego, CA) as described previously (4). The size distribution and concentration of the amplicon libraries were determined using the 2200 TapeStation system (Agilent Technologies) and a Qubit 2.0 fluorometer (Invitrogen), respectively, and the resulting quantification values were used to pool the libraries. The pooled library was diluted, denatured, and sequenced using Illumina HiSeq technology, with a paired-end read length of 2 × 151 bp.

Totals of 16,831,182 and 15,960,819 HTS raw reads were obtained from isolates CarVY-Vic and CarVY-2-22, respectively; after quality trimming using Trim Galore (version 0.6.5) (5), these numbers were reduced to 16,712,236 and 15,641,254, respectively. *De novo* assembly using the SPAdes (version 3.13.0) genome assembler (6) with default settings resulted in 3,416 contigs for the CarVY-Vic isolate and 2,827 contigs for the CarVY-2-22 isolate. A BLASTn search of the GenBank database (7) showed a 9,609-nt contig of the CarVY-Vic isolate and a 9,603-nt contig of the CarVY-2-22 isolate, with 100% and 98% nucleotide identity, respectively, with respect to the original 1,754-nt sequence for CarVY-Vic (NCBI RefSeq accession number NC_043142.1). No other virus

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contigs were detected by BLASTn analysis of contigs from the two samples. The assembled CarVY-Vic and CarVY-2-22 near-complete genome sequences had GC contents of 38.6% and 39.1% and average coverages of 1,698× and 1,931×, respectively. The CarVY-Vic and CarVY-2-22 near-complete genomes shared 98% nucleotide identity, and both isolates had a complete potyvirus polyprotein coding region with a typical 5′ untranslated region (UTR) and a poly(A) tail 3′ UTR (8). The polyproteins of both isolates translated correctly into a single complete polyprotein coding sequence, and the amino acid similarity was 99%. Overlapping primer pairs were designed (available upon request) for RT-PCR confirmation of only the assembled HTS CarVY genome sequences. Sanger sequencing of the amplicons confirmed the genome sequence arrangements for the CarVY-Vic and CarVY-2-22 isolates. This study reports the first near-complete genomes of two CarVY isolates and provides valuable genome data to facilitate further studies of the genetic diversity of CarVY.

Data availability. The genome sequences of the CarVY isolates CarVY-Vic and CarVY-2-22 have been deposited in GenBank under accession numbers LC511903 and LC511904, respectively. The CarVY-Vic and CarVY-2-22 raw data were deposited in the SRA under BioSample accession numbers SAMN14089764 and SAMN14089765, respectively, as part of BioProject PRJNA606340.

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