


The first complete sequence and genome structure of daphne virus Y

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Abstract From *Daphne odora* Thunb., an ornamental shrub in the Republic of Korea, a potyvirus was identified that has an RNA genome of 9,448 nucleotides (excluding the 3'-terminal poly(A) tail) encoding a polyprotein of 3,065 amino acids, with nine putative protease cleavage sites producing ten proteins. Since this potyvirus shared the highest nucleotide sequence identity (91 %; query coverage 5 %) with the available partial sequence of daphne virus Y (DVY) from New Zealand (EU179854), it was considered a Korean isolate of DVY. This is the first molecular characterization of the complete genome sequence of a DVY isolate.

Introduction

The family *Potyviridae* is one of the largest and most important groups of plant viruses, being economically significant for a wide range of agricultural crops. *Daphne*

odora Thunb. (family Thymelaeaceae) is an evergreen ornamental shrub, native to regions of East Asia (China, Japan, Korea), where it is cultivated for its beautiful fragrant flowers. At present, there are several reports of potyviruses infecting *Daphne* spp.: daphne virus Y (DVY) in New Zealand in 1975 [6], a second potyvirus, also named DVY in Germany in 1991 [11, 12], daphne mosaic virus (DapMV, DQ299908) in the Czech Republic in 2006 [11], and a potyvirus referred to as daphne mottle virus (DapMoV, LC123273, LC123274), which was isolated in the Republic of Korea in 2016 [10]. DVY has been detected in daphne in Korea in 2006 and 2009 [8, 9]. There is a DVY sequence available in the GenBank database from New Zealand in 2007 (EU179854), which includes the 3' half of the coat protein gene (400 nt) plus 91 nucleotides of the 3' untranslated region sequence (3'UTR). In this study, the complete genome sequence of an isolate of DVY from *D. odora* in the Republic of Korea was determined and described.

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Provenance of virus material

A total of 97 plant samples with virus-like symptoms were randomly collected from different places in Korea during 2014. For diagnostic purposes, all of the samples were pooled for high-throughput paired-end RNA sequencing. Total RNA was isolated from the pooled sample using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA), followed by removal of ribosomal RNAs using a Ribo-Zero rRNA Removal Kit (Plant Leaf) (Epicentre, Madison, WI, USA), both according to the manufacturers' instructions. The library was constructed using a TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA) following the protocols provided by the manufacturer.

Sequencing was performed by the Theragen Bio Institute (Suwon, Korea) using an Illumina HiSeq 2500 sequencer, which produced 425,280,472 reads for a total of 42,953,327,672 bp. All reads were filtered to remove adaptor sequences and low-quality bases. *De novo* assembly was performed with Trinity (v2.1.1) software and processed by SeqGenesis (Daejeon, Korea). Assembled contigs were analyzed using Basic Local Alignment Search Tool (BLAST) searches to identify sequence similarities to reference viral genomes in the GenBank database. One long contig (9434 bp in length) of a virus resembling a potyvirus in nucleotide sequence and genome organization was identified. BLASTn search analysis revealed that the contig shared the highest nucleotide sequence identity of 91 % with the partial sequence (491 nt) of a DVY isolate from New Zealand (EU179854). A total of 71,185 reads mapped to the final contig (depth of coverage across the contig: maximum, 6,362; minimum, 16; mean, 662). Therefore, to confirm the paired-end RNA sequencing result of the contig sequence and to find the sample infected with DVY, eight specific primer pairs were designed based on the contig sequence (Supplementary Table 1). Total RNA was isolated from each of the 97 plant samples, using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA). cDNA was synthesized using the degenerate 25-mer primer N25 [7] with RevertAid Reverse Transcriptase (Thermo Scientific, Waltham, MA, USA), following the protocol provided by the manufacturer. The cDNA was subjected to PCR using eight specific primer pairs with AccuPower ProFi Taq PCR PreMix (Bioneer, Daejeon, Korea). The eight specific primer pairs successfully amplified the expected PCR fragments from a *D. odora* sample. Among the 97 samples, only one sample from a *D. odora* plant was infected with DVY. In June 2014, when this sample was collected from a private garden in Muju County in the Republic of Korea, the *D. odora* plant showed yellowing and mosaic symptoms. PCR products were cloned into an RBC TA Cloning Vector (RBC Bioscience, Taipei, Taiwan) and sequenced by GenoTech (Daejeon, Korea). Sequencing results were shown to be completely consistent with the original contig and confirmed that the *D. odora* sample was infected with DVY. In addition, using the two specific reverse primers based on the DVY contig sequence, the 5'-terminal sequence was determined by 5'-RACE (Rapid Amplification of cDNA Ends; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The 3'-terminal sequence was determined by cDNA synthesis from total RNA using an oligo(dT) primer [4]. The specific forward primer was designed from the DVY contig sequence with an oligo(dT)₂₀ primer for PCR amplification of the 3'-terminal nucleotide sequences. All partial sequence assemblies were performed using the DNAMAN

5.0 program (Lynnon Biosoft, Quebec, Canada). The complete DVY genome sequence from the *D. odora* sample was assembled from overlapping PCR products. Phylogenetic relationships of DVY to 32 other members of the family *Potyviridae* were analyzed using MEGA6.06 [13] with 1000 replicates of the neighbor-joining algorithm (Fig. 2).

Sequence properties

The full-length genome of DVY is 9,448 nucleotides (nt) long excluding the 3' poly(A) tail, with a 5' UTR of 116 nt and a 3' UTR of 137 nt (Fig. 1). The sequence has been deposited in the GenBank database under the accession number KU556609. The genome organization of DVY is typical of a potyvirus, containing a single large ORF. The putative ORF has an AUG start codon and an UAA stop codon, located at positions 117–119 and 9,312–9,314, respectively. The ORF comprises 9,195 nt and encodes a polyprotein of 3,065 amino acid (aa) residues with an estimated molecular mass of 345.8 kDa. The polyprotein of DVY is processed into ten mature functional proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa, NIB, and CP), and the nine putative protease cleavage sites were identified [1, 14] (Fig. 1). Several conserved functional potyvirus motifs were identified in the DVY polyprotein, including the ²⁰³H-8X-D-34X-S²⁴⁷ motif in P1, the ³⁴⁹K-I-T-C³⁵² and ⁶⁰⁷P-T-K⁶⁰⁹ motifs in HC-Pro that are involved in aphid transmission, the ¹²³⁹GSGKS-3X-P¹²⁴⁷ and ¹³²⁵D-E-C-H¹³²⁸ motifs in CI, and the ²⁶²³GDD²⁶²⁵ motif in NIB [14]. Recent studies have demonstrated a putative protein PIPO with the conserved motif G₁₋₂A₆₋₇ [²⁸³²GAAAAAAA²⁸³⁹], which was also found within the P3 protein gene [5]. The complete nucleotide sequences and the deduced amino acid sequences of DVY and other related potyviruses were aligned and compared using the DNAMAN software (Supplementary Table 2). Percent identities of the full-length DVY sequence compared with those of members of the genus *Potyvirus* varied from 46.33 to 61.37 % at the nt level and from 37.83 to 62.50 % at the aa level. DVY was most similar to DapMV in nt (61.37 %) and aa (62.50 %) sequence identities. In addition, in phylogenetic analysis using the complete polyprotein aa sequence, DVY was most closely related to DapMV (Fig. 2). Its 5'-UTR shared highest identity (53.91 %) with that of narcissus late season yellows virus (NLSYV, JQ326210), while the 3'-UTR shared highest identity (92.31 %) with the partial DVY sequence (EU179854). At the aa level, the proteins P1, HC-Pro, P3, 6K1, 6K2, VPg, NIa, and NIB of DVY had the highest identities to those of DapMV (25.0, 75.6, 34.5, 59.6, 54.7, 69.1, 65.4 and 75.1 %, respectively); and CI and CP, to those of DapMoV

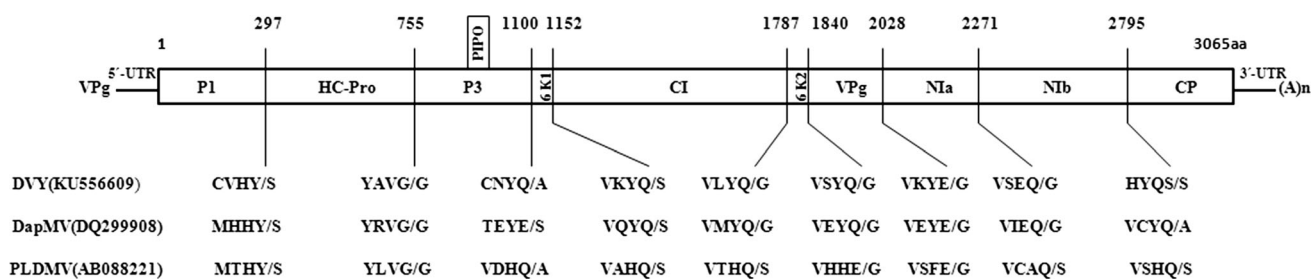


Fig. 1 Schematic representation of the genome organization of DVY. The 5' and 3' untranslated regions (UTR) are represented as bold lines, and the large ORF is depicted by an open box. The putative cleavage sites in the polyprotein are indicated and compared to two closely related viruses, DapMV and papaya leaf distortion mosaic

virus (PLDMV), below the genome. The numbers above the diagram indicate the predicted amino acid position for each mature protein in the DVY polyprotein. The boxed figure above the P3 protein indicates the position of PIPO

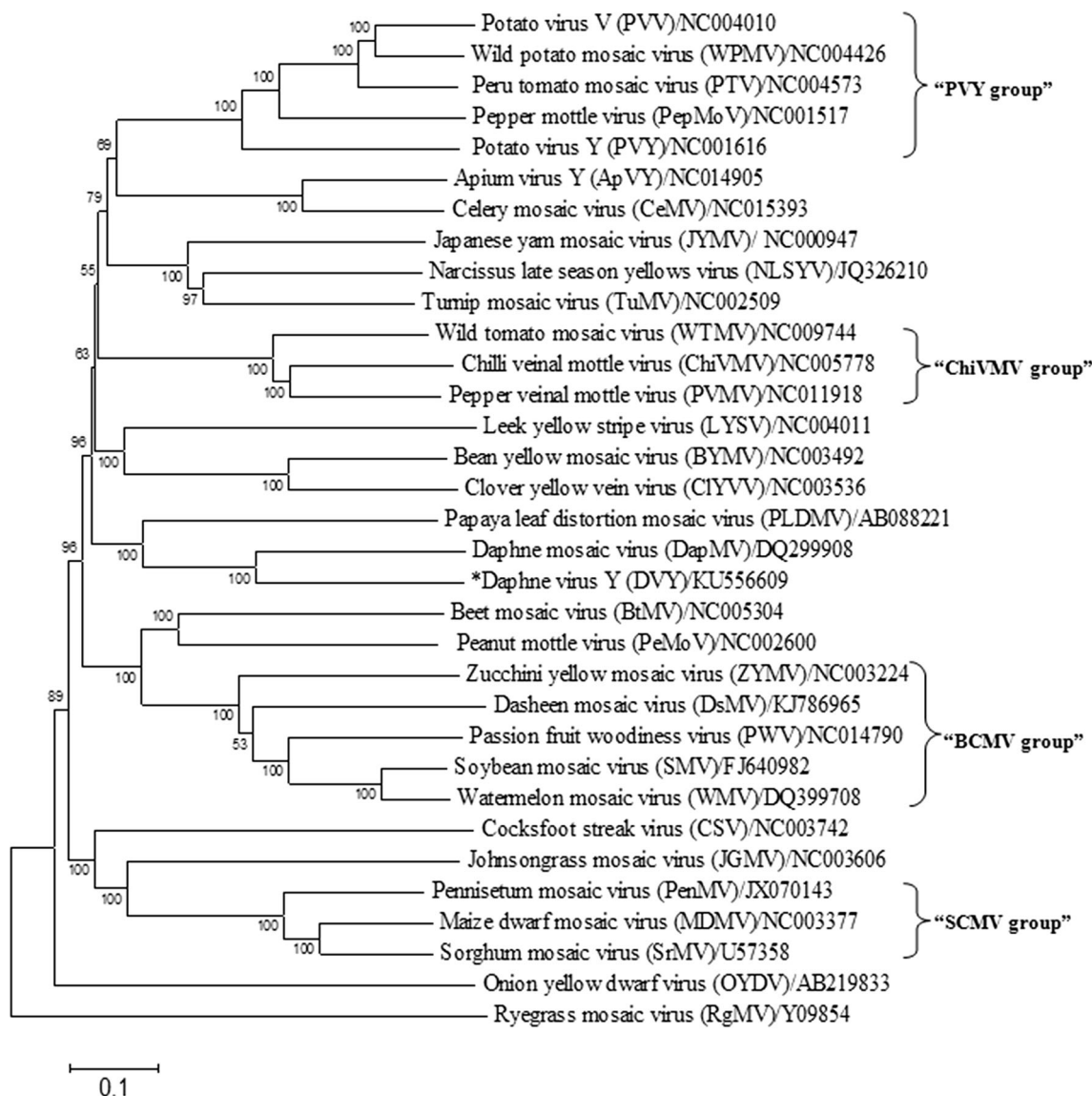


Fig. 2 Phylogenetic tree based on the polyprotein amino acid sequences of DVY and 32 representative members of the family *Potyviridae* (virus name/accession number). The tree was constructed by the neighbor-joining method using the MEGA6.06 program.

Bootstrap analysis was applied using 1000 replicates. Ryegrass mosaic virus (RgMV, genus *Rymovirus*, family *Potyviridae*) was used as an out-group

(partial sequences) (96.69 and 96.86 %). The CP aa sequence identity of DVY was above the current potyvirus species demarcation criteria set by the ICTV [2, 3]. Previously described partial CP sequence regions of DVY (EU179854) and DapMoV (LC123274) were 94 % identical at the aa level. A BLASTn search with the complete sequence of DVY revealed the highest shared nt sequence identity (91 %) with DVY (EU179854). For these reasons the daphne-infecting virus from South Korea (KU556609) was regarded as a DVY isolate, and this is the first report of the complete nucleotide sequence of DVY.

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Compliance with ethical standards

Conflict of interest This paper is in compliance with ethical standards for research. The authors declare that they have no conflict of interest.

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