Characterization of Lagenaria mild mosaic virus, a New *Potexvirus* from Bottle Gourd in Myanmar

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ABSTRACT

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A putative *Potexvirus* was detected from bottle gourd (*Lagenaria siceraria*) showing mosaic and mottle symptoms in Myanmar in 2007. The virus was designated Lagenaria mild mosaic virus (LaMMoV) and was further characterized. In artificial inoculation tests, infectivity of LaMMoV was limited to two families: *Chenopodiaceae* and *Cucurbitaceae*. The host range of LaMMoV differs from those of the two cucurbit-infecting potexviruses, *Alternanthera mosaic virus* (AltMV) and *Papaya mosaic virus* (PapMV). Sequence analyses of LaMMoV showed that the C-terminal 3,859 nucleotides, excluding the poly-A tail, includes the C-terminal region of an RNA-dependent RNA polymerase (RdRp), a triple gene block (TGB), a coat protein (CP), and a 3′ untranslated region (UTR), all of which are typical of potexviruses. Although LaMMoV is related closely to AltMV and PapMV, its nucleotide sequences differ from those of other previously reported potexviruses. Therefore, we report LaMMoV as a new species of the genus *Potexvirus* that occurs in the cucurbit bottle gourd.

Lagenaria siceraria (Mol.) Stand. is a widely distributed edible and medicinal cucurbit. It is commonly known as bottle gourd, because its fruits are used as bottles or containers (24). A bottle gourd plant with mosaic and mottle symptoms was collected in Myanmar in March 2007. Electron microscope analyses showed that this plant contained two viruses: a putative *Potexvirus* and a *Tobamovirus*. The *Tobamovirus* was identified as *Cucumber green mottle mosaic virus* (CGMMV) by serological and molecular analyses (11,12).

Viruses in the genus *Potexvirus* (family *Flexiviridae*) have filamentous and flexuous virions that are 470 to 580 nm in length. Each virion contains a single linear molecule of positive-sense RNA encoding five open reading frames (ORFs): an RNA-dependent RNA polymerase (RdRp) gene, three genes known as the triple gene block (TGB), and a coat protein (CP) gene (2,27). The 5′ end has a methylguanosine cap and the 3′ end has a poly-A tail (15). The ICTV database lists 28 known and 18 tentative member species of the genus *Potexvirus* (1). These viruses have diverse host species, including cactus (family *Cactaceae*), cymbidium (family *Orchidaceae*), potato (family *Solanaceae*), and strawberry (family *Rosaceae*). Although some of these viruses cause mosaic or ringspot symptoms in their hosts, others cause little visible damage (1).

There are a few reports of potexviruses that naturally infect cucurbits. Trichosanthes virus (TV) was isolated in 1988 from *Trichosanthes dioica* in the United States. TV is a possible potexvirus that is serologically related to *Papaya mosaic virus* (PapMV) (22,23), which infects *Carica papaya* and *Cucurbita pepo* (17). In addition, unidentified potexvirus-like virions were observed in one sample of *Momordica charontia* by electron microscopy (19). *Alternanthera mosaic virus* (AltMV) from *Alternanthera pungens* is a distinct potexvirus that is closely serologically related to PapMV and infects some cucurbit hosts (6). So far, artificial inoculation tests have found 11 potexviruses (excluding AltMV) that infect cucurbits: *Clover yellow mosaic virus* (ClYMV), *Daphne virus X* (DVX), *Foxtail mosaic virus* (FoMV), *Hydrangea ringspot virus* (HdRSV), *Narcissus mosaic virus* (NMV), PapMV, *Pepino mosaic virus* (PepMV), *Potato virus X* (PVX), *Tulip virus X* (TVX), *White clover mosaic virus* (WClMV), and Viola mottle virus (VMoV, a tentative species in genus *Potexvirus*) (3,9,10,15,16,18,21,25).

In this paper, we report the occurrence and molecular characterization of a putative *Potexvirus* isolated from bottle gourd in Myanmar, which we designated Lagenaria mild mosaic virus (LaMMoV). The taxonomic relationship of this virus with other potexviruses is also discussed.

MATERIALS AND METHODS

Virus isolates and maintenance. CGMMV and LaMMoV were obtained from a naturally infected bottle gourd with mosaic and mottle symptoms in Myanmar in 2007. To separate these two viruses, the sample was inoculated onto *Chenopodium quinoa*, which is the nonsystemic host of CGMMV (13). LaMMoV systemically infected the host plant, whereas CGMMV was restricted to the inoculated leaves. Therefore, LaMMoV was isolated from upper leaves of the hosts. In back-inoculation of bottle gourd, LaMMoV infected the host systemically. The infection was largely symptomless, but some mild mosaic symptoms were visible. The virus was propagated by artificial inoculation for further studies.

Electron microscopy. A drop of sap from the infected plant was placed on a copper grid with carbon-coated collodion film (Nisshin EM Co., Ltd., Tokyo, Japan). The sap was negatively stained with 2% phosphotungstic acid (pH 6.0), and the grid was examined under an electron microscope (H-7600, Hitachi Ltd., Tokyo, Japan).

Host range and symptomatology. To establish the experimental host range of LaMMoV, sap extract was macerated in 0.1 M potassium phosphate buffer (pH 7.0) (1:10 wt/vol), Carborundum was added as an abrasive, and this mixture was used to inoculate 14 species in six families. Inoculated plants were grown in an incubator at 24 to 27°C with a 16-h light/8-h dark photoperiod, and symptom development was observed for at least 4 weeks after inoculation. In several cases, symptomless or indistinct infections were identified by specific reverse-transcription polymerase chain reaction (RT-PCR).

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*The *e*-Xtra logo stands for "electronic extra" and indicates that Figure 1 appears in color in the online edition.

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Enzyme-linked immunosorbent assay (ELISA). Antisera against PVX and PapMV (Agdia Inc., Elkhart, IN) were used to determine serological relationships with LaMMoV. Antiserum against PVX was provided by T. Maoka (National Agricultural Research Center for Hokkaido Region, Japan). A commercial antiserum kit against CGMMV (Agdia) was used to determine the presence of CGMMV. Absorbance at 405 nm was measured using a microplate reader (Model 680 Microplate Reader, Bio-Rad, Hercules, CA). Reactions were designated as positive when the absorbance value was three times greater than that of the corresponding control after 30 min incubation with the substrate at room temperature.

RNA extraction, RT-PCR, and 3′ rapid amplification of cDNA ends (RACE). Total RNAs were extracted from bottle gourd infected by LaMMoV using Trizol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Total RNAs were used as a template for first-strand cDNA synthesis and for PCR analyses. The use of degenerate primers specific to the genus *Potexvirus* (26) resulted in PCR-amplification of a 593-bp fragment, which confirmed the presence of a potexvirus. Then, a pair of specific primers to amplify the partial CP was designed based on previously reported sequences of AltMV (AY863024, AF080448, AY850928, AY850931, AY850930, and AY566288) and PapMV (D13957, AY017188, and AY017187). First-strand cDNAs were synthesized using a ReverTra Ace -α- kit (TOYOBO Co., Ltd., Osaka, Japan) with AltPap-R (25 pmol) for specific detection. Second-strand cDNAs were synthesized by PCR using TaKaRa Ex Taq PCR buffer (Takara Bio Inc., Otsu, Japan) with amplification primers (Alt-F and AltPap-R, 25 pmol each). The reactions were carried out in a PTC-100 Peltier Thermal Cycler (MJ Research, Inc., Waltham, MA) at a PCR annealing temperature of 50°C. We used several other combinations of PCR primers (Table 1) and obtained specific cDNA amplification products from LaMMoV. To sequence the C-terminal region, a LaMMoV-specific primer (M17PX-3RACE, 10 pmol) was used in the 3′ RACE System for Rapid Amplification of cDNA ends (Invitrogen).

To further examine the relationship between LaMMoV and other potexviruses, and to confirm that LaMMoV was a new potexvirus, we designed several sets of specific primers (Table 1) and determined the sequences of the C-terminal region of ORF 1 and complete ORFs 2, 3, 4, and 5.

Cloning and sequencing. Amplification products were separated on agarose gels, and each band was then purified from the gel using the Wizard SV Gel and PCR Clean-Up system (Promega Corp., Madison, WI). Purified PCR fragments were ligated into the pGEM T-vector (Promega) following the manufacturer's protocol, and the vector was introduced into competent cells of *Escherichia coli* JM109 (Takara Bio). Plasmids were isolated from recombinant *E. coli* using the LaboPass Plasmid Mini kit (Cosmo Genetech Co., Ltd., Seoul, Republic of Korea), and those containing an insert of the expected size were sequenced with an Applied Biosystems 3130/3130xl Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Sequence analyses. Sequence analyses were conducted using the program on the NCBI homepage (http://www.ncbi.nlm.nih.gov/) and CLC Free Workbench 4.5 software (http://www.clcbio.com/index.php?id=206). For comparison with LaMMoV, Clustal W (version 1.83) was used to align the equivalent sequences of other potexviruses available in the GenBank DNA database (Table 2). Molecular weights of proteins were predicted using the Bioedit sequence alignment editor (version 7.0.9). Pairwise sequence alignments of nucleotide and amino acid sequences were analyzed using MacVector 7.2.2 software (Accelrys Software Inc., San Diego, CA). The phylogenetic tree was constructed from the distance matrix by the neighbor-joining (NJ) method. Reliability of the tree was estimated using bootstrap with 1,000 replicates.

RESULTS

Epidemiology and morphology. Bottle gourds showing mosaic and mottle symptoms were observed in Myanmar in 2007 (Fig. 1A). Electron microscope analyses showed that sap from these gourds contained 300-nm-long rod-shaped particles that were characteristic of a *Tobamovirus*. This was identified as CGMMV by ELISA and RT-PCR analyses. The sap also contained filamentous particles approximately 550 nm in length, which were characteristic of viruses in the genus *Potexvirus*. The virus was designated as LaMMoV (Fig. 1B).

Symptomatology and serology. LaMMoV were separated from CGMMV on upper leaves of an inoculated *C. quinoa* plant by mechanical transmission of a sample containing both viruses. LaMMoV was propagated in bottle gourd. The experimental host range of LaMMoV is shown in Table 3. Among the tested cucurbit plants, LaMMoV caused mosaic and yellowing in *Benincasa hispida*, but was asymptomatic in *Citrullus lanatus*, *Cucumis sativus*, and *Cucurbita pepo*. *Cucumis melo* and *Luffa cylindrica* were not susceptible host species.

Serological testing demonstrated that LaMMoV was closely related to PapMV, but not to PVX (*data not shown*).

RT-PCR detection, sequence analyses, and comparison with other potexviruses. We determined the sequence of 3,859 nucleotides (nt) excluding the poly-A tail (GenBank accession no. AB546335). This sequence included the coding regions for the C-terminal region of RdRp, the TGB, a CP gene and a 3′ UTR.

The C-terminal region contained the RdRp (ORF 1) gene, which encoded 650 amino acid (aa) residues including a highly conserved RNA replication motif with the consensus sequence 'S/TGEX$_2$TFDANT... GDD'. We also identified a TGB structure composed of overlapping TGB 1 (ORF 2: 690 nt), TGB 2 (ORF 3: 333 nt), and TGB 3 (ORF 4: 204 nt). TGB 1, 2, and 3 encoded polypeptides of 229, 110, and 67 aa, respectively, with calculated molecular weights of approximately 25,538, 11,785,

Table 1. Primers used for detection and sequencing of Lagenaria mild mosaic virus (LaMMoV)[a]

Primer	Sequence (5′ to 3′)	Target sequences	Position	Amplification regions
Potex U1	ACNNTNGTNYTNCCNVYNGAR	Genus *Potexvirus*	ORF 1	Partial ORF 1
M17PX1	TCAATTGTGGCCCATAAGAGG	LaMMoV	ORF 1	
Potex 5[b]	CAYCARCARGCMAARGAYGA	Genus *Potexvirus*	ORF 1	Partial ORF 1
Potex 2RC[b]	AGCATRGCNSCRTCYTG	Genus *Potexvirus*	ORF 1	
M17PX-TGBf	ATGACTTCACAGCCTTCGACC	LaMMoV	ORF 1	ORF 2, 3 and 4
M17PX-TGBr	TGTGGCGCTCATTGTGACACG	LaMMoV	ORF 5	
M17PX-T1f	CTGACAGAAGGAATCTGGAG	LaMMoV	ORF 2	Partial ORF 2
M17PX-T1r	CCCGTCAATAAGTCCTCGGTG	LaMMoV	ORF 2	
Alt-F	CCGGGCTTAGGTTTTAGCA	AltMV[c]	ORF 4	Partial ORF 4 and ORF 5
AltPap-R	TGGCCYTTGGTGATGAA	AltMV and PapMV[c]	ORF 5	
M17PX-3RACE	TCGCTTCCAACTCCTCCTTCATAAC	LaMMoV	ORF 5	Partial ORF 5 and 3′ UTR

[a] ORF = open reading frame. Underlined primers indicate antisense direction.
[b] Potex 5 and Potex 2RC are from van der Vlugt and Berendsen (26).
[c] AltMV = *Alternanthera mosaic virus*; PapMV = *Papaya mosaic virus*.

and 7,036 Da, respectively. We identified conserved potexvirus sequences in LaMMoV, such as 'X_2AGXGKS/T' (an NTP binding helicase motif in the TGB 1 region), and 'GDX_2HX_2PXGGXYXDGT KX_3Y' in the TGB 2 region. Comparisons between the deduced amino acid sequences of LaMMoV and those of other potexviruses indicated that TGB 1 and TGB 2 had the highest identity with the corresponding TGBs of AltMV, while TGB 3 showed highest identity to PapMV (Table 4). In phylogenetic trees based on TGB 1 and TGB 2, LaMMoV formed a single cluster with AltMV and PapMV. In the tree based on TGB 3, however, the phylogenetic relationship among LaMMoV and other potexviruses conflicted with their identity percentage, because of the variable lengths and high diversity of the TGB 3 sequences (*data not shown*). This result suggested that the TGB 3 sequence is too inconsistent to determine relationships among potexviruses.

The CP region of LaMMoV was 627 nt in length, and was predicted to encode a 208 aa protein with an ATG start codon and a TAA stop codon. The 3′ UTR consisted of 119 nt excluding the poly-A tail. The palindromic sequences in this region suggested that it forms stem-loop structures, like those found in other potexviruses. The CP region of LaMMoV contained the core sequence KFAAFD FFDGV, which is highly conserved in potexviruses.

The CP region of LaMMoV showed 69% homology to those of several AltMV isolates at the nucleotide level and 76% homology with the CP of PapMV (AY017187) at the amino acid level (Table 5). The sequence of the 3′ UTR of LaMMoV showed 67 to 72% identity with those of AltMV isolates, but only 59% identity with that of PapMV (D13957) (Table 4).

A phylogenetic tree was constructed to determine the relationships between LaMMoV and other potexviruses based on the alignments of CP amino acids (Fig. 2). LaMMoV was most closely related to PapMV, and LaMMoV, AltMV, and PapMV formed a single cluster. In addition, the 3′ UTR nucleotide sequence of LaMMoV showed the highest similarity to that of AltMV, and the next highest similarity to that of PapMV (Table 4). These data indicated that LaMMoV was more closely related to AltMV and PapMV than to the other potexviruses.

DISCUSSION

We have isolated a new potexvirus, designated as LaMMoV, from a bottle gourd plant in Myanmar, and determined its biological, serological, and molecular characteristics. In artificial inoculation tests, LaMMoV infected plants in only two families: the *Chenopodiaceae* and *Cucurbitaceae*. LaMMoV can be distinguished from AltMV, which causes mottle symptoms on tomato, after artificial inoculation. LaMMoV does not infect papaya, which distinguishes it from PapMV. Although LaMMoV showed close serological relationships with PapMV in this study and AltMV was known to be closely related to PapMV (6), the three viruses have differential effects on host plants (6,8,14). LaMMoV has a narrow host range, and many of the infected plants show very mild or no visible symptoms. The original host that showed mosaic and mottle symptoms was infected with both LaMMoV and CGMMV; therefore, the observed symptoms were probably a result of a mixed infection. LaMMoV may cause different symptoms when present as a single infection.

LaMMoV reacted positively with As-PapMV but not with As-PVX. In general, distinct species of *Potexvirus* do not show serological cross-reactions, although there are a few reports of cross-reactions occurring. For example, *Hosta virus X* (HVX) reacted positively with As-ClYMV (5), and AltMV was reported to react positively with As-PapMV, leading to a possible misdiagnosis (8). Thus, the positive reaction of LaMMoV against As-PapMV was not sufficient experimental evidence to confirm its identity.

To qualify as a distinct species in the family *Flexiviridae*, which includes the existing genus *Potexvirus*, the CP or replication protein genes of the virus must show less than 72% identical nucleotides or less than 80% identical amino acids

Table 2. *Potexvirus* sequences used in this study

Virus species	Host and country of origin	GenBank accession
Alternanthera mosaic virus (AltMV)	*Phlox stolonifera*; USA	AY863024
	Alternanthera pungens; Australia	AF080448
	Phlox stolonifera; USA	AY850928
	Phlox stolonifera; USA	AY850931
	Portulaca grandiflora; USA	AY850930
	Portulaca spp.; Italy	AY566288
Bamboo mosaic virus (BaMV)	*Bambusa oldhamii*; Taiwan	D26017
Cactus virus X (CVX)	*Hylocereus undatus*; Taiwan	AF308158
Cassava common mosaic virus (CsCMV)	*Manihot esculenta*; Brazil	U23414
Clover yellow mosaic virus (ClYMV)	*Vicia faba*; Canada	D29630
Cymbidium mosaic virus (CymMV)	*Cattleya* sp.; Singapore	U62963
Foxtail mosaic virus (FoMV)	*Hordeum* sp.; Canada	M62730
Lily virus X (LVX)	Unknown[a]; Netherlands	AJ633822
Narcissus mosaic virus (NMV)	*Narcissus* sp.; Netherlands	D13747
Papaya mosaic virus (PapMV)	Unknown	D13957
	Cucurbita spp.; Mexico	AY017188[b]
	Carica papaya; Mexico	AY017187[b]
Pepino mosaic virus (PepMV)	*Lycopersicon esculentum*; Spain	AF484251
Plantago asiatica mosaic virus (PlAMV)	*Plantago asiatica* L.; Russia	Z21647
Potato aucuba mosaic virus (PAMV)	*Solanum tuberosum*; Canada	S73580
Potato virus X (PVX)	Unknown	X05198
Strawberry mild yellow edge virus (SMYEV)	Unknown	D12517
Tulip virus X (TVX)	*Tulipa gesneriana*; Japan	AB066288
White clover mosaic virus (WClMV)	Unknown	X06728

[a] Insufficient evidence of natural host species and/or country of origin.
[b] 3′ Untranslated region (3′ UTR) sequences unavailable.

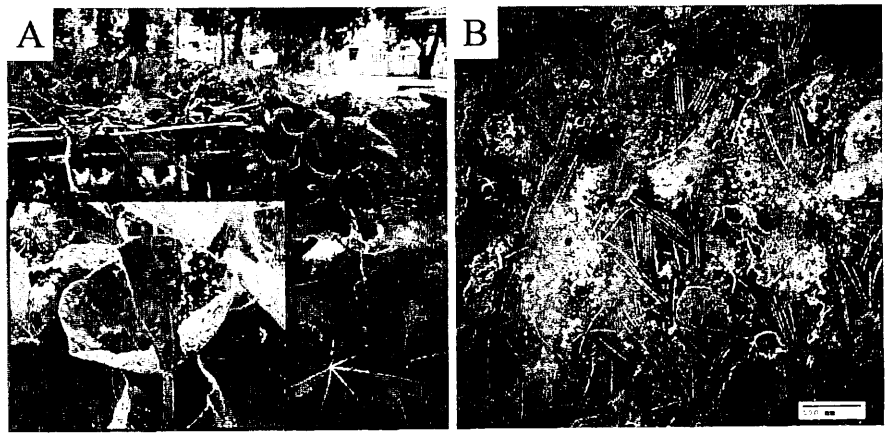

Fig. 1. A, Field symptoms on a diseased bottle gourd caused by Lagenaria mild mosaic virus (LaMMoV) and *Cucumber green mottle mosaic virus* (CGMMV). **B,** Transmission electron micrograph showing rod shaped particles of CGMMV approximately 300 nm in length, and filamentous particles of LaMMoV approximately 550 nm in length.

compared with their respective genes in other potexviruses (1,2). The CP gene of LaMMoV showed less than 69% identity with those of AltMV and PapMV isolates at the nucleotide level, and 75% identity with that of AltMV and 73 to 76% identities with those of PapMV isolates at the amino acid level. These results support the finding that LaMMoV is a distinct *Potexvirus* species. In addition, the sequence data confirm that LaMMoV is a potexvirus, because it contains several characteristic

Table 3. Comparison of host reactions from infection by Lagenaria mild mosaic virus (LaMMoV), *Papaya mosaic virus* (PapMV), and *Alternanthera mosaic virus* (AltMV)

Host					
Family	**Species**	**LaMMoV**	**PapMV**[a]	**AltMV**[b]	**AltMV**[c]
Amaranthaceae	*Spinacia oleracea*	–[d]	S/+	S/mosaic	S/necrotic fleck, leaf curl
Caricaceae	*Carica papaya*	–	S/mottle, mosaic	–	nt
Chenopodiaceae	*Chenopodium amaranticolor*	S/+	S/mottle, mosaic	S/mosaic	nt
	Chenopodium quinoa	S/+	S/mottle, mosaic	S/interveinal yellowing	L/necrotic
Cucurbitaceae	*Benincasa hispida*	S/mosaic, yellowing	nt	nt	nt
	Citrullus lanatus	S/+	nt	S/mosaic	nt
	Cucumis melo	–	nt	nt	nt
	Cucumis sativus	S/+	S/+	S/+	–
	Cucurbita pepo	S/+	-	L/+	nt
	Lagenaria siceraria	S/+, mild mosaic	nt	nt	nt
	Luffa cylindrica	–	nt	nt	nt
Fabaceae	*Vicia faba*	–	-	S/mosaic	S/chlorotic fleck
Solanaceae	*Solanum lycopersicum*	–	-	S/mottle	S/mild mottle, leaf curl
	Nicotiana benthamiana	–	nt	S/mosaic, rugosity, epinasty	S/mosaic, rugosity

[a] Symptoms of PapMV infection adapted from Margaret and Jeffrey (14).
[b] Symptoms of AltMV infection adapted from Geering and Thomas (6).
[c] Symptoms of AltMV infection adapted from Hammond et al. (8).
[d] S denotes systemic infection of the host, followed by symptom (if visible) on upper leaf. L denotes local infection, followed by symptoms on inoculated leaf: – = no infection, + = symptomless infection, nt = not tested.

Table 4. Nucleotides and amino acids identity (%) of Lagenaria mild mosaic virus (LaMMoV) (GenBank accession no. AB546335) with other *Potexviruses*[a]

Virus species	GenBank accession	TGB 1[b]		TGB 2		TGB 3		CP[b]		3′ UTR[b]
		aa	nt	aa	nt	aa	nt	aa	nt	nt
AltMV	AY863024	50	52	53	56	28	50	75	69	68
BaMV	D26017	36	43	31	42	13	48	22	32	27
CVX	AF308158	45	53	37	43	26	39	42	50	24
CsCMV	U23414	42	55	33	41	18	25	41	45	23
ClYMV	D29630	43	51	41	49	8	23	32	39	20
CymMV	U62963	24	35	31	43	13	21	29	41	30
FoMV	M62730	36	44	36	40	17	28	23	33	25
LVX	AJ633822	33	41	35	43	11	28	36	41	24
NMV	D13747	27	35	26	35	12	24	23	37	8
PapMV	D13957	45	52	52	58	39	48	73	67	59
PepMV	AF484251	24	37	25	37	13	27	27	35	18
PlAMV	Z21647	44	52	37	43	9	16	44	49	20
PAMV	S73580	22	37	26	41	12	25	23	34	27
PVX	X05198	36	44	34	45	14	29	32	42	16
SMYEV	D12517	28	38	37	41	14	25	30	38	25
TVX	AB066288	46	51	36	41	8	22	46	52	19
WClMV	X06728	30	41	31	37	16	25	31	40	28

[a] Underlined figures indicate the highest identity percentages between LaMMoV and other *Potexviruses* for each region.
[b] TGB = triple gene block; CP = coat protein; 3′ UTR = 3′ untranslated region.

Table 5. Identity of nucleotides (shaded) and amino acids (nonshaded) based on coat protein sequences[a]

		AltMV						PapMV			
	PVX	**AY850931**	**AY850928**	**AY863024**	**AF080448**	**AY850930**	**AY566288**	**D13957**	**AY017188**	**AY017187**	**LaMMoV**
PVX		33	33	33	33	34	34	33	34	34	33
AltMV											
AY850931	39		100	97	97	95	95	73	74	75	75
AY850928	39	99		97	97	95	95	73	74	75	75
AY863024	41	96	96		97	95	94	73	74	75	75
AF080448	39	93	93	93		98	97	73	75	76	75
AY850930	40	93	93	94	94		99	73	74	75	75
AY566288	40	94	93	95	94	97		72	73	74	75
PapMV											
D13957	40	67	66	66	67	66	67		89	89	73
AY017188	40	66	66	67	68	66	67	78		99	75
AY017187	40	67	67	67	68	67	67	77	93		76
LaMMoV	40	69	69	69	68	69	69	67	66	66	

[a] Pairwise analysis performed with *Alternanthera mosaic virus* (AltMV) and *Papaya mosaic virus* (PapMV) isolates (most closely related to Lagenaria mild mosaic virus [LaMMoV]; AB546335), and *Potato virus X* (PVX; X05198, only distantly related to LaMMoV).

conserved regions. Geering and Thomas (6) first identified the core region of CP in potexviruses, which is equivalent to amino acid residues A_{54} to E_{178} in PVX strain XA. Based on those findings, we aligned the core region of the CP to construct a phylogenetic tree. The result was consistent with those obtained when the CP amino acids were compared (*data not shown*). Accordingly, our results also suggest that the core region of the CP is a useful marker for genetic relationships among potexviruses.

The 3′ UTR of single-stranded positive-sense RNA viruses has essential roles in viral RNA amplification, such as stabilization of viral RNA and enhancement of translation of viral genes (4,7). Secondary or tertiary structures of the 3′ UTR of a few potexviruses have been characterized, and include such elements as stem-loop structures, a hexanucleotide motif (5′-ACATAA), and a U-rich region, the latter two of which are required for RNA accumulation in PVX (20). The ability of WClMV to infect host plants was affected by decreasing the length of the poly-A tail and mutating the hexanucleotide motif (5′-AATAAA), which is a putative polyadenylation signal (7). A hexanucleotide motif (5′-ACTTAA) in ClYMV is essential for accumulation of progeny RNA (28), and stem structures of the *Bamboo mosaic virus* (BaMV) play an important role in the initiation of minus-strand RNA synthesis (4). In the 3′ UTR of LaMMoV RNA, we found hexanucleotide motif-like sequences (5′-ACCTAC) located 80 nucleotides downstream from the stop codon of the CP gene. Hexanucleotide sequences in the form of 5′-ACTTAA or 5′-ACCTAA have been found in almost all potexviruses, and similar sequences have been reported in PVX (5′-ACATAA) and PapMV (5′-ACTTAC) (20,28). Taken together, these studies identified conserved residues at position 1 (A), 2 (C), 4 (T), and 5 (A), although White et al. (28) showed that a nucleotide substitution at position 5 did not affect accumulation of viral RNA. Thus, it is possible that the 5′-ACCTAC sequence containing at least four conserved residues may be associated with synthesis of viral RNA. In contrast, the 3′ UTR of LaMMoV RNA did not contain a polyadenylation signal. In light of this, a structural analysis of the 3′ UTR of LaMMoV RNA should be carried out to identify sequences associated with RNA accumulation, and to compare LaMMoV with other potexviruses. Information obtained from such studies will be useful for designing effective control strategies.

In summary, our analyses indicated that LaMMoV is closely related to, but distinct from, AltMV and PapMV. Our results also suggested that these three viruses may share common ancestry. To our knowledge, this is the first report of sequence information for a cucurbit-infecting potexvirus. Sequence data and serological analyses indicated that LaMMoV is a new species of the genus *Potexvirus*.

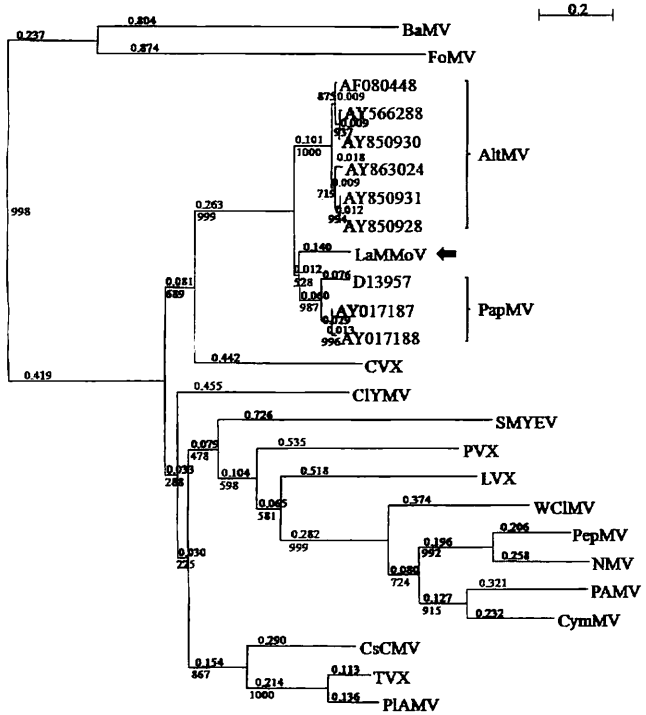

Fig. 2. Phylogenetic relationships among potexviruses based on coat protein amino acid sequences (Table 1). Tree was constructed using the neighbor-joining method as implemented in Clustal W (version 1.83). Numbers represent bootstrap values with 1,000 replications. Value on the scale bar represents 0.2 nucleotide substitutions per site. Virus species are designated by their abbreviation. Compound isolates were used for *Alternanthera mosaic virus* (AltMV) and *Papaya mosaic virus* (PapMV). GenBank accession numbers are shown.

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