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Biological characterization and complete genomic sequence of *Apium virus Y* infecting celery

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ABSTRACT

A celery isolate of *Apium virus Y* (ApVY-Ce) from diseased plants in a commercial field in California was characterized. The experimental host range of the virus included 13 plant species in the families *Apiaceae*, *Chenopodiaceae* and *Solanaceae*. Almost all infected plant species showed foliar chlorosis and distortion or severe stunting and systemic chlorosis. ApVY-Ce was transmitted to all 10 host species in the *Apiaceae* by green peach aphids. It reacted with the potyvirus group antibody and *Celery mosaic virus* (CeMV) antiserum. The complete genomic sequence of ApVY-Ce was determined to be 9917 nucleotides, excluding the 3′ poly(A) tail, and it comprises a large open reading frame encoding a polyprotein of 3184 amino acid residues. Its genomic organization is typical of potyviruses, and contains conserved motifs found in the genus *Potyvirus*. Comparisons with available genomic sequences of other potyviruses indicate that ApVY-Ce shares 26.1–52.9% identities with species of the existing genera and unassigned viruses in the *Potyviridae* at the polyprotein sequence level. Extensive phylogenetic analysis based on the 3′-partial sequences confirms that ApVY-Ce is most closely related to CeMV and is a distinct species of the genus *Potyvirus*.

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1. Introduction

Plants in the family *Apiaceae* are affected by many viruses (Davis and Raid, 2002), including seven belonging to the genus *Potyvirus* (Latham and Jones, 2004; Moran et al., 2002; Murant et al., 1970; Orilio et al., 2009; Robertson, 2007; Yan et al., 2010). Among them, *Celery mosaic virus* (CeMV) is one of the first identified potyviruses (Severin and Freitag, 1938) and causes serious diseases in apiaceous crops worldwide (Raid and Zitter, 2002). Six of these potyviruses, *Angelica virus Y* (AnVY), *Apium virus Y* (ApVY), *Arracacha mottle virus* (AMoV), *Carrot virus Y* (CarVY), *Carrot thin leaf virus* (CTLV) and Panax virus Y (PanVY), have only been recognized recently as distinct potyviruses based on their partial genomic sequences and/or serological reactions.

ApVY was first found in sea celery (*Apium prostraum*), poison hemlock (*Conium maculatum*) and parsley (*Petroselinum crispum*) in 2002 during a potyvirus survey of native and weed species of *Apiaceae* in Australia (Moran et al., 2002). The virus has since been reported to cause diseases in bishop's weed (*Ammi majus*), celery (*A. graveolens*), cilantro (*Coriandrum sativum*) and parsley in the United States and New Zealand (Baker et al., 2008; Eastwell et al., 2008; Tang et al., 2007; Tian et al., 2008). The ApVY isolates from Florida and Washington states in the USA reacted to the general potyvirus antibody (Baker et al., 2008; Eastwell et al., 2008), and the isolates from Australia and New Zealand did not react with the CeMV antiserum (Moran et al., 2002; Tang et al., 2007). Sequence comparison of the 3′-partial sequences showed that ApVY was a distinct species in the genus *Potyvirus* (Moran et al., 2002).

Phylogenetic comparisons of the CP sequences did not clearly separate ApVY from CeMV and CarVY as distinct species; however, the comparisons of the available 3′-partial sequences indicated that ApVY was distinct from others (Adams et al., 2005b; Moran et al., 2002). These three potyviruses are important viral pathogens of the apiaceous crops, but their complete genome sequences have not been available. Determination of the complete genomic sequence of ApVY is essential to distinguish it from other potyviruses as well as to advance our understanding of the potyviruses infecting the apiaceous plants. Furthermore, characterizations of biological and serological properties of ApVY would provide more information for the disease management. We report here the experimental host range, aphid transmission, serological reactions and complete genomic sequence of ApVY. Our results provide conclusive evidence that ApVY is a distinct species in the genus *Potyvirus*.

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2. Materials and methods

2.1. Virus survey

During the 2007–2009 growing seasons, leaf samples were collected from diseased celery plants, along with poison hemlock plants without obvious symptoms adjacent to the celery fields, in three locations of Monterey and Santa Clara counties in California. Total nucleic acids were extracted and used for the ApVY detection by RT-PCR using a pair of virus-specific primers (Tian et al., 2008).

2.2. Virus source

An isolate of ApVY (ApVY-Ce) was obtained from a celery plant showing ring spot and line pattern symptoms in Santa Clara County, California in 2007. The 3′-partial genome of 1.7 kb of this isolate has been obtained previously, and the sequence is available as EU515126 in the GenBank. The virus was maintained in celery plants through mechanical inoculation or aphid transmission using green peach aphids (*Myzus persicae*) in an insect-proof greenhouse of the USDA-ARS facilities at Salinas, California.

2.3. Host range study

A host range study was conducted by mechanical inoculation of ApVY-Ce onto 42 plant species/cultivars from 7 families (Table 1). Fresh leaves of the infected celery plants were ground in a mortar and pestle with five volumes (w/v) of cold 0.1 M sodium phosphate buffer, pH 7.0, containing 0.02 M sodium sulfite and a small amount of celite. The plant sap was gently rubbed onto leaves of five plants of each test species/cultivar using a cotton swab. Inoculated plants were maintained in a greenhouse under natural lighting with a temperature range of 24–30 °C for symptom expression. The viral infection was confirmed by the RT-PCR assay.

2.4. Aphid and seed transmission

Non-viruliferous green peach aphids were allowed to feed on the infected celery plants for 24 h. Ten to fifteen aphids were then transferred onto each of five plants of each species/cultivar for a 24-h transmission feeding before being killed by insecticide Orthene. The inoculated plants were maintained in the conditions described above for symptom observation, and infection was confirmed by the RT-PCR assay.

Seeds were collected from the ApVY-infected poison hemlock plants near the celery fields. Total nucleic acids were prepared from 0.1 g of the seeds from each of several plants immediately after harvest and tested by the RT-PCR assay. A portion of the RT-PCR-positive seeds from each plant were planted in soil. One hundred seedlings geminated from these seeds were then tested by RT-PCR for the ApVY detection.

2.5. Serological analyses

Serological relationships of ApVY with several potyviruses were studied by indirect ELISA as described by Li et al. (1998). Polyclonal antisera against CeMV-Ce, *Lettuce mosaic virus* (LMV), *Potato virus Y* (PVY), *Sugarcane mosaic virus* (SCMV), *Watermelon mosaic virus* and *Wheat streak mosaic virus* were obtained from American Type Culture Collection (Manassas, VA, USA). Polyclonal antiserum against AMoV was kindly provided by Dr. A. K. Inoue-Nagata of University of Brasilia in Brazil. Polyclonal antiserum against *Dasheen mosaic virus* (DsMV) was obtained from Dr. F. W. Zettler of University of Florida in the USA. The universal potyvirus group monoclonal antibody was purchased from Agdia, Inc. (Elkhart, IN, USA). Optical densities of virus samples were recorded at 405 nm by the Multiskan Spectrum (Thermo Scientific, Waltham, MA, USA). Samples showing optical density (OD) values higher than twice the average of the negative control were considered positive.

Western blot analysis was conducted to determine the molecular weight of the coat protein (CP). Total proteins were prepared from leaf tissues of healthy celery, ApVY-infected celery and CeMV-infected parsley and were resolved on 12% Ready Gel (Bio-Rad, Hercules, CA, USA). The gel was then electroblotted onto 0.45-μm nitrocellulose membranes, and the blot was treated as previously described (Li et al., 1998). Protein molecular weight was determined by the use of prestained SDS-PAGE standards (Bio-Rad).

2.6. RT-PCR, cloning and sequencing

Total nucleic acids were extracted from both infected celery and inoculated plants by a CTAB method described by Li et al. (2008) and used as templates for RT-PCR amplifications. RT-PCR was carried out using the One Step RNA PCR Kit (Clontech Laboratories, Inc., Madison, WI, USA) following the manufacturer's instructions in a Peltier thermal cycler (Bio-Rad).

Approximately 1.5 kb of the 3′-partial genome was amplified using a virus-specific forward primer (5′-GAGCGAACTCTTCAGACAGCTAG-3′) designed according to the available ApVY sequence (EU515126) and M4T (Chen et al., 2001). Two sequential RT-PCR assays were performed to obtain a CI-NIb region of 3367 nucleotides (nt) each using a degenerate forward primer, designed according to the alignment of genomic sequences of several potyviruses with high identity scores to ApVY, and a specific reverse primer. The two pairs of the primers used were PotyF1 (5′-GYGATTTTYGGAAACAYTGGAT-3′)/ApR1 (5′-ATCTATGCTCCGGTTCTGATGA-3′) and PotyF2 (5′-GTGARATTTGATGGCTCHATGCACCC-3′)/ApR2 (5′-TCATTGAGGCTTTCGAGTGTGTC-3′), respectively. To amplify HC-Pro-CI region, a 686-bp fragment within the HC-Pro was first obtained as described by Ha et al. (2008) and used to design a specific forward primer (5′-ATGGATGTTGCGACTGCTTGTC-3′) which paired with a specific reverse primer (5′-AACAGTTGTCCTTCTTGTGCTC-3′) to produce the 2998-bp target fragment. The 5′-partial genome of 2263 nt was obtained by a semi-nested RT-PCR using a forward primer containing a 17mer M4 tag (Chen et al., 2001) and a 10mer (5′-AAAATAAAAA-3′) found in the 5′-end of some potyviruses and two specific reverse primers, Ap5R1 (5′-AACTAGTATCCGTGGTAACTCAGC-3′) and Ap5R2 (5′-AATTGCGATTCGATGGGTTTTCCC-3′). For the 5′ terminal sequence, 5′ RACE was conducted by the GeneRacer® Kit (Invitrogen, Carlsbad, CA, USA) using the GeneRacer 5′ primers and two specific reverse primers, Ap5R3 (5′-TACCTGCTTAGGCTTGTCCTCG-3′) and Ap5R4 (5′-CAAATTCAGCTTCAGCTCGCTC-3′).

All amplified PCR products were purified by precipitation with 1/10 volume of 5 M ammonium acetate, pH 5.0 and one volume of isopropanol and subsequently cloned by the pGEM®-T Easy Vector System II (Promega, Madison, WI, USA) or the TOPO® TA Cloning Kit (Invitrogen) following the manufacturer's instructions. Plasmid DNA was isolated from overnight culture using the FastPlasmid® Mini Kit (5 Prime, Inc., Gaithersburg, MD, USA). Five clones from each ligation were sequenced on both strands using M13 forward and M13 reverse primers as well as specific sequencing primers if necessary (MacrogenUSA, Rockville, MD, USA).

2.7. Sequence analysis and phylogeny

The complete sequence of the ApVY-Ce genome was assembled from seven overlapping RT-PCR clones by Sequence Assembly in the DNAStar 5.01 package (DNAStar Inc., Madison, WI, USA). The putative cleavage sites on the ApVY polyprotein were determined