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ORIGINAL ARTICLE

The genomic sequence and biological properties of Pennisetum mosaic virus, a novel monocot-infecting potyvirus

C. L. Deng · W. J. Wang · Z. Y. Wang · X. Jiang · Y. Y. Cao · T. Zhou · F. R. Wang · H. F. Li · Z. F. Fan

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Abstract The complete nucleotide sequences of two isolates of Pennisetum mosaic virus (PenMV) were determined. The viral genome comprised 9,611 nucleotides (nt) excluding the 3′-terminal poly(A) sequence, with the capacity of encoding a single polyprotein of 3,065 amino acids. The large open reading frame is flanked by a 172-nt 5′-untranslated region (UTR) and a 244-nt 3′-UTR. Sequence comparisons and phylogenetic analyses of the complete genome and polyproteins suggest that PenMV is closely related to other monocot potyviruses such as *Maize dwarf mosaic virus*, *Sorghum mosaic virus* and *Sugarcane mosaic virus* (SCMV), and thus represents a distinct potyvirus within the SCMV subgroup. The host range of PenMV is limited to Gramineae, and the virus naturally infects maize, sorghum and some wild grasses, causing mosaic symptoms on the leaves. This virus could be transmitted by both mechanical inoculation and by at least four species of aphids.

C. L. Deng · W. J. Wang · Z. Y. Wang · X. Jiang · Y. Y. Cao · T. Zhou · H. F. Li · Z. F. Fan (✉)
Department of Plant Pathology and State Key Laboratory of Agro-biotechnology, China Agricultural University, 100094 Beijing, China
e-mail: virology@cau.edu.cn; plpath@cau.edu.cn; fanzf@cau.edu.cn

W. J. Wang · F. R. Wang
Maize Research Institute, Shanxi Academy of Agricultural Sciences, Xinzhou 034000, Shanxi, China

Present Address:
C. L. Deng
Plant Quarantine Laboratory, Beijing Exit–Entry Inspection and Quarantine Bureau, 100026 Beijing, China

Introduction

Potyvirus is the largest virus genus of the family *Potyviridae*, including nearly 200 definitive and tentative members [4]. Most potyviruses infect dicots and a few infect monocots. To date, only six grass-infecting potyviruses have been recognized: *Maize dwarf mosaic virus* (MDMV), *Sorghum mosaic virus* (SrMV), *Sugarcane mosaic virus* (SCMV), *Johnsongrass mosaic virus* (JGMV), *Zea mosaic virus* (ZeMV) and *Cocksfoot streak virus* (CSV), and the first five viruses forming the SCMV subgroup in the genus *Potyvirus* [1, 4, 16].

An isolate (isolate A) of a novel potyvirus from whitegrass (or flaccid pennisetum) (*Pennisetum centrasiaticum*) has been characterized and was tentatively designated as Pennisetum mosaic virus (PenMV) [7]. This virus not only infects whitegrass, but also infects maize (*Zea mays*) in the field and causes mosaic symptoms on the leaves of infected plants [10]. The variation in both nucleotide (nt) and deduced amino acid (aa) sequences of the capsid protein (CP) of some isolates from both whitegrass and maize has been investigated, and it was found that the extent of variation in maize isolates was slightly less than in those from whitegrass [9]. Although the available 3′ partial sequence data are helpful for the classification of PenMV, they are not sufficient to confirm its taxonomic status as a member of a distinct species in view of the fact that the potyviral CP cistron is not the best representative of the whole genome [3] and that there is the possibility of interspecific and intraspecific viral RNA recombination events, as reported recently [1, 6, 18]. The determination and analysis of the complete genomic RNA sequence of PenMV would be essential to discriminate it from other closely related potyviruses as well as to advance our understanding of its evolutionary history. In this paper, we

report the complete nucleotide sequence of PenMV and compare the genomic and deduced amino acid sequences of this virus with those of other closely related potyviruses, and we also investigate the host range and major transmission vectors of this virus.

Materials and methods

Virus sources and maintenance

The virus isolates (B and C) were from the same batch of diseased whitegrass plants as the previously reported isolate (A) in Xinzhou city of Shanxi province in northern China [7] and maintained in sorghum (*Sorghum bicolor*) in an insect-proof greenhouse. Virus purification and mechanical and aphid transmission were performed as previously reported [7, 8].

RT-PCR amplification and molecular cloning

Virus purification was conducted according to methods described previously with some modifications, and viral RNA was extracted from the purified viral particles by the phenol method [7, 8, 12]. The primers for RT-PCR amplifications were designed based on conserved regions of the sequences previously determined for other related potyviruses or by previous sequencing results for this virus. The viral RNA extracted from purified virions was used as template, and oligo (dT) as the primer for reverse transcription. Reverse transcription and PCR were performed in a thermal cycler (Hybaid Sprint)programmed to give 1 cycle at 42°C (60 min); 1 cycle at 94°C (3 min), 5 cycles at 94°C (30 s), 45°C (30 s) and 72°C (3–4 min), 30 cycles at 94°C (30 s), 55°C (30 s) and 72°C (3–4 min); and a final cycle at 72°C (7 min) for amplifying most parts of the viral genome. The primers used for 3′RACE are 5′-GAT CAA CTT TTT TTT TTT TTT-3′ (reverse) and 5′-CCG AGC TCC ACT AGC AGA GAA CGT GTG TAG-3′ (forward), and those for 5′RACE are 5′-TGT GGT CTG CTA CTT TCA CCC A-3′ (reverse) and 5′-CAG GAT CCA AGC GGI GIG GGI GGG-3′ (forward) after the addition of a poly (C) tail to the 3′ terminus of the cDNA using terminal deoxyribonucleotidyl transferase (TdT) (Promega). The procedures for purification of PCR products, molecular cloning and transformation of plasmids to *Escherichia coli* DH5α cells were as previously reported [7, 8].

Phylogenetic analysis

The software DNAMAN (Lynnon BioSoft, Quebec, ver. 5.2.2) and ScanProsite (http://cn.expasy.org/tools/scanprosite) were used to analyze the nucleotide and amino acid sequences. The genomic sequence of PenMV was assembled and analyzed using the DNAMAN software. A BlastP analysis of the putative polyprotein deduced from the single large ORF was performed on GenBank sequences to obtain the closely related viral sequences. Comparisons were performed using the multiple sequence alignment and two sequence alignment programs (with a gap-opening penalty of 50 and a gap-extension penalty of three for nt comparisons and corresponding values of eight and two, respectively, for aa alignments) [3]. The former program was used to align all the sequences selected and to calculate the percentage identities between two sequences by using optimal full alignment. The previously reported potyviral genomic sequences (listed in the legend to Fig. 1) were used for the nucleotide and amino acid sequence comparisons.

Aphid transmission

Four aphid species *Myzus persicae*, *Macrosiphum avenae*, *Rhopalosiphum padi* and *Schizaphis graminum* (provided by Prof. Xifeng Wang of the Plant Protection Institute, Chinese Academy of Agricultural Sciences) were used for transmission tests. The aphids were raised on healthy cabbage (for *M. persicae*) or wheat plants (for the three other aphid species) free of viruses. The aphids were placed onto PenMV-infected whitegrass (and sorghum in another set of tests) leaf pieces for acquisition feeding of the virus isolate C for 1 h in a Petri dish after 2 h fasting, and then five aphids were moved onto each healthy two to three true-leaf seedling of sorghum (cv. Xin-Liang 52) (and maize cv. Ye-Dan 2 in another set of tests) overnight for inoculation feeding before being killed by insecticide. A total of 20 sorghum (or maize) plants were inoculated for each species of aphid. Mosaic symptoms on plants were observed 10 days post-inoculation.

Host range determination

Natural host range: The leaves of some graminaceous plants (maize, sorghum, whitegrass and *Setaria viridis*) showing mosaic symptoms in the suburban fields of Xinzhou city, Shanxi province, were collected and used as inoculum after being ground in a mortar with 0.05 m phosphate buffer (pH 7.0) to infect sorghum (cv. Xin-Liang 52) seedlings. These samples were also tested by both ELISA [5] and RT-PCR (using a pair of primers specific for the coat protein cistron of PenMV) to confirm the natural infection of field plants by PenMV [7, 10]. Antigen-coated plate ELISA was performed using polyclonal antibodies against the procaryote-expressed coat protein of PenMV (raised in our laboratory). A protein-A-alkaline phosphatase conjugate (Sigma) was used for binding to the antibodies, and *p*-nitrophenyl-phosphate (Sigma) was used

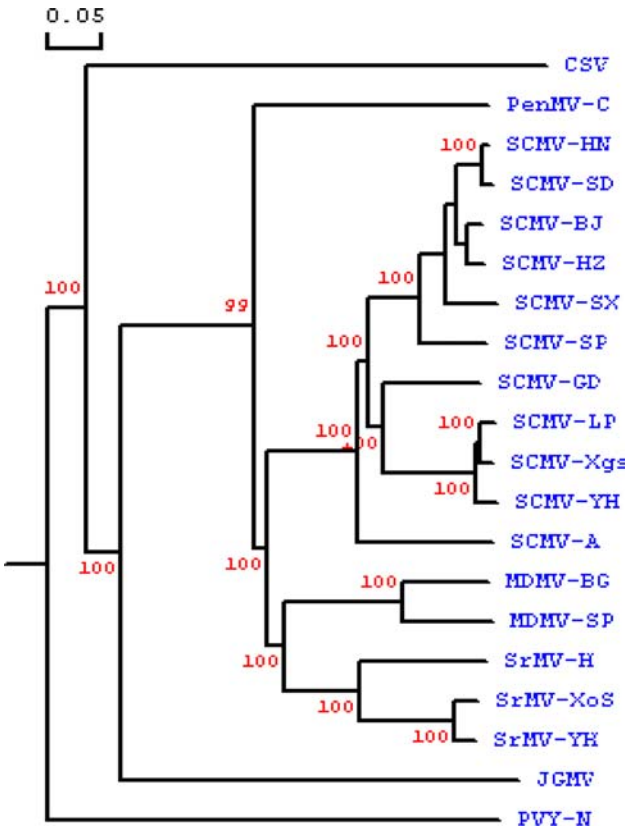

Fig. 1 Phylogenetic tree illustrating the relationships of PenMV with other potyviruses. This phylogram was generated by using the neighbour-joining method of DNAMAN ver. 5.2.2 from multiple alignment of genomic sequences. Horizontal distances are proportional to sequence distances (see scale bar), and vertical distances are arbitrary. The number at each branch indicates the percentage of 1,000 bootstraps, which support the grouping at that node. Bootstrap scores exceeding 90% are shown at major nodes. PVY was used as an outgroup in this phylogram. The acronyms present in the phylogram represent *Cocksfoot streak virus* (CSV, AF499738), *Johnsongrass mosaic virus* (JGMV, Z26920), *Maize dwarf mosaic virus*-BG (MDMV-BG, AJ001691), MDMV-SP (AM110758), *Potato virus* Y (PVY-N, D00441); *Sugarcane mosaic virus*-A (SCMV-A, AJ278405), SCMV-BJ (AY042184), SCMV-GD (AJ310105), SCMV-HN (AF494510), SCMV-HZ (AJ297628), SCMV-LP (AJ310102), SCMV-SD (AY149118), SCMV-SP (AM110759), SCMV-SX (AY569692), SCMV-XgS (AJ310103), SCMV-YH (AJ310104), *Sorghum mosaic virus*-H (SrMV-H) U57358, SrMV-XoS (AJ310197) and SrMV-YH (AJ310198)

as the substrate in the reaction. The absorbance was measured in a microplate reader (Bio-Rad) at 405 nm. The value for each sample is the average of two independent measurements. The reaction was considered a positive when the *I/H* value (the average absorbance of a sample minus that of the buffer control) was greater than or equal to three, and considered negative when *I/H* was less than three.

Experimental host range: Young leaves of a sorghum plant infected by PenMV-C (kept in an insect-proof greenhouse) showing typical mosaic symptom were used as inoculum after ground being ground in a mortar with phosphate buffer to infect the two true-leaf stage seedlings of 26 plant species/cultivars (23 plants each species/cultivar) belong to four families: Gramineae (or Poaceae) (15), Solanaceae (6), Leguminosae (3) and Chenopodiaceae (2) (see Table 4). Mosaic symptoms on plants were observed 10 days after inoculation, and all plants were tested by ELISA to confirm the infection by PenMV.

Assay of in vitro stability

The whitegrass leaves infected by PenMV-C showing mosaic symptom were ground in a mortar in 0.05 m phosphate buffer (pH7.0) and centrifuged briefly, and the supernatant was collected for the determination of the dilution end-point (DEP), thermal inactivation point (TIP) and longevity in vitro (LIV) of this virus isolate. Three replicates were performed for each treatment, and symptom development of inoculated plants was observed 10 days post-inoculation. The plant species used for inoculation after the below-mentioned treatments were maize (cv. Ye-Dan 2) and sorghum (cv. Xin-Liang 7). Five different temperatures in a water bath were used for the determination of DEP: 51, 52, 54, 56 and 58°C, and the crude virus sap samples in thin-walled Eppendorf tubes were kept separately at each of these temperatures for 10 min and then put into ice immediately before inoculating maize and sorghum. The three dilution gradients utilized for DEP determination were: 10^{-2}, 10^{-3} and 10^{-4} -fold dilution in 0.05M phosphate buffer (pH 7.0). LIV was determined by inoculating plants with the sap after being kept at room temperature (20–25°C) for 0, 48, 60 and 72 h.

Results

Genome organization

The complete nucleotide sequences of the genome RNA of isolates B and C of PenMV were deposited into GenBank under the accession numbers NC_007147 and DQ977725, respectively. The genomic sequences of the two isolates are both composed of 9,611 nucleotides with 5′- and 3′-UTRs of 172 and 241 nt, respectively, excluding the 3′-terminal poly(A) tail. The base compositions of the genome of two isolates, C/B, are: adenine 35.1/34.8%, cystosine 18.8/18.5%, guanine 21.1/21.3%, and uracil 25.0/25.4%, which are similar to those of other potyviruses. There are three (four for isolate B) in-frame AUG triplets within the first 260 nt of the long single open reading frame (ORF), and the first triplet context CGCA**AUG**GC (TGTG**AUG**GC for isolate B) is the closest to the consensus sequence (AACA**AUG**GC) for translational initiation in plants [13].

Therefore, the first AUG triplet at nucleotide positions 173–175 is most likely to be the start codon of the PenMV polyprotein. The termination condon UGA is located at nt 9,368 to 9,370. Thus, this ORF consists of 9,198 nucleotides and has the capacity of encoding a polyprotein of 3,065 amino acids with a calculated molecular mass (M_r) of 348,894 (349,101 for isolate B).

Nine putative protease cleavage sites were identified by comparing the putative coding region of PenMV with the consensus protease recognition motifs in other potyviruses (Table 1) [2]. The polyprotein is therefore highly likely to be processed into 10 smaller proteins by the three viral-encoded proteases P1, HC-Pro and NIa-Pro [2, 4, 15, 17]. This was supported by the presence of conserved motifs in this sequence that are required for the proteolytic processing of the potyviral polyprotein, such as the serine-type protease domain H_{166}-X_8-D_{175}-X_{31}-G_{207}-D-S-G_{210} and the proteolytic domain F_{220}IIRGR$_{225}$ in P1, C_{592}-X_{72}-H_{665} in HC-Pro, and H_{2046}-X_{34}-D_{2081}-X_{69}-C_{2151} in NIa-Pro [2]. Among the nine putative cleavage sites, the HC-Pro/P3 junction (G_{706}–G_{707}) for this potyvirus is identical to those of other potyviruses sequenced to date, and the other cleavage sites were similar to those of MDMV, SCMV and SrMV, except for the junction between P3 and 6K1, which is E/H, a rare dipeptide recognition site for the potyviral protease NIa-Pro (Table 1) [2].

The motif $M_{1873}YG_{1875}$, which contained a tyrosine for NIa-VPg to be linked to the 5′-end of the viral RNA, was also found. Two putative phosphorylation sites T_{2883}RAE$_{2886}$ and T_{2930}MMD$_{2933}$ of protein kinase CK2, conforming to the motif T/S-X_2-D/E [14], were found in the middle of the CP sequence, one of which is presumably involved in the cell to cell and long-distance movement of this virus as is the case with another potyvirus, *Potato virus* A [11].

Variation in isolates of PenMV

The genomic (9,611 nt) and polyprotein (3,065 aa) sequences of PenMV-C are most closely related to those of PenMV-B, with 88.7% nt and 96.8% aa identities, respectively. The 3′-UTR sequences of isolates B and C are 99.6 and 96.7% identical, respectively, compared with that of isolate A (AY172336) [7], and the CP amino acid sequences of isolates B and C are both 98.7% identical to that of isolate A. Thus, the three isolates undoubtedly belong to one virus species according to the current classification criteria for potyviruses [4].

Similarity of PenMV with other potyviruses

A BLAST search of GenBank (27 August 2007) using the polyprotein sequence (3,065 aa) of PenMV-C revealed that it was most closely related to SrMV, SCMV and MDMV in addition to PenMV-B. A phylogenetic tree was constructed from an alignment of the complete nucleotide sequences of monocot-infecting potyviral genomes with *Potato virus* Y (PVY) as an outgroup (Fig. 1), and this showed that PenMV clusters with SCMV, MDMV and SrMV. A similar phylogenetic tree was obtained from an alignment of the polyprotein aa sequences of the same potyviruses (data not shown). These results confirmed a relationship proposed previously on the basis of coat protein and 3′-UTR sequence data that PenMV is a member of the SCMV subgroup [7].

The pairwise sequence comparisons of the genomic nucleotide and polyprotein amino acid sequence identities showed that the PenMV was most closely related to SrMV-H (69.5% whole-genome and polyprotein ORF nt identity, and 77.5% CP and 72.1% polyprotein aa identity) (Table 2, Fig. 1). The current species demarcation criteria in relation

Table 1 Putative proteins obtained after cleavage of the polyprotein of PenMV

UTR/Putaive protein	Coding/non-coding region	Cleavage site (C-terminus) [a]	
		Isolate C	Isolate B
5′-UTR	1–172 (172 nt)	–	–
P1 (246 aa)	173–910 (738 nt)	LDID**HY/A**	LDIN**HY/A**
HC-Pro (460 aa)	911–2,290 (1,380 nt)	RD**Y**IV**G/G**	RD**Y**IV**G/G**
P3 (347 aa)	2,291–3,331 (1,041 nt)	TG**V**IH**E/H**	TG**V**IH**E/H**
6K1 (67 aa)	3,332–3,532 (201 nt)	KN**V**VH**Q/S**	RN**V**VH**Q/S**
CI (638 aa)	3,533–5,446 (1,914 nt)	NT**V**IH**Q/G**	NT**V**IH**Q/G**
6K2 (53 aa)	5,447–5,605 (159 nt)	QD**V**TH**Q/G**	QD**V**TH**Q/G**
VPg (189 aa)	5,606–6,172 (567 nt)	EG**V**TH**E/A**	EG**V**TH**E/A**
NIa-Pro (242 aa)	6,173–6,898 (726 nt)	DD**V**ME**Q/G**	DD**V**QE**Q/G**
NIb (521 aa)	6,899–8,461 (1,563 nt)	ED**V**YH**Q/S**	ED**V**YH**Q/S**
CP (302 aa)	8,462–9,367 (906 nt)	–	–
3′-UTR	9,368–9,611 (244 nt)	–	–

[a] The cleavage sites were deduced by comparison with those of other potyviruses

Important amino acids for the cleavage by the respective viral-encoded proteases are indicated in *boldface*, and those different for the two PenMV isolates are *underlined*

Table 2 Percentage identity in nucleotide (numbers before /) and the corresponding amino acid (numbers after /) sequences between PenMV isolate C and other monocot-infecting potyviruses.

Virus[a]	5′-NTR	P1	HC-Pro	P3	6K1	CI	6K2	NIa-VPg	NIa-Pro	NIb	CP	3′-NTR	Polyprotein	Genomic
SrMV	50.4	**49.8/40.3**	**72.0/79.1**	66.5/**64.5**	**71.5**/67.7	**73.6/80.1**	**71.5/64.2**	**67.9/75.7**	**70.7/75.2**	70.4/76.6	72.6/**77.5**	50.7	**69.5/72.1**	**69.5**
SCMV	55.9	48.4/38.6	71.5/77.2	66.8/61.6	68.7/**70.1**	69.3/77.0	68.7/60.4	67.5/72.5	68.3/71.5	**72.2/78.3**	70.8/74.8	**59.8**	67.8/69.8	68.1
MDMV	**57.4**	46.4/34.8	69.7/78.9	**67.1**/63.6	69.2/65.7	72.1/79.5	69.2/62.3	66.1/66.1	67.2/68.2	70.2/77.5	**73.1**/76.6	46.7	67.7/70.9	67.6
JGMV	30.8	38.0/27.2	55.3/47.8	47.5/32.1	57.3/48.1	58.4/56.4	57.3/43.5	58.2/58.2	53.1/47.1	62.9/63.3	56.7/57.7	31.3	54.8/47.5	55.1
CSV	34.7	38.5/16.1	50.5/40.3	45.0/30.8	54.5/46.2	53.3/53.0	54.5/35.8	55.9/47.8	52.9/45.2	63.1/61.7	55.4/49.5	25.2	51.4/38.6	51.9

The highest values are indicated in *boldface*

[a] The viruses used in this table are: *Cocksfoot streak virus* (CSV) AF499738, *Johnsongrass mosaic virus* (JGMV) Z26920, *Maize dwarf mosaic virus* (MDMV-BG) AJ001691, *Sugarcane mosaic virus* (SCMV-XgS) AJ310103, and *Sorghum mosaic virus* (SrMV-H) U57358

to the genome sequence relatedness given by ICTV for the genus *Potyvirus* include CP aa identity of less than about 80%, complete genomic nt identity of less than 85%, and different polyprotein cleavage sites [4]. Another study showed that a value of 76% nt identity for either the complete ORF or the CP cistron is the most appropriate for separating species in the genus [3]. Therefore, whichever criteria we might employ, PenMV should be classified as a distinct potyvirus.

Aphid transmission

Four to five out of 20 sorghum/maize seedlings developed mosaic symptom 10 days after transmission inoculation by each of the four aphid species *M. avenae*, *M. persicae*, *R. padi* and *S. graminum*, thus confirming the transmissibility of PenMV by these four aphid species.

Host range

Natural host range: All of the tested plants of maize, sorghum, whitegrass and *S. viridis* showing mosaic symptoms were confirmed to be infected by PenMV in the field by both ELISA and RT-PCR (Table 3). The band of predicted size of 0.9 kb of the amplified cDNA of the coat protein cistron of PenMV was visualized under UV light in agarose gel after electrophoresis (data not shown). Since only one plant of *S. viridis* was found to be infected in the field in this survey, the importance of this annual wild grass as a reservoir of the virus may be negligible. In contrast, most whitegrass plants were infected in the suburban fields of Xinzhou city; thus, this perennial wild grass could be the main source of primary infection of maize and sorghum by PenMV in that region.

Experimental host range: The results of inoculation of the test plants by PenMV are listed in Table 4. These wild grasses infected by PenMV are potentially the natural hosts of the virus in the field and thus could be found to be the reservoirs of this virus in the future.

Table 3 The assay for natural host range of PenMV

Plant species	Symptom	Plant[a]	ELISA[b]	RT-PCR[c]
Pennisetum centrasiaticum	SM	10	+	+
Setaria viridis	SM	1	+	+
Sorghum vulgare	SM	5	+	+
Zea mays	SM	10	+	+

SM systemic mosaic

[a] The number of plants collected from fields

[b] Positive reaction (*I*/*H* value $\geq$ 3) in ELISA

[c] The coat protein cistron of PenMV was amplified

Table 4 The experimental host range of PenMV

Plant family	Species (cultivars)	Symptom	Diseased plants[a]	ELISA[b]
Gramineae	*Avena sativa* (Huabei 2)	–	0	–
	Arthraxon prionodes	SM	4	+
	Chloris virgata	SM	9	+
	Eleusine indica	SM	8	+
	Hordeum vulgaris (Zhe 88-23)	–	0	–
	Oryza sativa (Liming)	–	0	–
	Panicum glaucum	SM	13	+
	Setaria vulgare	SM	13	+
	Sorghum bicolor (Atlas)	SM	22	+
	Sorghum vulgare (Xin-Liang 7)	SM	22	+
	Sorghum vulgare (Xin-Liang 52)	SM	23	+
	Sorghum halepense	–	0	–
	Spodiopogon sibiricus	SM	7	+
	Triticum aestivum (Mingxian 169)	–	0	–
	Zea mays (Ye-Dan 2)	SM	22	+
Solanaceae	*Capsicum annuum*	–	0	–
	Datura stramonium	–	0	–
	Nicotiana glutinosa	–	0	–
	Nicotiana tabacum	–	0	–
	Nicotiana tabacum (Xanthi-nc)	–	0	–
	Physalis floridana	–	0	–
Leguminosae	*Phaseolus vulgaris*	–	0	–
	Phaseolus radiatus	–	0	–
	Vigna sinensis	–	0	–
Chenopodiaceae	*Chenopodium amaranticolor*	–	0	–
	Chenopodium quinoa	–	0	–

SM systemic mosaic

– No symptom

[a] The total number of inoculated plants for any plant species/cultivar is 23

[b] Reactions are considered as positive (+) when the *I*/*H* value ≥ 3, and negative (–) when $I/H < 3$

Stability in vitro

The infectivity test of PenMV-C inoculum after various treatments showed that the virus isolate C had a thermal inactivation point of 53°C and a dilution end-point of 10^{-2}, and its infectivity was retained in vitro for up to 48 h. The values of these in vitro properties of this virus are more or less similar to those previously reported for other members of the SCMV subgroup [16], indicating that the stability of this virus isolate is not very different from those of other closely related viruses.

Discussion

In the present study, we found that the homologous gene products have differing degrees of sequence identity and that PenMV is most closely related to MDMV, SrMV and SCMV, members of the SCMV subgroup of potyviruses. The cistron of the CI was remarkably conserved among the nt and aa sequences of PenMV and other members of the SCMV subgroup and was distinct from that of dicot-infecting potyviruses, while the NIb-coding region was the most conserved. Most parts along the genome of PenMV were most closely related to SrMV (such as P1, 6K1, CI, NIa-VPg, NIa-Pro and complete genomic sequences). It was noteworthy that some coding regions/UTRs (or proteins) of PenMV were most closely related to those of SCMV-XgS (such as NIb and 3′-UTR), and some parts were most closely related to the corresponding parts of MDMV-BG (such as P3, CP and 5′-UTR)(Table 2). Since the genomic sequences of PenMV are significantly different from those of all other known potyviruses, it should be assigned to the genus *Potyvirus* as a distinct species. In view of the similarities of the genomic and polyprotein sequences, the host range, and some other properties of PenMV to the SCMV subgroup, we believe that PenMV is a new member of this subgroup.

Since PenMV is now restricted to a small region (Shanxi province) in China, and the symptoms on its hosts are usually a relatively mild mosaic compared to that of SCMV [8, 10], its importance in agriculture has been relatively limited to date. With the change of cultural practices and continuing monoculture of a few cultivars of maize and sorghum in production in addition to the efficient transmission of the virus by four species of common aphids and

the abundance of wild grasses serving as virus reservoirs, PenMV as an emerging virus has the potential to become a major pathogen in maize as SCMV [8, 10, 18] or, in the future, in sorghum.

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