Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Scopolia Mild Mottle Tobamovirus: a New Tobamovirus Isolated From *Scopolia japonica* Plant in Japan

Tamaki Uehara-Ichiki (tuehara@affrc.go.jp)
NARO Genebank https://orcid.org/0000-0003-1124-6885
Ayaka Uke
JIRCAS
Kaoru Hanada
National Agriculture and food research organization
Atsuyuki Hishida
Tokyo University of Agriculture
Eiko Nakazono-Nagaoka
University of Tsukuba
Eiichi Kodaira
Kitasato university

Research Article

Keywords: Scopolia japonica, tobamoviruses

Posted Date: October 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-997459/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

A tobamovirus was isolated from leaves of *Scopolia japonica* that had mild yellowing. Back-inoculation of healthy *Scopolia japonica* with the isolated virus induced mild mottle on upper leaves. Phylogenetic analysis of the coat protein and replicase protein revealed that the newly isolated tobamovirus is related to yellow tailflower mild mottle virus. The highest identity of the 6350-nucleotide sequence of the whole genome shared with that of other tobamoviruses was 71%. According to species demarcation criterion for the genus *Tobamovirus*, our result suggests that scopolia mild mottle tobamovirus is a new species within the genus *Tobamovirus*.

Main Text

Scopolia, a genus with two species in the family Solanaceae, is native to Europe and Asia [14]. *Scopolia japonica* Maxim (Japanese belladonna) is distributed in Honshu to Kyushu in Japan and Korean Peninsula in moist forests along mountainous valleys. It forms an inflorescence at the leaf axil in April to May [7] and becomes dormant during the hot season; therefore, aerial parts grow only in late winter and early spring. The roots and rhizomes of Japanese belladonna, known as “scopolia rhizome”, provide an herbal medicine containing atropine, scopolamine, scopoletin and scopoline, and other compounds that have analgesic and spasmolytic activities [5, 10].

In 2017, leaves with a slight roughness and mottling were collected from a Japanese belladonna plant in Takeda Garden for Medicinal Plant Conservation, Kyoto in western Japan (Fig. 1A). leaves samples from a single *S. japonica* plant was homogenized with 0.1 M phosphate buffer (pH 7.2) containing 0.1% v/v 2-mercaptopropanol and used to mechanically inoculate indicator plants *Nicotiana benthamiana* and *Chenopodium quinoa*. After single-lesion isolations were repeated three times using inoculated leaves of *C. quinoa*, isolated viruses were propagated in *N. benthamiana* plants.

To sequence the viral genome, total RNA was extracted from the leaves of *N. benthamiana* with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Ribosomal RNA was removed using a RiboMinus Plant Kit for RNA-Seq (Thermo Fisher Science, Carlsbad, CA, USA), and the library was prepared using an Ion Total RNA-Seq Kit v2 (Thermo Fisher Science) and the manufacturer’s protocol and sequenced using the Ion GeneStudio S5 System. De novo assembly of sequence reads was performed after removing sequences shorter than 50 nucleotides or longer than 180 nucleotides using CLC Genomics Workbench version 20.0.1. (Qiagen, Valencia, CA, USA). The 5’-terminal sequence of Scopolia mild mottle tobamovirus (SMMoV) was obtained using Takara 5’-Full RACE Core set (cat. 6122) and the manufacturer’s instructions. The 3’-terminal sequence of SMMoV was obtained by adding a poly(A) tail to the RNA using poly(A) polymerase (New England Biolabs, cat. M0276S) and SMARTer RACE 5’/3’ kit (Takara Bio, Kusatsu, Japan).

Phylogenetic analysis of SMMoV and other tobamoviruses was conducted in MEGA X [8]. Multiple sequence alignments and removal of ambiguous alignments including gaps were done using the Clustal

W algorithm [9]. Phylogenetic trees were constructed by the neighbor-joining (NJ) method (1,000 bootstrap replications; substitution model, Dayhoff model) [2]. Open reading frames and molecular weights of the predicted proteins were determined using the NCBI ORF Finder [15] and the Protparam tool of the Expert Protein Analysis System (ExPASy) [3]. *N. benthamiana* plants that were inoculated with sap from the infected *S. japonica* plant developed mosaic and *C. quinoa* developed necrotic spots by 1 week after inoculation. By sequencing analysis using the CLC Genomics workbench, we obtained 850 contigs that were assembled from 1,020,138,918 reads. Annotation of these contigs in BLASTn searches of the NCBI Nucleotide Database showed that the longest one in the obtained contigs corresponded to yellow tailflower mild mottle virus (Genebank accession KJ683937, E-value = 0, Query cover 49%, Percent identity 74%), which belongs to genus *Tobamovirus*. Other contigs were matched to some viruses, but their query cover ratio was under 40%. Using the ORF finder, we found four ORFs that were predicted to be ORFs of *Tobamovirus* in BLASTp searches. The ORF1 contains methyltransferase and helicase1, the ORF2 encodes RNA-dependent RNA polymerase, the ORF3 encodes a movement protein, and the ORF4 encodes coat protein, which matches the genome organization of *Tobamovirus*. The genome sequence of the isolated virus (MAFF accession 260298; DDBJ LC643028) is 6350 nt long. In the genome, four ORFs were identified, as in other tobamoviruses (Fig. 2A). The first gene, p126 (ORF1), extends from nucleotides 70 to 3,429 and encodes a 126-kDa protein. Readthrough translation from the p126 stop codon at nt 3427-3429 to an opal stop codon at nt 4927-4929 was predicted to encode a p183 protein (ORF1-RT). ORF2 (nt 4945-5742) is predicted to encode a 30-kDa protein. ORF3 (nt 5746-6219) encodes a 17.5-kDa protein. GENETYX v13 (GENETYX, Tokyo, Japan) analysis showed that the nucleotide sequence of the full genome of our isolated virus shares 50-71% identity with those of other tobamoviruses and that the range in amino acid sequence identity shared between our virus and other tobamoviruses was 43-70% for replicase protein genes, 30-79% for movement protein genes, 33-73% for coat protein genes (Supplementary table 1). The species demarcation criterion for the genus *Tobamovirus* is less than 90% whole genome nt sequence identity shared with the other species; thus, our result suggests that scopolia mild mottle tobamovirus (SMMoV) is a new species in genus *Tobamovirus*. Phylogenetic analysis showed that SMMoV was most closely related to yellow tailflower mild mottle virus [13], which belongs to subgroup I (host-plant family: Solanaceae) (Fig. 2B and C). To understand its host range, we mechanically inoculated several species of plants with SMMoV and incubated the plants at 25°C until viral symptoms appeared. From 1 to 2 weeks after inoculation, we sampled inoculated plants to detect the CP region of SMMoV using RT-PCR (Takara PrimeScript One Step RT-PCR Kit Ver.2 Dye Plus), SMMoV-specific primers SMMoV-F4938 (GTT ACC TAT GGC TTT GTC ACT) / SMMoV-R5676 (ACC ACT ATT CTT CTT ACC ATT TAC) and the following thermocycling conditions: reverse transcription at 50°C for 30 min and 94°C for 2 min; 30 cycles of PCR at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. Among the inoculated plants, SMMoV was detected from *Nicotiana rustica*, *N. glutinosa*, *C. quinoa*, *C. amaranticolor*, *Tetragonia tetragonioides*, *Solanum lycopersicum* (cvs. Momotaro and Rutgers) and *Physalis floridana* (Fig. 1B and C). But *Capsicum annuum* (cv. Kagayaki), *Cucumis melo* (cv. Andesu) and *Gomphrena globosa* were not infected. Japanese belladonna developed slight yellowing and roughness on upper leaves (Fig. 1D) about 2 weeks after SMMoV inoculation. These results suggested that the host range of SMMoV was mainly confined to Solanaceae plants.

Interaction of secondary metabolites of plants and plant viruses has been studied, and several alkaloids have been suggested to have anti-phytoviruses activity [1, 16]. Since there are few reports of viral disease of genus *Scopolia* plants [11, 12], our result may provide helpful information for screening for novel phytochemicals to control plant viral diseases.

Since Japanese belladonna is vegetative propagated using the rhizome, any viruses in plant will remain in the infected host for a long period. Several reports have shown deformation of harvested rhizomes and decreased yields after long periods of cultivation [4, 6]. In addition, some tobamoviruses are known to be transmitted through soil. Therefore, the infection cycle and the effect of SMMoV on rhizome quality and yield need further analysis.

Declarations

Acknowledgements

This research was supported by a Genebank project, National Agriculture and Food Research Organization (NARO) and AMED under grant number 21ak0101105h0002.

Compliance with ethical standards

The authors declare that they have no conflict of interest.

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

Funding

This study was founded by Genebank Project, NARO, Japan. NARO; National Agriculture and Food Research Organization and AMED under Grant number 21ak0101105h0002.

References

1. Daniel P, C Cheng K, and Katalin AH (2005) Pokeweed Antiviral Protein Inhibits Brome Mosaic Virus Replication in Plant Cells. 280: 20069–20075
2. Dayhoff MO (1978) Survey of new data and computer methods of analysis. In: Dayhoff MO (ed) Atlas of Protein Sequence and Structure vol.5. National Biomedical Research Foundation, Washington DC, p29
3. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A (2005) *Protein Identification and Analysis Tools on the ExPASy Server.* In: Walker JM (ed) The Proteomics Protocols Handbook, Humana Press, pp 571–607
4. Hatakeyama Y, Kumagai T, Kanno E, Li Y, Maehira Y, Yoneda K (1993) Studies on the cultivation and breeding of wild medical plants (II) On the cultivation of Scopolia japonica in Hokkaido.

Syoyakugaku Zassi 47(1): 22-26

5. Harashima H (2017) *Scopolia japonica*. Shoyakutan the 3rd edition: Word book of botanical and Latin names with etymological memory aids (Svs) Ito M, Kitamura T. Maruzen-Yushodo (Tokyo) 266–267
6. Hisata Y, Kawamura T, Okuda K, Noro Y, Tanaka T, Sakai E, Hatakeyama Y (1989) Studies of scopoliae rhizoma (1) Variation of alkaloid contents of cultivated Scopolia japonica. Syoyakugaku Zassi 43(2): 159-162
7. Katsuyama T (2017) Solanaceae. Wild flowers of Japan (Eds) Ohashi H, Y, Kadota, J, Murata, K, Yonekura. Heibonsha (Tokyo) 5: 33–46
8. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35: 1547–1549
9. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinfomatics 23: 2947–2948
10. Mimaki Y (2020) Solanaceae. Partner pharmacognosy (Eds) Takeya K, F, Kiuchi, K, Komatsu. Nankodo (Tokyo) 289–294
11. Pelikanova J, Petrzik K and Honetslegrova J (1992) The Discovery of Belladonna Mottle Virus in Czechoslovakia and its Comparison with a Hungarian Isolate of this Virus J. Phytopathology 134, 10–16
12. Salamon P, Salánki K (2001) Virus diseases and viruses of cultivated and wild-growing Solaneaous plants in Hungary.4. Spontaneous occurrence of cucumber mosaic virus (CMV) on Scopolia carniolica Jacq. Növényvédelem 37, 123–127. ISSN: 0133-0829
13. Stephen JW, Hua L, Michael GKJ (2014) Yellow tailflower mild mottle virus: a new tobamovirus described from Anthocercis littorea (Solanaceae) in Western Australia Arch Virol 159:791–795
14. WFO (2021) *Scopolia*. World Flora Online. Published on the Internet. http://www.worldfloraonline.org. Accessed on: 07 Jun 2021
15. Wheeler DL, Church DM, Federhen S, Lash AE, Madden TL, Pontius JU, Schuler GD, Schriml LM, Sequeira E, Tatusova TA, Wagner L (2003) Database resources of the National Center for Biotechnology. Nucleic Acids Res 31: 28–33
16. Wenhui G, Xiang L, Bin L, He Y, Juntao F (2021) Anti-TMV activity and mode of action of three alkaloids isolated from Chelidonium majus Pest Manag Sci: 77: 510–517

Figures

Ichiki et al. Fig. 1

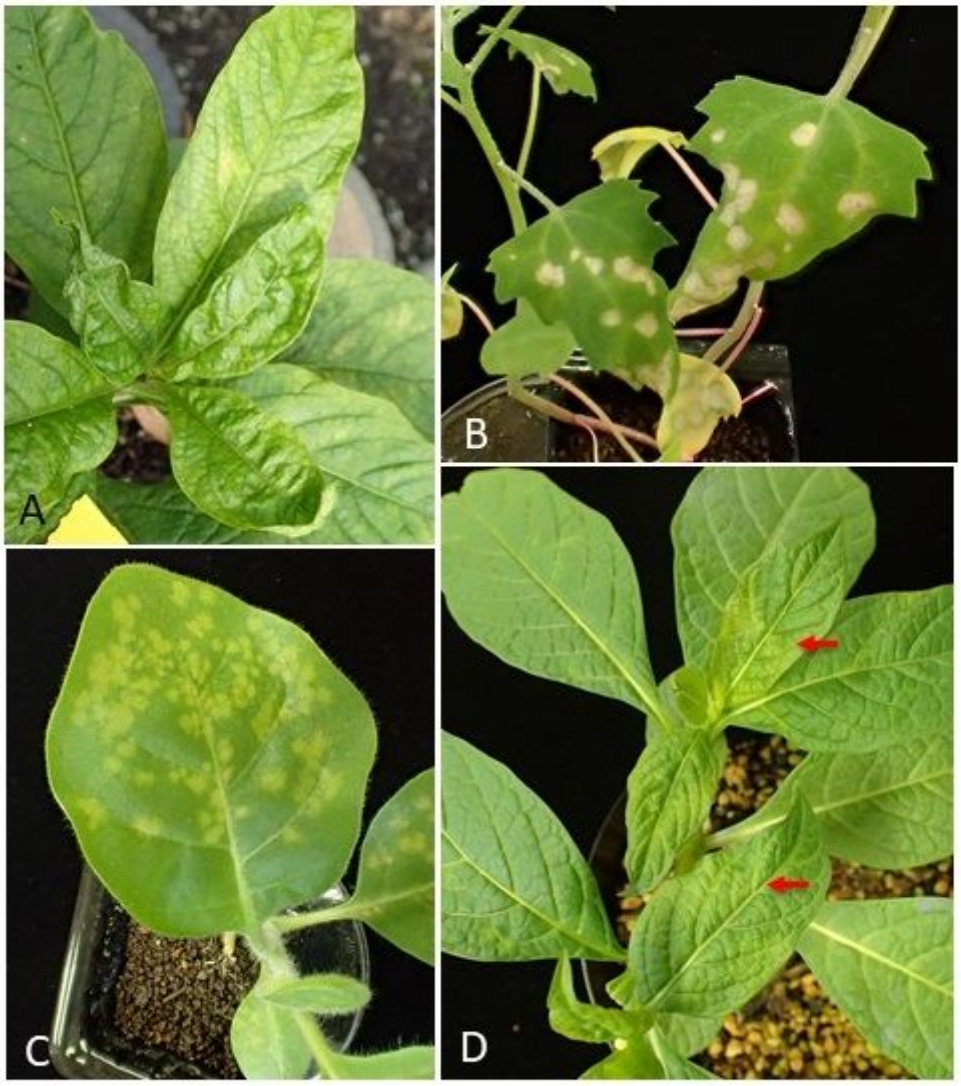


Figure 1

Symptoms on leaves of Scopolia japonica, Chenopodium quinoa, and Nicotiana rustica induced by scopolia mild mottle tobamovirus. A. Mild mottle and roughness on leaves of naturally infected Scopolia japonica plant. B. Necrotic local lesions on inoculated leaves of Chenopodium quinoa plant 1 week after inoculation with scopolia mild mottle tobamovirus (SMMoV). C. Yellow lesions on inoculated leaves of Nicotiana rustica plant 1 week after inoculation with SMMoV. D. Slight yellowing and roughness (red arrows) on upper leaves of Scopolia japonica 2 weeks after back-inoculation with SMMoV.

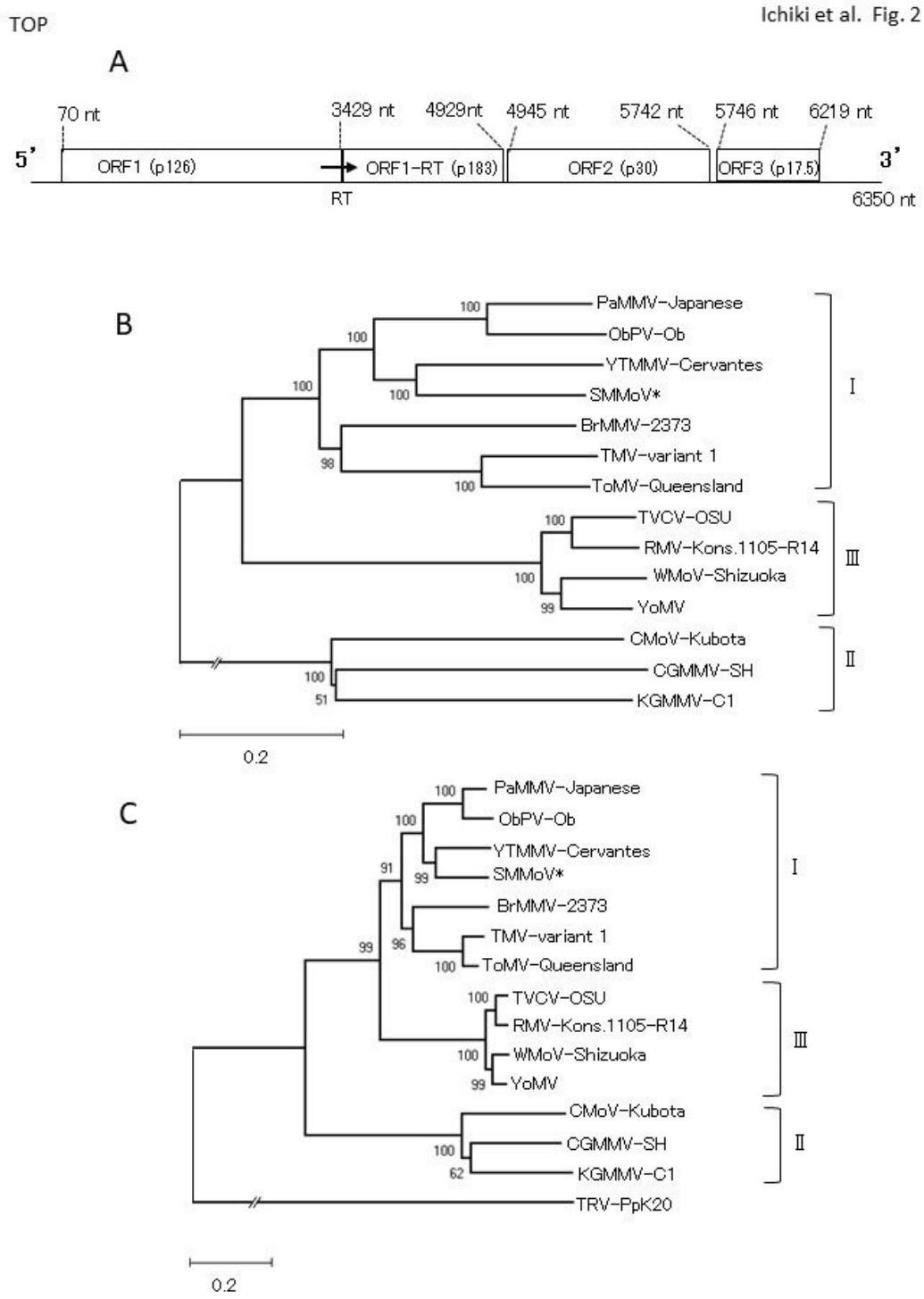


Figure 2

Genome organization of scopolia mild mottle tobamovirus (SMMoV) (MAFF accession 260298) and phylogenetic trees of tobamoviruses including SMMoV. A. Genome organization of SMMoV. Genes are denoted by open rectangles. Arrow between open reading frame ORF1 and ORF2 indicates a readthrough amber stop codon. RT, translational readthrough of the termination codon. B. Phylogenetic tree based on the genomic nucleotide sequence of viruses in the genus Tobamovirus including SMMoV. Scale bars

correspond to substitutions per nucleotide. I, II and III are subgroups according to their host-plant family: Solanaceae, Cucurbitaceae, and Brassicaceae. C. Phylogenetic tree based on the amino acid sequence of the replicase protein of the genus Tobamovirus including SMMoV. Scale bars correspond to substitutions per amino acid residue. Abbreviations: BrMMV-2373, Brugmansia mild mottle virus (accession AM398436); CGMMV-SH, cucumber green mottle mosaic virus (D12505); CMoV-Kubota, cucumber mottle virus (AB261167); KGMMV-C1, kyuri green mottle mosaic virus (AJ295948); ObPV-Ob, Obuda pepper virus (D13438); PaMMV-Japanese, paprika mild mottle virus (AB089381); RMV-Kons.1105-R14, ribgrass mosaic virus (HQ667979); SMMoV* Scopolia mild mottle tobamovirus* (LC643028); TMV-variant 1, tobacco mosaic virus (V01408); ToMV-Queensland, tomato mosaic virus (AF332868); TVCV-OSU, turnip vein-clearing virus (U03387); WMoV-Shizuoka, wasabi mottle virus (AB017503); YTMMV-Cervantes, yellow tailflower mild mottle virus (KF495564); YoMV, youcai mosaic virus (U30944); TRV-PpK20, tobacco rattle virus (outgroup, AF166084).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S1table.xlsx
- S2SMMoVntfull.txt