

The complete nucleotide sequence and genomic characterization of tropical soda apple mosaic virus

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Abstract We report the first complete genome sequence of tropical soda apple mosaic virus (TSAMV), a tobamovirus originally isolated from tropical soda apple (*Solanum viarum*) collected in Okeechobee, Florida. The complete genome of TSAMV is 6,350 nucleotides long and contains four open reading frames encoding the following proteins: i) 126-kDa methyltransferase/helicase (3354 nt), ii) 183-kDa polymerase (4839 nt), iii) movement protein (771 nt) and iv) coat protein (483 nt). The complete genome sequence of TSAMV shares 80.4 % nucleotide sequence identity with pepper mild mottle virus (PMMoV) and 71.2–74.2 % identity with other tobamoviruses naturally infecting members of the Solanaceae plant family. Phylogenetic analysis of the deduced amino acid sequences of the 126-kDa and 183-kDa proteins and the complete genome sequence place TSAMV in a subcluster with PMMoV within the Solanaceae-infecting subgroup of tobamoviruses.

The genus *Tobamovirus* of the family *Virgaviridae* is composed of 35 species [1]. Tobamoviruses are characterized by having rigid rod-shaped virions and single-stranded positive-sense RNA genomes. These viruses are easily transmitted by mechanical inoculation and plant-to-

plant contact and are distributed worldwide [2]. Genomes of all characterized tobamoviruses contain four open reading frames encoding the following proteins: a 124- to 132-kDa replicase protein with methyltransferase and RNA helicase domains, a 181- to 189-kDa replicase protein with a polymerase domain, a movement protein (MP), and a coat protein (CP) [2, 7, 8].

Tropical soda apple (*Solanum viarum* Dunal) is a noxious solanaceous weed native to South America [9] that has spread throughout Florida and the southern United States since being introduced in the 1980s [4, 5]. Tropical soda apple mosaic virus (TSAMV) was originally isolated from tropical soda apple plants displaying foliar mosaic symptoms that were collected in Okeechobee, FL [3]. Host range analysis of TSAMV demonstrated that it infected 16 species of plants in the family Solanaceae. Molecular and phylogenetic analysis of TSAMV MP and CP indicated high sequence similarity to pepper mild mottle virus (PMMoV) and other Solanaceae-infecting tobamoviruses [3]. However, the complete genome sequence of TSAMV has not been reported.

The original *Nicotiana tabacum* cv Xanthi nc local-lesion-passaged TSAMV isolate [3] was used to inoculate *Nicotiana benthamiana*, from which virions were partially purified from systemically infected tissue using a typical tobamovirus protocol [13]. TSAMV genomic RNA extraction, RNA-seq library preparation, and sequencing on an Ion Torrent Personal Genome Machine (Life Technologies) were as described previously [6]. The complete genome consensus sequence was generated using the Torrent Mapping Alignment Program (TMAP version 4.21), Geneious R8 version 8.1.4 (Biomatter, Auckland, New Zealand) and Integrative Genomic Viewer (IGV) version 2.3 [10, 12]. MEGA6 was used for multiple sequence alignments of the nucleotide and deduced amino

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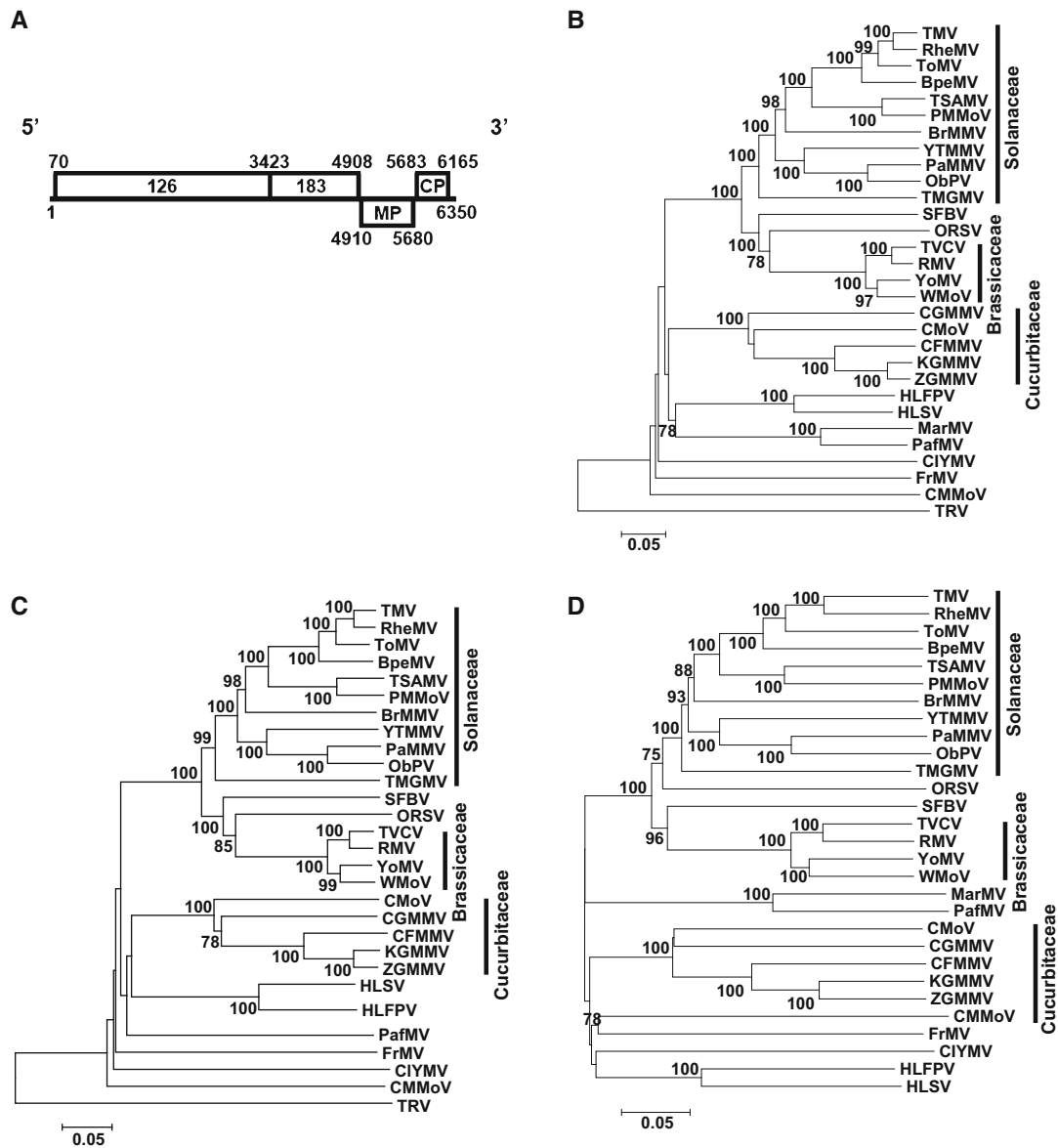


Fig. 1 Genome organization (A) and phylogenetic analysis of TSAMV and recognized members of the genus *Tobamovirus* based on the deduced amino acid sequences of the 124- to 132-kDa protein (B), 181- to 189-kDa protein (C), and complete genome sequence (D). Genomic RNA is shown as a solid line, with nucleotides indicated at 5' and 3' termini. Predicted open reading frames are shown as boxes containing protein names and nucleotides (shown above or below) indicating start and stop codons. Unrooted, neighbor-joining trees were inferred using MEGA6 [11], with tobacco rattle virus (TRV; NP_620669.1 and NP_620669.2) as an outgroup in B and C. Branch lengths indicate the number of amino acid or nucleotide differences per site, and the numbers at the nodes indicate bootstrap values greater than 70 based on bootstrap analysis of 1000 replicates. The following viruses were included (listed with abbreviation and GenBank accession no. for nucleotide sequence): bell pepper mottle virus (BpeMV, NC_009642), brugmansia mild mottle virus (BrMMV, NC_010944), cactus mild mosaic virus (CMMoV, NC_011803), clitoria yellow mottle virus (CIYMV, NC_016519), cucumber fruit mottle mosaic virus (CFMMV, NC_002633), cucumber green mottle mosaic virus (CGMMV, NC_001801), cucumber mottle virus

(CMoV, NC_008614), frangipani mosaic virus (FrMV, NC_014546), hibiscus latent Fort Pierce virus (HLFPV, NC_025381.1), hibiscus latent Singapore virus (HLSV, NC_008310.2), kyuri green mottle mosaic virus (KGMMV, NC_003610.1), maracuja mosaic virus (MarMV, NC_008716), Obuda pepper virus (ObPV, NC_003852), odontoglossum ringspot virus (ORSV, NC_001728), paprika mild mottle virus (PaMMV, NC_004106), passion fruit mosaic virus (PafMV, NC_015552) pepper mild mottle virus (PMMoV, NC_003630), rehmannia mosaic virus (RheMV, AB628188), ribgrass mosaic virus (RMV, NC_002792.2), streptocarpus flower break virus (SFBV, NC_008365), tobacco mild green mosaic virus (TMGMV, NC_001556), tobacco mosaic virus (TMV, NC_001367), tomato mosaic virus (ToMV, KR537870.1), tropical soda apple mosaic virus (TSAMV, KU659022), turnip vein clearing virus (TVCV, NC_001873), wasabi mottle virus (WMoV, NC_003355), yellow tailflower mild mottle virus (YTMMV, KF495564), youcai mosaic virus (YoMV, NC_004422), and zucchini green mottle mosaic virus (ZGMMV, NC_003878). Solanaceae-, Brassicaceae- and Cucurbitaceae-infecting subgroups are indicated

Table 1 Percent identity of the deduced amino acid (aa) sequences of the 124- to 132-kDa protein, 181- to 189-kDa protein, MP and CP, and the nucleotide (nt) sequence of the complete genome of TSAMV and other tobamoviruses

Virus	124-132 kDa (aa)	181-189 kDa (aa)	MP (aa)	CP (aa)	Genome (nt)
BpeMV	74.6	76.8	71.0	81.3	74.2
BrMMV	68.5	71.8	65.5	70.6	71.5
CFMMV	45.3	48.8	39.4	42.9	65.7
CGMMV	45.0	49.5	38.8	39.9	65.9
CIYMV	44.8	47.4	35.7	41.6	65.8
CMMoV	40.2	45.0	33.9	41.6	66.1
CMoV	45.2	49.4	37.9	45.4	65.7
FrMV	42.0	46.8	32.7	45.5	66.0
HLFPV	45.7	50.1	34.8	45.3	66.0
HLSV	45.5	50.3	36.5	44.8	66.4
KGMMV	45.1	48.7	40.9	42.9	65.4
MarMV	46.3	46.5	33.2	42.0	66.3
ObPV	66.0	69.0	62.9	69.1	71.3
ORSV	58.4	62.8	58.4	74.2	70.5
PafMV	47.3	50.2	32.2	43.6	66.0
PaMMV	66.0	69.5	63.4	70.8	71.2
PMMoV	90.2	90.1	80.9	84.8	80.4
RheMV	73.8	76.2	67.1	73.8	73.7
RMV	59.3	63.4	44.4	53.5	69.2
SFBV	59.4	62.2	46.4	47.4	68.7
TMGMV	62.7	65.4	64.6	70.2	71.5
TMV	73.8	76.1	69.2	72.5	73.0
ToMV	74.9	77.2	69.9	76.9	73.9
TVCV	58.8	63.0	43.7	53.5	68.8
WMoV	58.2	62.4	45.6	51.6	68.8
YoMV	60.1	63.9	44.4	52.2	69.3
YTMMV	64.9	69.1	66.5	63.4	71.6
ZGMMV	43.4	47.9	40.8	37.4	65.7

Virus abbreviations and accession numbers are as shown in the legend to Fig 1

acid sequences, and for phylogenetic analysis [11]. Pair-wise comparisons of TSAMV and other tobamoviruses were made with DNA Master (available from <http://cobamide2.bio.pitt.edu/>).

The complete genome of TSAMV (KU659022) is 6,350 nucleotides (nt) long and includes a 5' untranslated region (UTR, nt 1-69), genes encoding a 183-kDa polymerase (nt 70-4908), 126-kDa methyltransferase/helicase (nt 70-3423), MP (nt 4910-5680), and CP (nt 5683-6165), and a 3' UTR (nt 6166-6350) (Fig. 1A). Comparison of the TSAMV 126-kDa, 183-kDa, MP and CP amino acid sequences with those of other tobamoviruses showed sequence identities ranging from 40.2 to 90.2 %, 45.0 to 90.1 %, 32.2 to 80.9 % and 37.4 to 84.8 %, respectively (Table 1). Both the 126-kDa and 183-kDa proteins showed highest amino acid identity (90.2 % and 90.1 %, respectively) to the corresponding proteins of PMMoV, consistent with previous sequence analysis of the TSAMV MP and CP [3]. At the complete-genome level, TSAMV shared

80.4 % sequence identity with PMMoV. When comparing the TSAMV genome sequence with available complete genome sequences of 28 other tobamoviruses, the closest nucleotide sequence identities (71.2-80.4 %) were with other Solanaceae-infecting tobamoviruses.

Phylogenetic analysis of the deduced amino acid sequences of the 126-kDa and 183-kDa proteins (Fig. 1B and C), and the complete genome sequence (Fig. 1D), revealed the previously demonstrated clusters of the Solanaceae-, Brassicaceae- and Cucurbitaceae-infecting tobamovirus subgroups [2, 3, 7]. In all cases, TSAMV was placed in a subcluster with PMMoV within the Solanaceae-infecting subgroup of tobamoviruses, confirming that TSAMV is most closely related to, but distinct from, PMMoV.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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