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RESEARCH ARTICLE

Near-complete genomic characterisation and experimental host infection observations of pepper veinal mottle virus isolates from Taiwan

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Abstract

Pepper veinal mottle virus (PVMV; genus *Potyvirus*, family Potyviridae) primarily infects pepper but it also occurs in tomato and eggplant. Although several complete genome sequences of PVMV isolates have been found around the world, genomic information concerning the isolates preserved in Taiwan is still scarce. In this study, two isolates – Tom1 (derived from tomato) and EP1 (derived from eggplant), isolated in Taiwan, were examined using near-complete genome sequencing and infection assays. The near-complete genomic sequences of Tom1 and EP1, consisting of 9796 nucleotides, contained a 3073-amino-acid polyprotein, which is cleaved into the 10 general proteins found in potyviruses. A comparison of the genomic sequences of Tom1 and EP1 showed that the two have 94.3 % nucleotide sequence similarity. Phylogenetic analysis, using close to near-complete genomic sequences, placed the isolates of Tom1 and EP1, along with isolates from Taiwan, Japan and Korea, within a relatively conserved group. Gene-wise comparison showed high sequence conservation in the coat protein (CP), while moderate variation was observed in the P1, HC-Pro, CI and VPg regions. The two isolates exhibited isolate-dependent symptom expressions in selected experimental hosts, as observed qualitatively without quantitative assessment. No recombination signals were detected between Tom1 and EP1. This study provides updated genomic reference data for PVMV isolates from Taiwan and documents qualitative host infection outcomes, offering baseline information for future molecular, epidemiological and functional studies of PVMV.

Keywords: complete genome sequencing; genetic diversity; host infection; pepper veinal mottle virus

Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated vegetable crops worldwide and one of the most economically significant members in the family Solanaceae (1). In Taiwan, tomatoes are cultivated on approximately 4100 ha, yielding 98340 tonnes and contributing around USD 141 million to the national economy (2). In addition to tomato, pepper (*Capsicum* spp.) is another important solanaceous crop in the region. Both crops are under serious viral disease threats, such as pepper veinal mottle virus (PVMV), which represents an important and emerging constraint to tomato and pepper production (3). Within the genus *Potyvirus* (family Potyviridae), PVMV belongs to the species *Potyvirus capsivenae*. The 10 kb monopartite, single-stranded, positive-sense RNA genome of potyviruses encodes a polyprotein that is cleaved to produce multiple functional proteins needed for replication, mobility and host defense suppression (3, 4). Pepper veinal mottle virus is transmitted non-persistently by aphids and infects various solanaceous hosts, such as *Capsicum* species, tomato (*S. lycopersicum*) and eggplant (*Solanum melongena*), which have been reported in several regions, including Taiwan.

The wide host range of PVMV contributes to its persistence and spread in agricultural systems, increasing challenges for crop management and disease control. In Taiwan, PVMV leads to major economic losses, with severe outbreaks causing 30–50 % yield reduction and increased vector-management costs (5). An infected tomato crop usually shows chlorosis, mottling and necrosis (5). Such impacts are particularly challenging in mixed cropping systems, where management becomes more complex (6). Ecologically, widespread solanaceous hosts act as reservoirs, sustaining the virus. Epidemiologically, repeated outbreaks with high incidence are driven by aphid activity. These local impacts mirror global trends, underscoring PVMV as a continuing threat to solanaceous crops. Although the genome sequences of certain PVMV isolates have been reported, the detailed molecular characterisation of Taiwanese

isolates remains limited. In addition, comparative genomic analysis with isolates from different host species is scarce, which restricts a complete understanding of host specificity and viral evolutionary patterns. To address these gaps, this study examined two PVMV isolates maintained at the World Vegetable Center in Taiwan: Tom1 (from tomato) and EP1 (from eggplant), which were analysed using near-complete genome sequencing and comparative molecular approaches, including phylogenetic analysis and open reading frame (ORF) based sequence comparisons. In addition, experimental mechanical inoculation was performed on selected plant species to document qualitative host infection outcomes under controlled greenhouse conditions.

The primary objective of this work is to provide comparative genomic reference data for Taiwanese PVMV isolates, while presenting descriptive host infection observations without drawing quantitative or functional conclusions. The results provide updated molecular reference data that can support future studies on PVMV evolution, diagnostics and host-virus interactions in solanaceous crops in Taiwan.

Materials and Methods

Origin and detection of PVMV isolates

The two PVMV isolates, Tom1 and EP1, were obtained from the Taiwan Agricultural Research Institute (TARI) and National Chung Hsing University (NCHU), respectively (5). Under controlled greenhouse conditions at the World Vegetable Center, Taiwan, the EP1 isolate was propagated in eggplant and *Nicotiana benthamiana*, whereas the Tom1 isolate was propagated in tomato and *N. benthamiana*. Total RNA from infected leaf tissues was extracted using the GenMark total RNA purification kit (TR02-150, GenMark, USA), according to the manufacturer's instructions. Reverse transcription PCR (RT-PCR) analysis with PVMV-specific primers targeting the coat protein (CP) gene (Forward primer: 5′-AATGCAGGAACACAGGGGAC-3′ and Reverse primer: 5′-CGCGCTAATGACATATCGGT-3′), designed in this study based on the reference genome sequence of PVMV isolate Tn (OR355467) was used to confirm the presence of PVMV. Mechanical inoculation was performed to establish infection in tomato (*S. lycopersicum*) (WVC accession No. VI065301) at the 3–4 true leaf stage. The virus-inoculated plants were observed for the emergence of symptoms and RT-PCR was used to confirm the infection in tissues exhibiting symptoms. The amplified CP gene sequences were deposited in the NCBI GenBank database and the corresponding accession numbers are provided in the Results section.

Host infection and symptom observation

Isolates of PVMV (Tom1 and EP1) were mechanically inoculated into 17 plant species. For each species, a total of 24 plants were used: 10 inoculated with Tom1, 10 with EP1 and 4 uninoculated healthy controls. Both isolates were tested on all plant species. The inoculated plants within each entry were considered subsamples rather than independent biological replicates; therefore, the results are presented descriptively without inferential statistical analysis. Supplementary Table S1 lists the cultivar/accession information for all host species used in the host infection assay.

A 10 % (w/v) crude sap extract was made by grinding 1 g of infected leaf tissue in 10 mL of phosphate buffer (pH 7.0; 0.01 M Na_2HPO_4, 0.01 M KH_2PO_4). Two pieces of cheese cloth were used to filter the homogenate. After dusting the adaxial surface of the first two completely expanded leaves with 600-mesh carborundum, each leaf was gently rubbed with approximately 100 µL of inoculum using a delicate cotton swab. The same process was used to treat mock-inoculated control plants with phosphate buffer alone. To remove excess inoculum and abrasives, leaves were rinsed with distilled water after inoculation. A balanced NPK (20:20:20) fertiliser was applied every two weeks to the inoculated and control plants, which were kept in an insect-proof greenhouse at 25–30 °C and 50–60 % relative humidity. At 7-day intervals following inoculation, symptom manifestation was evaluated on the 7th, 14th, 21st and 28th day. Separate observations were made for upper, non-infected leaves (systemic symptoms) and inoculated leaves (local symptoms). According to the traditional *Potyvirus* symptom classification, systemic symptoms (mosaic, systemic chlorosis or necrotic patterns) appeared on upper non-infected leaves, whereas local symptoms were classified as chlorotic patches or mottling limited to inoculated leaves (5, 6). Observations of chlorotic spots, mottling and necrosis were recorded and compared with those of uninoculated control plants. Simple Direct Tube RT-PCR (SDT-RT-PCR) was used to establish the presence of PVMV in the affected plants after symptom observation, guaranteeing precise identification of virus-infected individuals.

Simple Direct Tube RT-PCR (SDT-RT-PCR) and gel electrophoresis

After homogenizing leaf samples from all 17 plant species at the 28 days post-inoculation (DPI) in 500 µL of 1X phosphate-buffered saline with Tween 20 (PBST) buffer, the extract was incubated for 30 min at 37 °C (7). To get rid of extra buffer, tubes were briefly centrifuged after being cleaned twice with 1X PBST. In accordance with the manufacturer's instructions, cDNA was also produced using M-MuLV Reverse Transcriptase (Invitrogen, Thermo Fisher Scientific) and a reverse primer specific to the CP gene. Next, Taq DNA Polymerase (Invitrogen, Thermo Fisher Scientific) was used for PCR amplification using CP gene primers. Initial denaturation at 95 °C was followed by 29 cycles of denaturation at 95 °C, annealing at 52 °C and extension at 72 °C, followed by a final extension at 72 °C for 5 min. PCR products were separated on a 1% agarose gel prepared in 0.5× TBE buffer (8) and electrophoresed at 100 V for 30 min. The gel was stained with SYBR Green and the expected ~840 bp amplicon was visualised under UV light using a 1 kb DNA ladder (Thermo Fisher Scientific, Cat. No. SM0311) as a molecular size marker. *Nicotiana benthamiana* was identified as a suitable experimental host for both isolates based on infection confirmation.

Complete genome sequencing and analysis of PVMV isolates Tom1 and EP1

RNA extraction, cDNA synthesis and PCR amplification

Using the GenMark plant total RNA purification kit (TR02-150, GenMark, USA), total RNA was extracted from 100 mg of fresh *N. benthamiana* leaf tissue infected with PVMV in accordance with the manufacturer's instructions. After grinding in liquid nitrogen, the tissues were lysed in a solution containing 2-mercaptoethanol. Following incubation at 60 °C for 3 min and centrifugation, the lysate underwent spin filter treatment and ethanol precipitation. Genomic DNA was eliminated by DNase I treatment (9). A NanoDrop spectrophotometer (Invitrogen, Thermo Fisher Scientific) was used to quantify the RNA after it was eluted in 30–50 µL of nuclease-free water and kept at −80 °C. As described above, cDNA synthesis was performed and overlapping PVMV genome fragments were amplified by PCR using Taq DNA polymerase

(Invitrogen, Thermo Fisher Scientific) and primers based on the reference genome sequence of the PVMV isolate Tn (OR355467). Information on primer sequences, annealing temperatures and expected product sizes is provided in Supplementary Table S2.

Gel purification, cloning and sequencing

The Wizard® SV Gel and PCR Clean-Up System (Promega) was used to clean the PCR products before they were ligated into the pGEM® -T Easy Vector. Heat shock was used to transform ligation reactions into *Escherichia coli* DH5α competent cells, which were then plated on LB agar containing ampicillin, IPTG and X-Gal (10). Colony PCR was used to identify white colonies, whereas, ampicillin and LB broth were used to cultivate confirmed positive clones. Sanger sequencing was performed on pure plasmid DNA (Genomics, Taiwan) (11). The ApE program was used to create consensus sequences for the two PVMV isolates (Tom1 and EP1) (12) and gene annotation was carried out with NCBI BLAST (13). This approach represents complete genome sequencing based on overlapping PCR fragments and sanger sequencing. These sequenced samples were deposited in GenBank (NCBI, USA) to obtain GenBank accession numbers. In addition, 16 publicly available complete genome accessions of PVMV from NCBI were retrieved and included for comparative genomic and phylogenetic analyses. ClustalW in BioEdit version 5.0.9 was used to create multiple sequence alignments. MEGA version 11 and the neighbor-joining method were used to create phylogenetic trees with 1000 bootstrap replicates. The K2P model was selected based on the lowest Bayesian information criterion (BIC) value in MEGA's find best DNA/protein models analysis, which identified it as the most suitable substitution model for this dataset.

Interactive Tree of Life (iTOL) version 6 was used to display and annotate the resultant trees. Sequence Demarcation Tool (SDT) (v1.3) for heatmap visualisation and sequence identity matrix for precise nucleotide and amino acid values were the two methods used to determine pairwise sequence identity. In order to determine nucleotide and amino acid identities and identify specific amino acid substitutions in functional areas, gene-to-gene comparisons between PVMV Tom1 and EP1 were performed by examining each coding region independently (14).

Recombination analysis

The Recombination Detection Program v4 (RDP4) was used to assess potential recombination events. Using MUSCLE, all available full-length or nearly complete PVMV genome sequences (n = 18), including Tom1 and EP1, were aligned. RDP4's seven detection techniques-RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq were then used to screen for recombination. A Bonferroni-corrected significance level of $p < 0.05$ was applied using default settings. Recombination events were deemed reliable only if they were identified by ≥5 independent methods and verified through eye examination of breakpoint plots.

Results

Propagation and confirmation of PVMV isolates

At 4–5 DPI, *N. benthamiana* plants infected with both PVMV isolates showed local symptoms (veinal chlorosis and mild mottling) on inoculated leaves. By 7–8 DPI, systemic symptoms (slight leaf deformation and chlorotic mottling on the upper leaves) had appeared. The presence of PVMV was first verified by RT-PCR using CP-specific primers, which produced amplicons of the anticipated size (~840 bp). Total RNA isolated from *N. benthamiana*-infected plants demonstrated good integrity. When both isolates were mechanically inoculated into tomatoes (*S. lycopersicum*), isolate-specific symptoms were observed. Local symptoms, including vein chlorosis and mild mottling, were observed in Tom1-infected plants at 5–6 DPI, followed by systemic symptoms such as leaf deformation and necrosis at 7–8 DPI. In EP1-infected plants, local symptoms appeared at 5–6 DPI, whereas systemic symptoms – characterised by mild mottling and occasional necrosis – were delayed until 14–15 DPI. In *N. benthamiana*, both isolates produced local symptoms at the same time, but in tomatoes, EP1 produced milder and delayed systemic symptoms than Tom1. The presence of PVMV was confirmed by RT-PCR analysis of symptomatic tomato leaves (Fig. 1).

The amplified CP gene sequences of both isolates were deposited in the NCBI GenBank database (PX119809 for PVMV Tom1 and PX119811 for PVMV EP1) as part of the initial molecular confirmation.

Experimental host infection observations of PVMV isolates Tom1 and EP1

The host-infection study was conducted as a single biological experiment to determine the susceptibility of selected plant species to two PVMV isolates, Tom1 and EP1. A total of 17 plant species were mechanically inoculated and symptom development was recorded at 7, 14, 21 and 28 DPI. The symptom data collected at 28th DPI were used to compare responses among different hosts (Table 1). Each treatment contained 10 plants, which were considered subsamples within this single experiment and results are presented descriptively.

According to SDT-RT-PCR results and apparent symptoms, 10 of the 17 species evaluated were infected with Tom1 and 8 in EP1. The presence of the virus was confirmed by amplifying a ~840 bp PVMV-specific segment from infected plants. *Chenopodium quinoa*, *Datura metel* and *Nicotiana rustica* all experienced systemic symptoms like extensive chlorosis and necrosis on the upper leaves, as well as local symptoms including vein clearing and moderate chlorotic patches. These hosts were infected by both isolates. In *S. lycopersicum*, Tom1 caused local symptoms (chlorotic spot and mottling) at 5–6 DPI and systemic symptoms (severe mosaic and necrotic spots) by 7 DPI, whereas EP1 produced delayed and milder systemic symptoms, with local symptoms appearing around 14 DPI.

All three *Capsicum* species (*C. annuum*, *C. chinense*, and *C. frutescens*) were susceptible to both isolates. Within 7 DPI, EP1 induced more severe systemic symptoms (leaf curling, vein clearing and mosaic) and earlier local symptoms (mild mottling and vein clearing), with the most pronounced effects observed in *C. annuum*. The systemic effects of Tom1 were milder and manifested later (13–14 DPI). Cowpea (*Vigna unguiculata*) and green gram variety KPS2 (*Vigna radiata*) showed local symptoms (vein clearing and chlorotic patches) by 6 DPI and systemic symptoms by 10–12 DPI, but only in response to Tom1 infection. Among the tested legumes, cowpea (*Vigna unguiculata*) exhibited more severe systemic effects following Tom1 infection. In contrast, black gram (*Vigna mungo*) tested negative for both isolates by SDT-RT-PCR and did not exhibit any local or systemic symptoms. Okra (*Abelmoschus esculentus*), bitter gourd (*Momordica charantia* L.), cucumber (*Cucumis sativus* L.),

Table 1. Host infection assay for PVMV Tom1 and PVMV EP1

S. No	Common name	Scientific name	PVMV Tom1	PVMV EP1	PVMV Tom1		PVMV EP1		PCR reaction*	
			Number of plants expressing symptoms/ total number of plants inoculated	Number of plants expressing symptoms/total number of plants inoculated	Local symptoms	Systemic symptoms	Local symptoms	Systemic symptoms	PVMV Tom1	PVMV EP1
1.	Tomato	*Solanum lycopersicum* (ANT22)	10/10	6/10	Cs	Sm, Ns	NVS	mM	+	+
2.	Petrapper	*Capsicum annuum* (VC27a)	8/10	10/10	NVS	Vmm	mm, Vc	Vc, Lc and mosaic	+	+
3.	Pepper	*Capsicum chinense* (AVPP1932)	8/10	10/10	NVS	Vmm	mm	Vc, Lc and mosaic	+	+
4.	Pepper	*Capsicum frutescens* (VI040881)	8/10	10/10	NVS	Vmm	mm	Vc, Lc and mosaic	+	+
5.	Cucumber	*Cucumis sativus*	0/10	0/10	NVS	NVS	NVS	NVS	–	–
6.	Luffa	*Luffa aegyptiaca*	0/10	0/10	NVS	NVS	NVS	NVS	–	–
7.	Bitter gourd	*Momordica charantia*	0/10	0/10	NVS	NVS	NVS	NVS	–	–
8.	Okra	*Abelmoschus esculentus*	0/10	0/10	NVS	NVS	NVS	NVS	–	–
9.	Cowpea	*Vigna ungiculata*	6/10	0/10	NVS	Vmm	NVS	NVS	+	-
10.	Green gram	*Vigna radiata* (KPS 2)	10/10	0/10	NVS	Ns	NVS	NVS	+	–
11.	Black gram	*Vigna mungo*	0/10	0/10	NVS	NVS	NVS	NVS	–	–
12.	Tobacco	*Nicotiana rustica*	8/10	8/10					+	+
13.	Tobacco	*Nicotiana tabacum* white burley	0/10	0/10	NVS	NVS	NVS	NVS	–	–
14.	Tobacco	*Nicotiana tabacum* var. Xanthi	0/10	0/10	NVS	NVS	NVS	NVS	–	–
15.	Quinoa	*Chenopodium quinoa*	10/10	10/10	Cs, Vc	Ns	Cs, Vc	Ns	+	+
16.	Lambs quarters	*Chenopodium amaranticolor*	10/10	10/10	Cs	Ns	Cs, Vc	Ns	+	+
17.	Indian thornapple	*Datura metel*	10/10	8/10	Cs, Vc	NVS	Cs, Vc	NVS	+	+

PVMV: pepper veinal mottle virus; mm: mild mottle; Vmm: very mild mottle; mM: mild mosaic; Vc: vein clearing; NVS: no visible symptoms; Cs: chlorotic spot; Ns: necrotic spot; Sm, Ns: severe mosaic with necrotic spots; Lc: leaf curling. *PCR reaction indicates RT-PCR confirmation of PVMV infection in symptomatic hosts. +: Positive reaction; −: Negative reaction in RT-PCR.

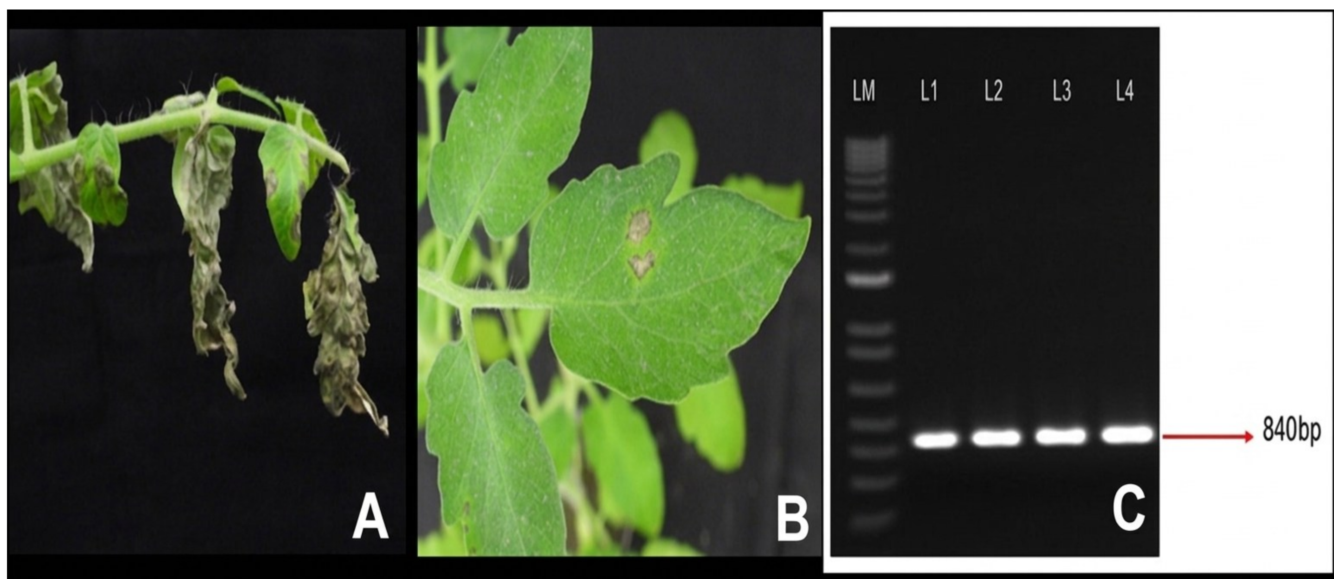

Fig. 1. Symptoms of pepper veinal mottle virus (PVMV Tom1 and PVMV EP1) and visualisation of PVMV fragments via gel electrophoresis. (A) Severe leaf blighting in PVMV Tom1 inoculated tomato on 28th DPI; (B) Necrotic spots in PVMV EP1 inoculated tomato on 28th DPI; (C) Lane M: 1 kb Ladder; Lane 1 and 2: PVMV Tom1; Lane 3 and 4: PVMV EP1 inoculated samples amplified at ~840 bp.

luffa (*Luffa aegyptiaca*) and two tobacco varieties (*Nicotiana tabacum* var. White burley and var. Xanthi) did not exhibit any symptoms up to 28 DPI. SDT-RT-PCR results verified that neither Tom1 nor EP1 was present (Fig. 2 and 3, Table 1).

Genome organisation of PVMV isolates Tom1 and EP1

ApE software was used to collect and assemble the almost full genome sequences for both PVMV isolates and NCBI BLAST was used to annotate them. These sequences were added to NCBI GenBank in the United States, where they were assigned accession codes PV476910 (Tom1) and PV476911 (EP1).

Each genome is 9796 nt in length, consisting of a 5′ untranslated region (UTR), a single large ORF of 9219 nt encoding a polyprotein of 3073 amino acids and a 3′ UTR (nt 9415–9796) followed by a poly(A) tail. The polyprotein is predicted to be processed into 10 functional proteins characteristic of the *Potyvirus* genus: P1 (nt 196–1095), HC-Pro (1096–2466), P3 (2467–3498), 6K1 (3499–3660), CI (3661–5589), 6K2 (5590–5748), VPg (5749–6321), NIa-Pro (6322–7047), NIb (7048–8604) and CP (8605–9414). In addition, the frameshift product P3N-PIPO (nt 2926–3144) is encoded within the P3 region, as reported for potyviruses (ICTV). The terminal untranslated regions (UTRs) of the PVMV genome were inferred based on the published genome sequence of isolate Tn (OR355467), as rapid amplification of cDNA ends (RACE) amplification was not performed in this study.

Comparative sequence analysis with global PVMV isolates

Twelve related potyviruses and 16 PVMV isolates were compared through genome-based phylogenetic analysis. The WorldVeg isolates Tom1 and EP1 showed very high nucleotide identity (>90 %) with Japanese and Taiwanese PVMV isolates, establishing a conserved East Asian cluster. Sequence alignment was performed using the MUSCLE algorithm and pairwise identity analysis was conducted using the Sequence Demarcation Tool v1.3 (SDT). Taiwanese isolates such as ns1 (FJ617225) and Tn (OR355467) showed similarly high identity values, indicating regional conservation rather than unique divergence. In contrast, isolates from China (KR002568, MN082715), Nigeria (OP722584) and Senegal (OK558747) displayed moderately reduced identity values (~70–82 %), forming distinct clusters that reflect geographic and evolutionary separation. The SDT heatmap shows Tom1 and EP1 grouping with the highly related East Asian isolates, while divergent isolates form separate clusters. The colour gradient from red (>90 %) to blue (~70 %) visually highlights these relationships (Fig. 4).

Sequence identity matrix

A high genetic relatedness was indicated by 94.3 % nucleotide sequence identity between PVMV Tom1 and PVMV EP1. Strong conservation in key protein-coding areas may indicate the identity of 91.4 % at the amino acid level. The Japanese isolates (LC438541 and LC438544) showed high nucleotide identity with Tom1 and EP1 (98.0 –99.5 %) and amino acid identities ranging from 95.1 % to 96.4 %, indicating potential host-associated or regional adaptation. Similarly, the Taiwanese isolate OR355467 showed high similarity, with nucleotide identities of 97.7 % (Tom1) and 97.5 % (EP1) and amino acid identities of 90.7 % and 90.5 %, respectively, indicating potential host-specific and regional evolutionary influences.

Significant divergence was observed when comparing the isolates with other *Potyvirus* species, such as chilli veinal mottle virus (ChiVMV), which showed significantly lower nucleotide (67.1 %) and amino acid (41.2 %) identities. Additionally, PVMV isolates' unique taxonomic position within the *Potyvirus* genus was confirmed by the fact that they shared less than 50 % nucleotide identity and fewer than 25 % amino acid identity with non-potyvirus RNA viruses (Table 2).

Phylogenetic analysis of near-complete genome PVMV isolates

Phylogenetic analysis based on near-complete genome nucleotide sequences of study isolates (PVMV Tom1 and EP1) and 16 global PVMV genomes, along with three other potyviruses and one outgroup, revealed that both study isolates clustered within the same homogeneous East Asian clade (Fig. 5). Tom1 and EP1 grouped together with isolates from Japan, Korea, Taiwan and China and showed minimal genetic differentiation (<1 %), reflecting expected intraspecific variability with no indication of phylogenetic separation. West African isolates formed a separate

Table 2. Sequence identity matrix and comparative analysis of PVMV Tom1, PVMV EP1 and related near-complete genomes

S. No.	GenBank accession	Host	Country	Virus-acronym	PVMV (complete genome)	
					NT (%)	AA (%)
1.	LC438540	*Capsicum annuum*	Japan	PVMV	99.5	99.2
2.	LC438541	*Capsicum annuum*	Japan	PVMV	99.5	99.2
3.	LC438542	*Capsicum annuum*	Japan	PVMV	98.6	97.3
4.	LC438543	*Capsicum annuum*	Japan	PVMV	98.2	96.2
5.	LC438544	*Capsicum annuum*	Japan	PVMV	99.3	98.7
6.	LC438545	*Capsicum frutescens*	Japan	PVMV	98.2	96.3
7.	FJ617225	*Solanum nigrum*	Taiwan	PVMV	97.9	96.3
8.	OR355467	*Solanum lycopersicum*	Taiwan	PVMV	97.7	95
9.	PV476911 (PVMV EP1)	*Solanum melongena*	Taiwan	PVMV	94.2	92.1
10.	PV476910 (PVMV Tom1)	*Solanum lycopersicum*	Taiwan	PVMV	94.5	92.8
11.	KR002568	*Capsicum annuum*	China	PVMV	98.2	96.5
12.	MN082715	*Capsicum chinense*	China	PVMV	97.8	95.3
13.	OR355466	*Capsicum annuum*	Taiwan	PVMV	97.3	94.6
14.	OP722584	*Solanum lycopersicum*	Nigeria	PVMV	94.5	87.6
15.	OK558747	*Solanum lycopersicum*	Senegal	PVMV	93.9	87.4
16.	OQ102061	*Capsicum annuum*	Nigeria	PVMV	93.8	87
17.	OK558746	*Solanum lycopersicum*	Senegal	PVMV	83.5	66.9
18.	NC011918	*Capsicum annuum*	Korea	PVMV	98.5	97.2
19.	PQ520507	*Capsicum annuum*	Ethiopia	EPMV	72.6	48.7
20.	NC043537	*Solanum aethiopicum*	Tanzania	EMV	67.8	43.8
21.	MK405594	*Nicotiana tabacum*	China	ChiVMV	67.1	41.2
22.	NC009744	*Solanum torvum*	Laichau	WTMV	64.9	39.7
23.	EU091075	*Saccharum officinarum*	Mexico	SCMV	49.7	21.9

NT: nucleotide; AA: aminoacid; PVMV: pepper veinal mottle virus.

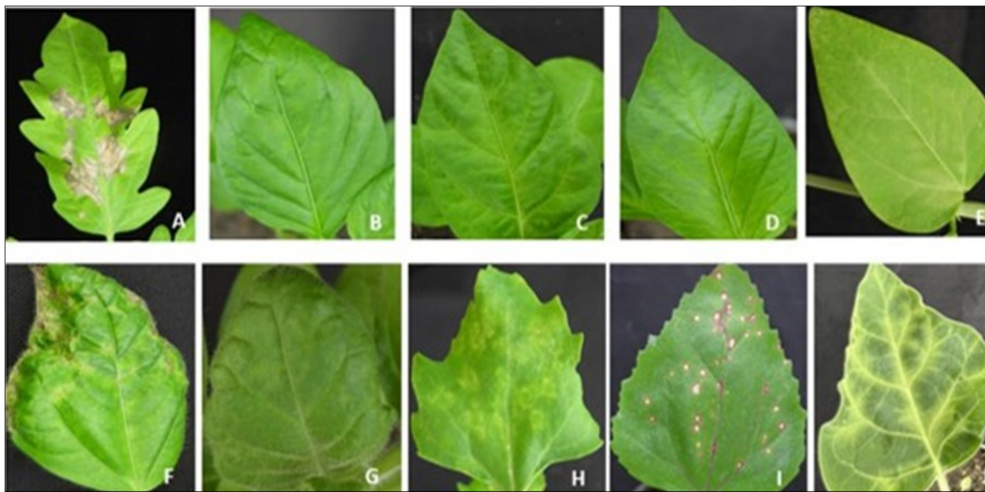

Fig. 2. Symptoms observed on various hosts inoculated with PVMV Tom1 at 28 DPI. (A) *Solanum lycopersicum* (severe mosaic with necrotic spots); (B) *Capsicum annuum* (mild mottle with slight leaf distortion); (C) *Capsicum frutescens* (mild mottle with leaf curling); (D) *Capsicum chinense* (very mild mottle); (E) *Vigna unguiculata* (very mild mottle); (F) *Vigna radiata* (necrotic spots); (G) *Nicotiana rustica* (mild mottle); (H) *Chenopodium quinoa* (chlorotic spots); (I) *Chenopodium amaranticolor* (necrotic spots); (J) *Datura metel* (mild mottle).

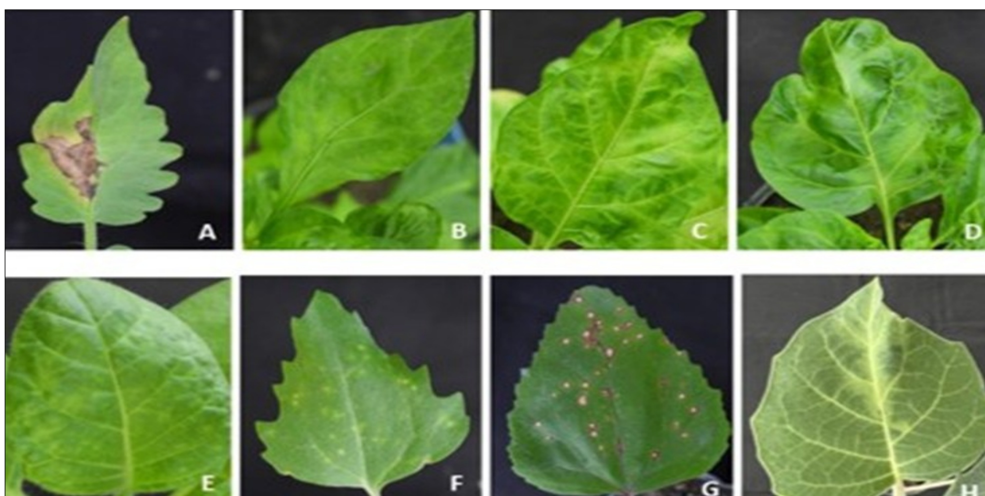

Fig. 3. Symptoms observed on various hosts inoculated with PVMV EP1 at 28 DPI. (A) *Solanum lycopersicum* (very mild mottle with necrotic spot); (B) *Capsicum chinense* (mild mottle); (C) *Capsicum frutescens* (mild mottle with leaf curling); (D) *Capsicum annuum* (mild mottle with slight leaf distortion); (E) *Nicotiana rustica* (mild mottle); (F) *Chenopodium quinoa* (chlorotic spots); (G) *Chenopodium amaranticolor* (necrotic spots); (H) *Datura metel* (mild mottle).

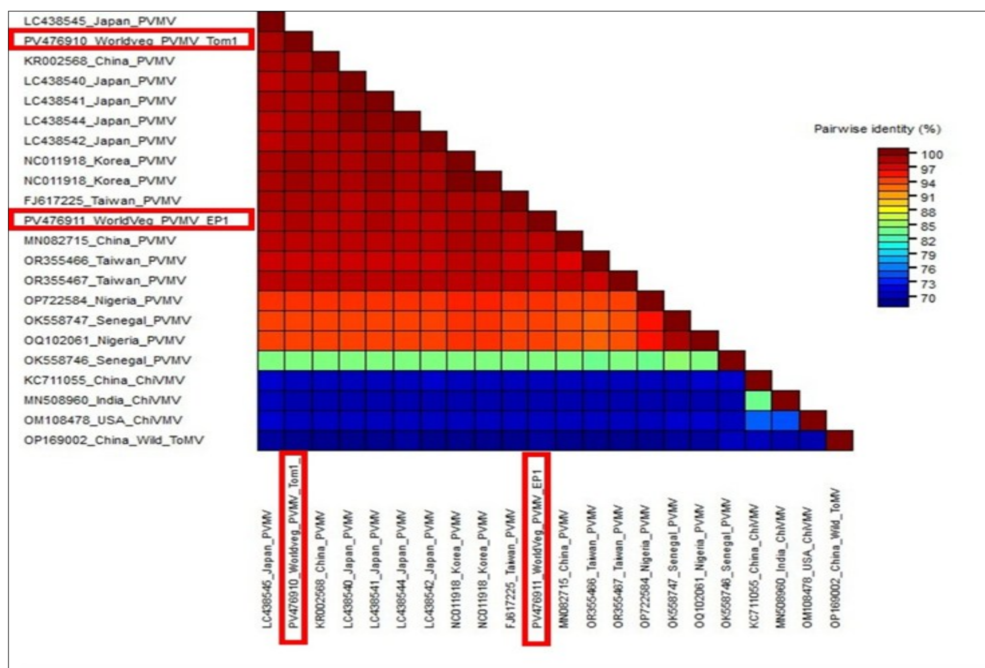

Fig. 4. Nucleotide identity comparison of PVMV isolates using SDT v1.3. The heatmap compares PVMV isolates Tom1 and EP1 with 20 global isolates. The colour gradient represents nucleotide identity percentages, with red (>90 %) indicating highest similarity and blue (<60 %) indicating lowest similarity.

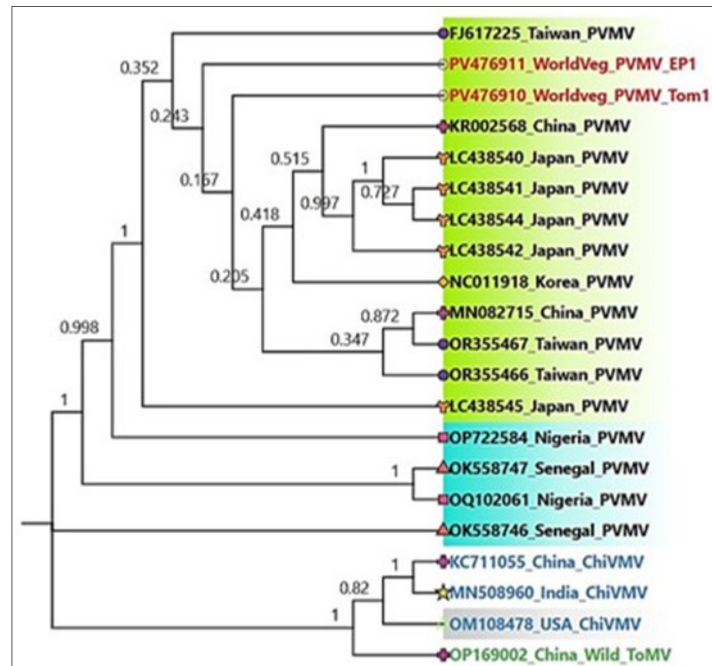

Fig. 5. Phylogenetic trees constructed using the neighbor-joining method with the Kimura 2-parameter model in MEGA version 11, with 1000 bootstrap replicates.

clade, ChiVMV, wild ToMV served as outgroups, confirming the monophyly of PVMV.

Comparative nucleotide and amino acid variations between PVMV Tom1 and EP1

The coding regions of PVMV isolates Tom1 and EP1 showed high nucleotide conservation across the polyprotein, with identities ranging from 97.6 % to 99.3 % per gene (Table 3). Because of its crucial function in virion assembly and transmission, the CP was the most conserved structural proteins. Non-structural proteins, such as P1, HC-Pro, CI and VPg, on the other hand, displayed somewhat lower nucleotide identities (97.6–98.8 %). Amino acid substitutions between PVMV Tom1 and PVMV EP1 at the protein level showed several differences between the viral polyprotein's several functional domains. Amino acid substitutions were observed across functional domains: P1 (D68E, A74V, E117G, H149R, R245K), HC-Pro (A319T, T488A, D503N), CI (M1200T, R1642P, T1667S), VPg (A1893S, K1969I, M2003V, A2008V, M2019V), NIa (N2152I, L2153C), NIb (R2612G, V2639I) and CP (S2972N, R3049Q) (Fig. 6).

Recombination analysis

Recombination analysis of PVMV isolates using RDP4 (RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq) revealed that the study isolates, Tom1 and EP1, showed no recombination between them, indicating genetic homogeneity within the East Asian clade. Some other East Asian isolates exhibited minor recombinant fragments supported by multiple methods ($p < 0.01$), suggesting historical recombination within the region.

African isolates displayed distinct recombination patterns and a few genomic regions of unknown parental origin likely represent recombination with unsampled lineages or highly conserved segments. Overall, recombination contributes to PVMV evolution, but Tom1 and EP1 remain largely non-recombinant (Fig. 7).

Discussion

Pepper veinal mottle virus remains a significant constraint on solanaceous crop production, as infected plants typically exhibit mottling, chlorosis, leaf deformation and reduced growth, resulting in substantial yield and quality losses (2, 5). Although PVMV infections in tomato and eggplant have been reported previously (15, 16), comparative biological and molecular characterisation of isolates maintained in Taiwan remains limited. The present study addresses this gap by providing a descriptive comparison of host infection patterns and genome-level variation between two Taiwanese PVMV isolates, Tom1 and EP1.

Host infection experiments revealed differences between Tom1 and EP1 in the timing and extent of symptom development

Table 3. Gene-wise comparison of PVMV Tom1 and EP1 isolates

Gene	Coordinates (NT)*	Coordinates (AA)**	Length (NT)	Length (AA)	Notable substitutions (Tom1, EP1)	NT identity (%)	AA identity (%)
P1	196–1095	1–300	900	300	D68E, A74V, E117G, H149R, R245K	97.6	96
HC-Pro	1096–2466	301–850	1371	457	A319T, T488A, D503N	97.9	99.1
P3	2467–3498	851–1140	1032	344	V872A, D923G, M980I, A984T, R986K	98.8	98.5
6K1	3499–3660	1141–1185	162	54	–	97.6	100
CI	3661–5589	1186–1835	1929	643	M1200T, V1303I, V1611I, R1642P, T1667S, V1788I	98.6	99.0
6K2	5590–5748	1836–1875	159	53	–	98.8	100
VPg	5749–6321	1876–2050	573	191	I1886V, A1893S, K1969I, N195S, M2003V, A2008V, M2019V	98.1	95.8
NIa-Pro	6322–7047	2051–2280	726	242	H2123Y, N2152I, L2153C	99.0	99.6
NIb	7048–8604	2281–2795	1557	519	I2351T, Q2421R, V2639I	98.6	98.7
CP	8605–9414	2796–3074	810	270	S2972N, R3049Q	99.3	100

NT: nucleotide; AA: aminoacid.

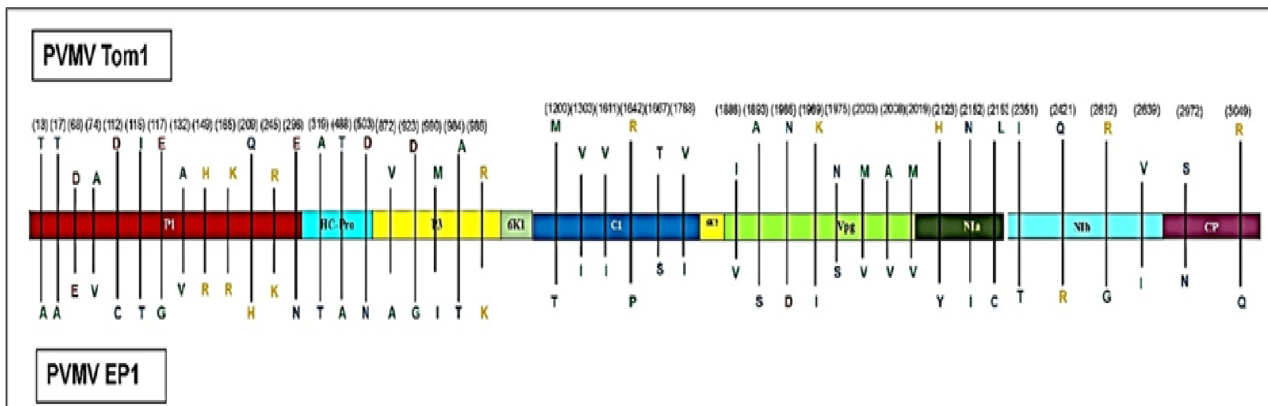

Fig. 6. Mapping amino acid variations based on hydrophobicity and charge. Amino acid differences are colour-coded according to their physicochemical properties: green for hydrophobic (non-polar), blue for polar (uncharged), yellow for positively charged (basic) and red for negatively charged (acidic) residues.

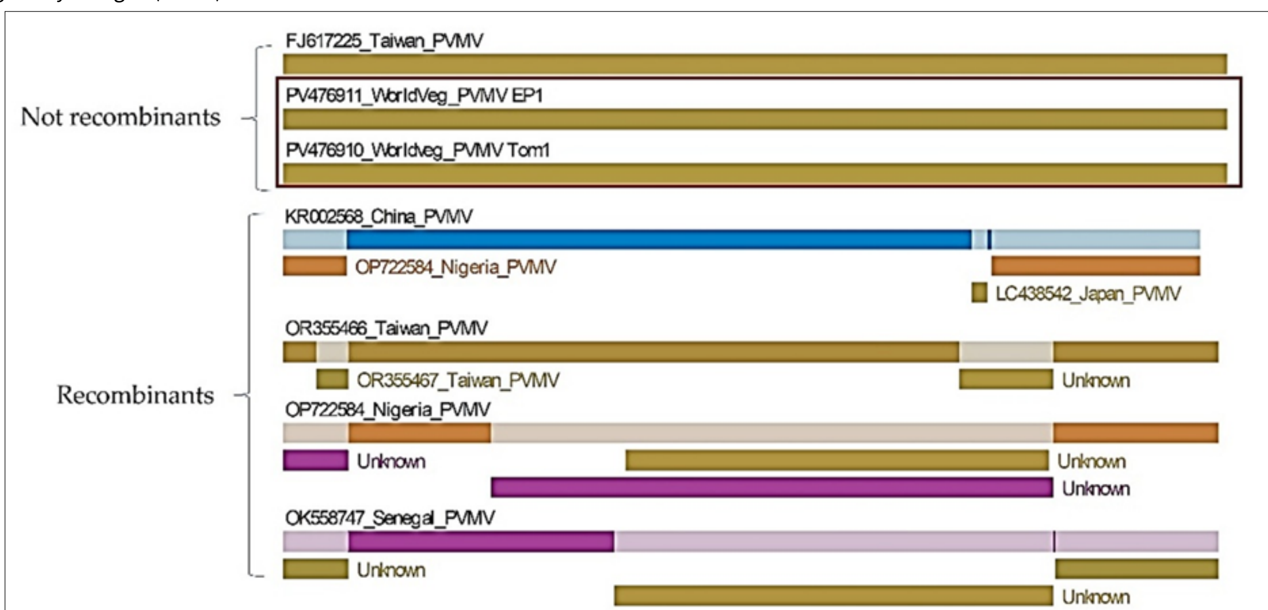

Fig. 7. Recombination breakpoint analysis of pepper veinal mottle virus (PVMV) isolates generated using RDP4. Each bar represents the genome of PVMV isolate, with coloured segments indicating predicted parental contributions. The study isolates EP1 and Tom1 (both from Taiwan) display uniform genome colouration with no detectable recombination, whereas several global isolates exhibit mosaic recombinant patterns.

across multiple plant species, despite their high nucleotide sequence identity. Host infection was evaluated qualitatively based on symptom observation and confirmed by SDT-RT-PCR and no quantitative assessments of disease severity, viral accumulation, or virulence were conducted. Therefore, differences observed between the two isolates should be interpreted as variation in host-virus interaction outcomes rather than differences in virulence.

Indicator hosts such as *Chenopodium quinoa*, *Datura metel* and *Nicotiana rustica* developed characteristic local and systemic symptoms following mechanical inoculation. Both isolates were able to establish infection in these hosts, although symptom intensity and progression differed. According to standard *Potyvirus* symptom classification (17), chlorotic spots and mottling restricted to inoculated leaves were considered local symptoms, whereas mosaic and necrotic patterns on upper leaves indicated systemic virus movement.

In tomato, Tom1 was associated with systemic symptoms earlier and more consistently than EP1, while EP1 caused delayed and comparatively milder symptom expression. These observations suggest differences in infection dynamics or movement efficiency within the host, rather than inherent differences in pathogenic strength. Similar isolate-dependent variation in symptom expression has been documented for other potyviruses, including turnip mosaic virus, where minor genomic differences influence symptom development without altering species identity (18).

All tested *Capsicum* species were susceptible to both isolates; however, EP1 produced more readily observable and earlier symptoms under the experimental conditions used, with symptom severity being more pronounced in *C. annuum* than in other *Capsicum* species. This finding is consistent with isolate-specific compatibility with particular hosts, a phenomenon commonly reported among potyvirus strains (19, 20). Such differences may reflect variation in viral replication efficiency, systemic movement, or interaction with host defense pathways, although direct functional associations cannot be established without targeted experimental assays.

Several plant species, including cucurbits, okra and selected tobacco cultivars, remained symptomless and tested negative by SDT-RT-PCR. However, latent infection or low-titer virus accumulation cannot be excluded and therefore non-host status cannot be conclusively determined (21, 22). Legume hosts exhibited isolate-specific susceptibility, with cowpea and green gram (KPS2) supporting infection by Tom1, but not EP1. This result indicates that Tom1 displayed a broader experimental host range

under the conditions tested, emphasizing the importance of evaluating multiple host species and cultivars when assessing virus adaptability and epidemiological potential (23, 24).

At the molecular level, Tom1 and EP1 shared >94 % nucleotide identity, confirming that both isolates belong to the same PVMV species and represent strain-level variation rather than divergent lineages, in accordance with ICTV species demarcation criteria. Their close genetic relationship with East Asian isolates from Japan and Taiwan suggests regional conservation, likely shaped by shared agricultural practices and historical virus movement, as reported for other potyviruses in Asia (25–27). Greater divergence observed among African and some Chinese isolates reflects broader geographic structuring, consistent with evolutionary patterns reported for potato virus Y and related viruses (28). The CP was highly conserved between Tom1 and EP1, underscoring its essential role in virion structure and its suitability for molecular diagnostics. Moderate sequence variation observed in P1, HC-Pro, CI and VPg likely reflects accumulated neutral or adaptive mutations during virus evolution.

Although these proteins have been implicated in host interaction and symptom development in other potyviruses (29, 30), their functional significance in PVMV cannot be inferred from sequence data alone.

Phylogenetic analysis placed Tom1 and EP1 within a single, homogeneous East Asian clade, showing minimal genetic differentiation. Recombination analysis detected recombination signals among some global PVMV isolates; however, no recombination events were identified in Tom1 or EP1 within the analyzed dataset, supporting their classification as non-recombinant isolates. Nevertheless, recombination detection is constrained by sequence availability and undetected historical events cannot be ruled out.

Overall, the biological and molecular differences observed between Tom1 and EP1 represent typical intraspecific variability within PVMV. This study provides baseline descriptive data on host infection patterns and genome organisation of Taiwanese PVMV isolates, without assigning functional significance to specific mutations.

Conclusion

This study provides a comparative genomic and biological characterisation of two Taiwanese PVMV isolates, Tom1 and EP1. Despite high nucleotide identity (94.3 %), the isolates differed in host infection responses, particularly in tomato and selected *Capsicum* species. The CP was highly conserved, supporting its continued utility in molecular detection, whereas moderate variation was observed in P1, HC-Pro, CI, VPg and other polyprotein regions, the functional significance of which remains unclear. Recombination analysis confirmed both isolates as non-recombinant East Asian lineages. Collectively, these results establish baseline genomic, biological and host-response data for PVMV isolates from Taiwan. Further studies involving selection pressure analysis, site-directed mutagenesis and quantitative pathogenicity assays will be essential to elucidate the role of sequence variation in virulence and host specificity. Further studies incorporating selection pressure analyses, functional mutagenesis and quantitative pathogenicity assays will be required to elucidate the biological significance of sequence variation in PVMV.

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Authors' contributions

HC contributed to conceptualisation, methodology, validation, formal analysis, investigation, resources, writing – review and editing and supervision. RO supported conceptualisation, investigation, resources and supervision. LL and SS assisted in methodology, validation and writing – review and editing. KS performed validation, formal analysis, writing – original draft preparation and writing – review and editing. SR handled investigation, resources, writing – review and editing, supervision and funding acquisition. RP oversaw supervision. JI, SA, IRC and SN performed review and supervision. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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