

# Identification, Partial Sequencing, and Detection of a Potyvirus from *Kalanchoë blossfeldiana*

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Accepted for publication 9 October 1993.

## ABSTRACT

Husted, K., Bech, K., Albrechtsen, M., and Borkhardt, B. 1994. Identification, partial sequencing, and detection of a potyvirus from *Kalanchoë blossfeldiana*. *Phytopathology* 84:161-166.

Flexuous, filamentous virus particles with an average length of 724 nm and showing a characteristic green island mosaic pattern were observed in *Kalanchoë blossfeldiana*. The virus was sap inoculated from naturally infected *K. blossfeldiana* plants to *Chenopodium quinoa* and purified from these plants. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the capsid protein size was estimated to be 33 kDa. Of 14 plant species inoculated, only *C. quinoa* and *C. amaranticolor* showed local

symptoms. Immunosorbent electron microscopy showed the virus to be serologically unrelated to 10 other potyviruses. The virus was aphid transmitted. This ability was lost, however, after serial mechanical transmissions. The intact virions were used for antiserum preparation, and RNA was purified from virions for cDNA cloning and sequencing. A database search showed the virus to be closely related to but distinct from turnip mosaic potyvirus. On the basis of these investigations, it is concluded that the virus is a hitherto undescribed potyvirus, and the name *Kalanchoë mosaic potyvirus* is suggested. The prepared antiserum was very specific and sensitive in enzyme-linked immunosorbent assays, and this method is recommended for routine testing.

A number of viruses have been described from *Kalanchoë* spp. Among these are *Kalanchoë* top spotting bacilliform virus (19,25,26), *Kalanchoë* latent carlaviruses 1 and 2 (17,18), an isometric viruslike particle (21), a potyvirus (16,29), a strain of tomato bushy stunt tomosvirus (31), and tomato spotted wilt tospovirus (35).

However, the causal agent of a disease that has been called *Kalanchoë* green island mosaic, because of its characteristic symptoms on *Kalanchoë blossfeldiana*, has until now been unidentified, although it was shown that the agent was graft, sap, and aphid transmitted (29,30). Earlier attempts to identify the agent led to the detection of tomato bushy stunt virus, but this virus failed to produce the characteristic symptoms by reinfection experiments (N. Paludan, *personal communication*). This research project was initiated to identify the causal agent of green island mosaic in *Kalanchoë* and to establish a routine detection method. In this report, a virus isolated from *Kalanchoë* is described along with methods of detection. We propose the name *Kalanchoë* mosaic potyvirus (KMV).

## MATERIALS AND METHODS

### Plant materials, mechanical inoculation, and diagnostic species.

Naturally infected *Kalanchoë* plants were received from growers. As an initial assay method, healthy *K. blossfeldiana* 'Attraction' were top-grafted to cuttings from infected plants. Crude sap inoculum was prepared from diseased plants by grinding leaves in 0.03 M phosphate buffer, pH 7.6, containing 4% polyethylene glycol (PEG 6000) with Carborundum added as an abrasive. Extract from *K. blossfeldiana* was sap inoculated to leaves of indicator plants *Chenopodium quinoa* and healthy *K. blossfeldiana* 'Attraction.' To test for other diagnostic species, either extracts from infected *C. quinoa* plants or purified virus was inoculated to leaves of *C. amaranticolor*, *Cucurbita pepo*, *Nicotiana tabacum* 'Xanthi NN,' *N. tabacum* 'Samsun,' *N. tabacum* 'White Burley,' *N. benthamiana*, *N. occidentalis* 'P1,' *N. clevelandii*, *Phaseolus vulgaris*, *Brassica campestris*, *B. napus*,

*Philodendron selloum*, *Saponaria vaccaria*, *Pisum sativum*, and *Beta vulgaris*. Inoculated plants were observed for at least 1 mo in a greenhouse maintained at 20–30 C. Symptoms were monitored visually, and the virus was monitored with serological tests.

**Aphid transmission tests.** Aphid transmission tests were conducted with aphids (*Myzus persicae* [Sulzer]). Nonviruliferous aphids were allowed to colonize infected plants of *C. quinoa* or *K. blossfeldiana*. After an acquisition period of 15 min, 20 aphids were transferred either directly to *K. blossfeldiana* plants that were not shown by enzyme-linked immunosorbent assays (ELISA) to not contain the virus or to the nonhost *N. tabacum* 'Samsun' for 1 or 4 h before transfer to *K. blossfeldiana*. After an access period of 24 h, the insects were killed by an insecticide. Inoculated plants were observed for 1 mo and finally tested by ELISA.

**Virus purification.** *C. quinoa* plants, 2–3 wk postinoculation, were used as source plants. Leaves with local lesions were homogenized in 2 volumes of extraction buffer (0.5 M potassium phosphate buffer, pH 7.2, 10 mM EDTA, and 0.1% [w/v]  $\text{Na}_2\text{SO}_3$ ), filtered through two layers of cheesecloth, and extracted with 1/3 volume of  $\text{CHCl}_3$  and 1/3 volume of  $\text{CCl}_4$ . The extract was centrifuged for 5 min at 1,250  $g_{\text{max}}$  in a Centricon A 8.24 rotor. The supernatant was removed, and the organic phase was reextracted with 1 volume of buffer and centrifuged. The supernatants were pooled and centrifuged for 20 min at 12,000  $g_{\text{max}}$  in the Centricon rotor. The supernatant was then centrifuged for 2 h at 120,000  $g_{\text{max}}$  in a Kontron TST 28.38 rotor. The resulting pellet was resuspended in 0.2 M potassium phosphate buffer, pH 8.2, containing 0.1% (v/v)  $\beta$ -mercaptoethanol and subjected to an isopycnic centrifugation in CsCl (start concentration 35% [w/v]) for 20 h at 110,000  $g_{\text{max}}$  in a Kontron TST 41.14 rotor. Virions in the opalescent zone were collected, precipitated with 6% PEG, centrifuged for 10 min at 10,000 rpm in a microcentrifuge (Biofuge A, Heraeus, Osterode, Germany), and finally resuspended in 0.2 M potassium phosphate buffer, pH 8.2, containing 0.1% (v/v)  $\beta$ -mercaptoethanol (0.5 ml per 100 g of plant material). Glycerol was added to a final concentration of 50% (v/v), and the virus was stored at –20 C. The concentration of the virus preparation was determined by measurement of the absorbance value at 280 and 260 nm.

**Electron microscopy.** Preparations of purified virus and extracts of infected leaves were negatively stained with 4% aqueous uranyl

acetate, and particle length measurements were performed on 77 particles.

Extracts of infected leaves were analyzed by leaf-dip preparation with the immunosorbent electron microscopy (ISEM) technique with decoration (28). Staining was performed with 4% aqueous uranyl acetate. Antisera against a range of potyviruses were tested: bean common mosaic virus (BCMV) (E. Luisoni, Consiglio Nazionale delle Ricerche, Torino, Italy), bean yellow mosaic virus (BYMV) (M. Albrechtsen, Danish Institute of Plant and Soil Science, Lyngby), carnation vein mottle virus (CVMV) (E. Luisoni), celery mosaic virus (CeMV) (E. Luisoni), dasheen mosaic virus (DsMV) (F. W. Zettler, University of Florida, Gainesville), leek yellow stripe virus (LYSV) (L. Bos, Institute of Phytopathological Research, Wageningen, The Netherlands), onion yellow dwarf virus (OYDV) (L. Bos), pea seedborne mosaic virus (PSbMV) (M. Albrechtsen), potato virus Y (PVY) (M. Christensen, Danish Institute of Plant and Soil Science, Lyngby), and watermelon mosaic virus I (WMV1) (syn. papaya ringspot virus [PRSV]) (D. E. Purcifull, University of Florida, Gainesville). These antisera are routinely used for diagnostic purposes in our laboratory.

**Serology.** KMV antiserum was prepared by intramuscular injection of rabbits with the purified virus emulsified with Freund's incomplete adjuvant (1:1). For each immunization, 100–200  $\mu$ g of virus was used. The immunization was repeated at 3-wk intervals, and serum was obtained 10 days after each immunization, beginning after the second immunization. For the experiments described here, the serum obtained after five immunizations was used. The antiserum was tested in ISEM, dot immunobinding assay, Western blots, and double-antibody sandwich ELISA (DAS-ELISA). For DAS-ELISA, the immunoglobulin G (IgG) fraction was purified by ammonium sulfate precipitation and

coupled with alkaline phosphatase (Boehringer GmbH, Mannheim, Germany) essentially as described by Clark and Bar-Joseph (9). DAS-ELISA was performed by standard procedure (8) with a coating IgG concentration of 1  $\mu$ g/ml and an alkaline phosphatase-IgG conjugate dilution of 1:2,000.

**SDS-PAGE and immunoblotting.** Purified virions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 12.5% slab gels (23). Gels were stained for protein with Coomassie brilliant blue (7). For immunoblotting of either purified virions or extracts of *K. blossfeldiana* or *C. quinoa* leaves (extracted in 2 and 3 volumes [w/w] of sample buffer, respectively, and loading an equal amount of proteins), the gels were electroblotted as described by Towbin et al (37), except that polyvinylidene difluoride membranes (Immobilon, Millipore Corp., Bedford, MA) were used instead of nitrocellulose and the transfer buffer contained only 15% (v/v) methanol. The membranes were blocked with 10% (v/v) horse serum in TST buffer (0.05 M Tris, 0.5 M NaCl, 0.5% [v/v] Tween 20, pH 10.2) for 1 h. Immunolabeling was performed by incubating the membranes overnight at 4 C with the primary antiserum diluted in TST buffer (1:2,000) plus 5% (v/v) horse serum, washing them in three changes of TST buffer for 10 min each, incubating them for 3–4 h at room temperature with alkaline phosphatase-conjugated secondary antibody in TST buffer, and washing as before. The enzyme reaction was developed with 5-bromo-4-chloroindolyl phosphate (3).

Protein molecular weight markers for SDS-PAGE were obtained from Sigma Chemical Co., St. Louis, MO: phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), and soybean trypsin inhibitor (20.1 kDa).

**Nucleic acid preparations.** Molecular work was performed essentially as described by Maniatis et al (27), unless otherwise stated. RNA was extracted from purified virus by incubation of the virus preparations in 20 mM Tris, pH 7.5, 50 mM NaCl, 1% SDS, 1.5 mM EDTA, and 400  $\mu$ g of proteinase K per milliliter for 90 min at 37 C. After a phenol-chloroform extraction, RNA was precipitated with 2.5 volumes of ethanol in the presence of 250 mM NaCl, dissolved in double distilled H<sub>2</sub>O, and stored at –80 C.

**cDNA synthesis and cloning.** cDNA synthesis was carried out with the cDNA Synthesis System Plus kit (Amersham, Buckinghamshire, U.K.). The synthesis was performed as described by the manufacturer; either random hexanucleotide or oligo dT was used as a primer. The cDNA was blunt-end ligated into the plasmid vector pGEM-3Z cut with the restriction enzyme *Sma*I and dephosphorylated. Competent *Escherichia coli* DH5 $\alpha$  cells were transformed according to Hanahan (15). Plasmid DNA was isolated with the CTAB (cetyltrimethylammonium bromide) method modified from Del Sal et al (10) and analyzed for the presence of recombinant cDNA by *Eco*RI restriction endonuclease digestion and agarose gel electrophoresis.

**Sequence analysis.** Two oligo dT-primed cDNA clones (cDNA34 and cDNA43) with insert sizes of 1.8 and 1.4 kbp, respectively, were selected for sequencing. Subclones containing progressive unidirectional deletions of the two KMV cDNA fragments were obtained with the Erase-A-base system (Promega, Madison, WI). Dideoxynucleotide sequencing was performed with the Sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, OH). Sequences were analyzed by the WORDSEARCH, FASTA, and PILEUP programs from the Genetics Computer Group Sequence Analysis software package, version 7.0 (11).

## RESULTS

**Symptomatology and diagnostic species.** *K. blossfeldiana* 'Attraction' exhibited systemic green island symptoms 14–25 days after mechanical inoculation with sap from diseased *K. blossfeldiana* or *C. quinoa* or with purified virus (Fig. 1A). In some cases, the symptoms were a more diffuse chlorotic mosaic. *C. quinoa* showed more distinct and larger local chlorotic spots 8–14 days after inoculation, and *C. amaranticolor* showed smaller chlorotic

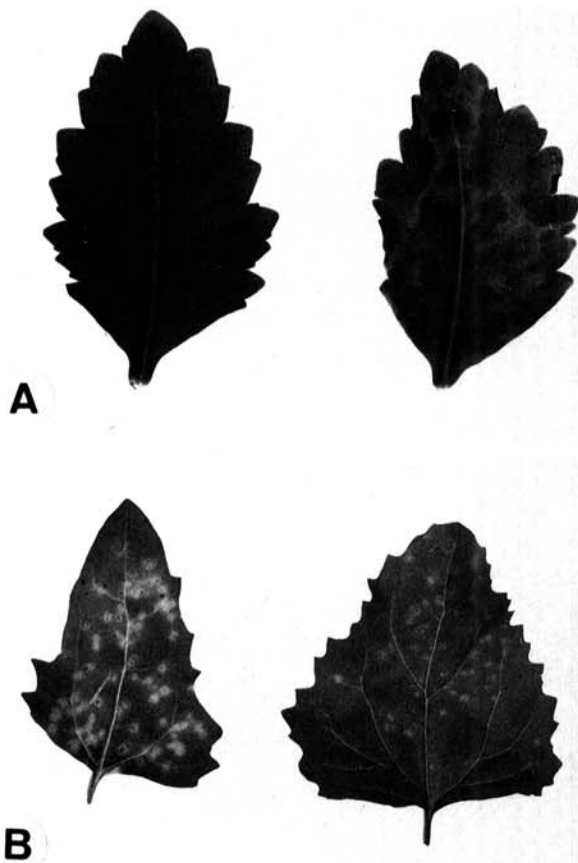


Fig. 1. Symptoms of Kalanchoë mosaic potyvirus 25 days postinoculation. A, Healthy (left) and systemically infected (right) *Kalanchoë blossfeldiana* 'Attraction'; B, locally infected *Chenopodium quinoa* (left) and *C. amaranticolor* (right).

spots. There were always fewer lesions on *C. amaranticolor* than on *C. quinoa* (Fig. 1B). The lesion centers became necrotic and were surrounded by mildly chlorotic tissue, which eventually became totally necrotic on *C. quinoa*. Sap transmission of KMV from *K. blossfeldiana* to *C. quinoa* gave rise only to a few lesions, but further sap transmission from these lesions to *C. quinoa* resulted in an increased number of lesions. Systemic infection in *C. quinoa* or *C. amaranticolor* was not detected. Mechanical inoculation of *C. pepo*, *N. tabacum* 'Xanthi NN,' *N. tabacum* 'Samsun,' *N. tabacum* 'White Burley,' *N. benthamiana*, *N. clelandii*, *N. occidentalis* 'P1,' *P. vulgaris*, *P. sativum*, *B. campestris*, *B. napus*, *P. selloum*, *S. vaccaria*, and *B. vulgaris* with either sap from KMV-infected *C. quinoa* or purified virus gave no symptoms, and no latent infections were revealed by serological tests.

**Aphid transmission tests.** The aphid species *M. persicae* has been used to transmit the virus in a nonpersistent way to *K. blossfeldiana* from infected plants of *C. quinoa* and *K. blossfeldiana*. Symptoms appeared 2–3 wk after transmission and appeared earlier when transmitted from *K. blossfeldiana*. No transmission was seen when aphids were transferred to a nonhost for 1 or 4 h before transfer to *K. blossfeldiana*, and there was no transmission to *N. tabacum*. The virus could not be transmitted when the aphid transmission test was made with a KMV isolate that had been mechanically transferred several times.

**Virus purification.** The virus was purified from locally infected plants of *C. quinoa*, because the only systemically infected test plant was *K. blossfeldiana* 'Attraction.' The virus yield was 3–5 mg per 100 g of plant material. Analysis of the purified virus by SDS-PAGE showed only one protein ( $M_r$  of 33 kDa), presumed to be the capsid protein (CP) (Fig. 2A). The virus preparation was used directly for production of KMV antiserum in rabbits.

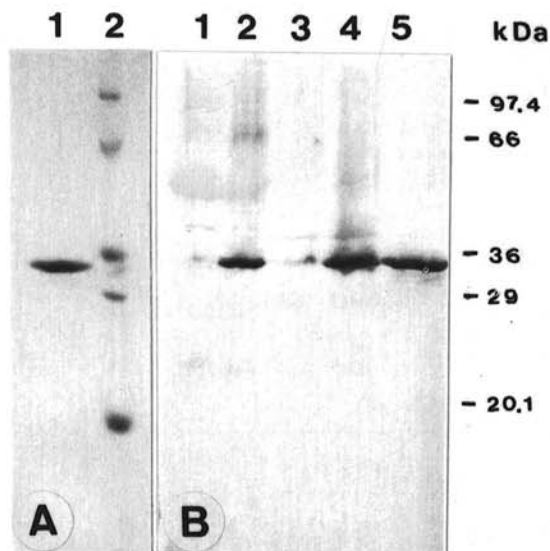
**Electron microscopy.** Flexuous, filamentous particles were consistently observed by electron microscopy in negatively stained purified virus preparations and in leaf extracts infected with KMV (Fig. 3A). Seventy-seven particles were measured. The mean length was 724 nm (sample standard deviation 71 nm); 91% of the measured particles were 625–825 nm.

**Serology.** In Western blots of KMV-infected *K. blossfeldiana* or *C. quinoa* plants, the KMV antiserum recognized a band corresponding to the virus CP. However, the sensitivity was quite

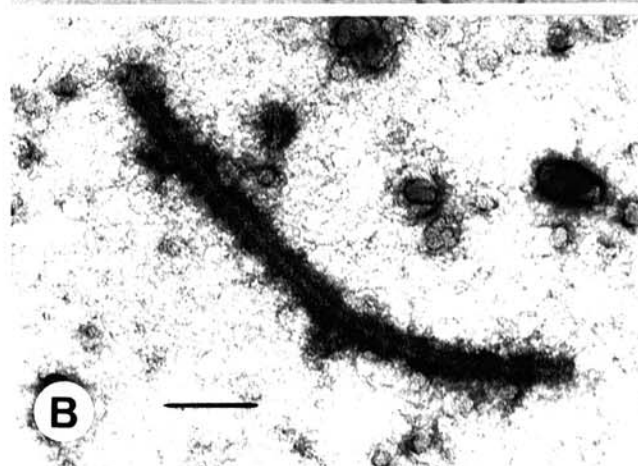
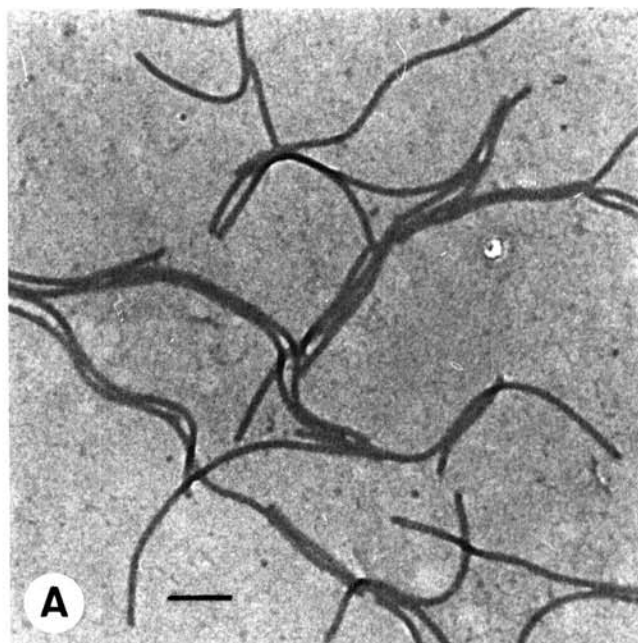
low; the result was negative for some plants, which by ELISA were shown to be weakly infected. Occasionally, the antiserum faintly recognized a protein in healthy plants of *K. blossfeldiana* and *C. quinoa* with a size similar to that of the CP (Fig. 2B).

In ISEM, the antiserum efficiently trapped and decorated KMV particles (Fig. 3B). Antisera raised against 10 other potyviruses were tested but did not react with KMV particles. In DAS-ELISA, there was no cross-reaction to healthy plant sap of either *K. blossfeldiana* or *C. quinoa*, and no cross-reaction was seen with Kalanchoë latent virus-infected *K. blossfeldiana*, BYMV-infected *N. benthamiana*, PVY<sup>N</sup>-infected *N. tabacum* 'Xanthi-NN,' or PSbMV-infected *P. sativum*. The virus could be detected in plant sap from infected *K. blossfeldiana* at a dilution of 1:3,125, and 1–2 ng of purified KMV could be detected.

**Molecular analysis of viral cDNA clones.** The C terminal portion of the KMV polyprotein and the complete 3' untranslated region are shown in Figure 4. This sequence was obtained by sequencing two oligo dT-primed cDNA clones, cDNA34 and cDNA43, and derived unidirectional subclones in both directions. The dipeptide QS located at amino acid position 45–46 has been selected as a putative cleavage site for the coat protein of KMV because cleavage of potyviral polyproteins has been shown to take place mainly between the dipeptide sequence Q/A, Q/S, or Q/G, and exceptionally between Q/V in the case of LMV



**Fig. 2.** A, Analysis of purified Kalanchoë mosaic potyvirus (KMV) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; lane 1, purified KMV capsid protein with an estimated size of 33 kDa; lane 2, protein markers (from bottom to top) 20.1, 29, 36, 66, and 97.4 kDa. B, Immunoblot of purified KMV and leaf extract with KMV antiserum; lane 1, healthy *Chenopodium quinoa*; lane 2, KMV-infected *C. quinoa*; lane 3, healthy *Kalanchoë blossfeldiana*; lane 4, KMV-infected *K. blossfeldiana*; lane 5, purified KMV.



**Fig. 3.** Particle morphology and immunosorbent electron microscopy of Kalanchoë mosaic potyvirus (KMV). A, Particles of KMV in a purified virus preparation negatively stained with uranyl acetate; B, particles of KMV trapped and decorated by KMV antiserum. Bar = 100 nm.

(12). Cleavage at the selected dipeptide generates a coat protein 32 kDa in size, which correlates well with the size of the CP determined by SDS-PAGE (33 kDa). No other potential cleavage site of the KMV polyprotein that corresponds to 38 kDa is present in the deduced amino acid sequence shown in Figure 4. Alignment of the deduced KMV CP sequence with the coat protein sequence of other potyviruses further supports this conclusion (data not shown).

A search of the EMBL data base with the nucleic and amino acid sequence of KMV CP showed the highest similarity with turnip mosaic potyvirus (TuMV) (72.2%) (38). Besides the TuMV CP, the nine most closely related CPs showed similarities of 60–63%: clover yellow vein virus (CIYVV) (39), bean yellow mosaic virus (BYMV) (4), potato virus Y (PVY) (36), pepper mottle virus (PepMoV) (13), pepper severe mosaic virus (PSMV) (32), lettuce mosaic virus (LMV) (12), plum pox virus (PPV) (24), papaya ring spot virus (PRSV) (41), and pea seedborne mosaic virus (PSbMV) (22). A dendrogram for the 11 CPs analyzed is shown in Figure 5.

## DISCUSSION

KMV was found to have the properties of a potyvirus. The virus has filamentous particles with a mean length of 724 nm

and a CP size of 33 kDa (6,20). Heaton (16) and Paludan (29) have mentioned a potyvirus in *Kalanchoë* that causes mild mosaic, but this is the first paper to give a detailed characterization of such a virus. Several attempts to identify the potyvirus as one of the already known potyviruses were performed: symptom expression in diagnostic hosts, ISEM with antisera against various potyviruses, and sequence analysis.

Diagnostic species for KMV were found to be very limited. In addition to systemically infected *K. blossfeldiana* 'Attraction,' *C. quinoa* and *C. amaranticolor* were infected, but only locally. Only two previously described potyviruses cause symptoms in *C. quinoa* and *C. amaranticolor* without causing systemic infection or infecting one or more of the other diagnostic species included in our tests: iris mild mosaic virus (IMMV) and onion yellow dwarf virus (OYDV) (2,5,14,34). Of these, antiserum against OYDV did not react with KMV in ISEM, and IMMV normally produces only a few circular necrotic lesions in *C. amaranticolor*; the lesions are different from those seen for KMV (5).

Antisera against potyviruses often show some cross-reaction to other potyviruses; the degree of cross-reaction depends on the extent to which the antibodies recognize the core protein region or the more virus-specific N terminus (40). The cross-reaction is generally more pronounced in Western blots than in ELISA, because the conserved core region is exposed in Western blots but is exposed only to a very limited extent in ELISA. ISEM

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1 TATATAGCGGAGAGCGCTCTAAGGAACTATACTGGATAAAGAGCCAGAAAATAATGAA
1 Y I A E S A L R K L Y L D K E P E N N E

61 ATTATCCGCTATCTGGATGCGATTTTCGAGGAGTTTGACAACGAGATGACACTCAGTAT
21 I I R Y L D A I F E E F D N E D D T Q Y

121 GATGTCTATCACCAATCTGGAACGGCAGAAGCTCGATGCTGAACGCAATGATAATCAGAAG
41 D V Y H Q S G T A E L D A E R N D N Q K
      ▲

181 AAACAAGACTCGAAGAATAAAGAGAGCATGGTAGTTCGCGCTCTGCGGATGTGGATAAA
61 K Q D S K N K E S M V V S R S A D V D K

241 CCTGAGTCATCAGGATAGGCAATTGAACACTGATAGAGACGTCACGCGAGCACAAT
81 P E S S R V G Q L N T D R D V N A G T T

301 GGTACGTTCTCAGTGCCAAAGACTCAAGGACTCGCCAGCAAAATGAATCTACCAAAATAT
101 G T F S V P R L K G L A S K M N L P K Y

361 AAAGGTAATTCAGCTTAAACCTGGATCATTGCTAGGTTACACCCCTAACCAAGTGGAC
121 K G N S A L N L D H L L G Y N P N Q V D

421 ATTTCAAACACACGTCGCAACGCATGCGCAATTAACACTGGTATGAGGGTGTAAAGAG
141 I S N T R A T H A Q F N T W Y E G V K E

481 GATTATGAATGTTGGATGACAAAATCAAATCATTCTCAATGATGATTAATGGTCTGGTGC
161 D Y E L L D D K M Q I I L N G L M V W C

541 ATTGAGAATGGAACCTCTCCAAACATAAAGCGTATGTTGGTATGATGGATGGGGACGAG
181 I E N G T S P N I N G M W V M M D G D E

601 CAAGTCGAGTTTCTCATCAAAACATTAATGATCAGCCAAACCCACATTTAGGCAGATA
201 Q V E F P I K P L I D H A K P T F R Q I

661 ATGGCCATTTTCAGTAACGTAGCTGAAGCGTATATTGAAAAGCGTAACCAAGACCGTCCA
221 M A H F S N V A E A Y I E K R N Q D R P

721 TATATGCCACGATATGGACTTCAGCGCACTTGACCGACATGAGCTTAGCCCGCTATGCT
241 Y M P R Y G L Q R N L T D M S L A R Y A

781 TTCGACTTCTATGAGATGACTTCAAAAACCTCTGTTCGAGCAGAGAAGCACACATTCAG
261 F D F Y E M T S K T P V R A R E A H I Q

841 ATGAAAGCAGCGCATTGCGAAGCGCCAAAATAATTTATTGGATTAGATGGCAACGTC
281 M K A A A L R S A K N N L F G L D G N V

901 GGCACAACGGAAGAGAACACCGGAAAGGCACACCACTGAAGATGTTAATCAGAAGATGCAT
301 G T T E E N T E R H T T E D V N Q K M H

961 AGTCTCCTTGGCGTAAGGGGATGTAATCTTGTGTGCCAGTACCAACAATAATGTTAC
321 S L L G V R G M *

1021 CGTACTCGTTAGTATTCACCTTCCTTAGAGCTATTTTATTATGATCATAGGTAGCCAGAT

1081 GGGTTTTACCAGCGTGCCTCCCTCGTCTAATATATTAGTGTCCGAACAGTTATTGGGC

1141 ATACTTGGTGGTGGTGGTCTTTGGCTCGTATACCTTATGCTTTGATAATGTCAAGGG

1200 Poly(A)

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Fig. 4. The nucleotide sequence of the 3'-terminal regions of *Kalanchoë* mosaic potyvirus and the deduced amino acid sequence. ▲ = The cleavage site for the coat protein; \* = the stop codon. The DAE sequence is underlined.

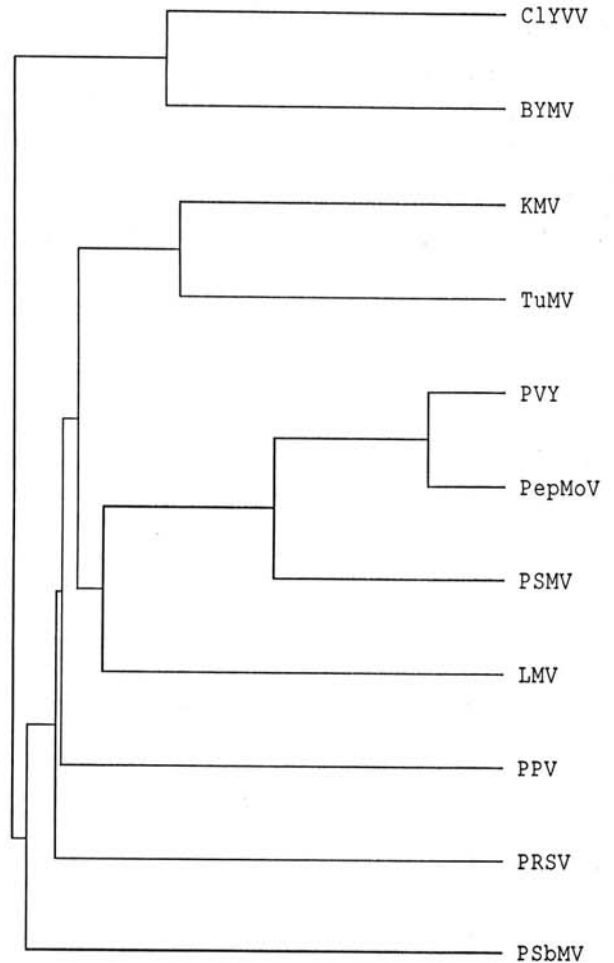


Fig. 5. Sequence relationship of the total coat protein amino acid sequence of *Kalanchoë* mosaic potyvirus (KMV) with the 10 potyviruses showing highest similarity to KMV. Sequences were analyzed by the PILEUP program from the Genetics Computer Group Sequence Analysis software package, version 7.0 (11). CIYVV = clover yellow vein virus; BYMV = bean yellow mosaic virus; TuMV = turnip mosaic virus; PVY = potato virus Y; PepMoV = pepper mottle virus; PSMV = pepper severe mosaic virus; LMV = lettuce mosaic virus; PPV = plum pox virus; PRSV = papaya ring spot virus; and PSbMV = pea seedborne mosaic virus.

failed to indicate any close serological relationship between KMV and a wide range of potyviruses, including TuMV.

Taken together, the limited number of host species for KMV and the result of the serological tests make it highly unlikely that KMV is identical to any of the previously known potyviruses. However, to support this conclusion, the sequence of the KMV CP was determined and compared to the previously sequenced potyviral CPs.

According to Ward and Shukla (40), the similarities of the complete CP amino acid sequence are 38–71% for distinct members of the potyvirus group and 90–99% between strains of the same virus. Thus, the sequence analysis showed KMV to be distinct from all the previously sequenced potyviruses, since the highest sequence similarity observed was 72.2% with the CP of TuMV.

The dendrogram produced by the program PILEUP is in accordance with dendrograms produced by the program CLUSTAL for the CP amino acid sequences of 40 potyviruses (33). The suggestion that KMV should be placed on a branch with TuMV is obvious and thereby diminishes the distance of TuMV from other potyviruses (33).

According to the CP sequence, KMV is not expected to be aphid-transmissible, since it does not have the DAG sequence in the N terminal portion of the CP typical for aphid-transmissible potyviral strains (1). Instead, it has the sequence DAE similar to the non-aphid-transmissible variant of tobacco vein mottling virus (TVMV-NAT) (1). Therefore, one can speculate on the reason for the successful aphid transmission tests seen in most cases. Between the first aphid transmission tests and the later ones, the KMV isolate was passed through repeated serial transmissions as well as through purification for cDNA cloning. It is therefore obvious to propose a loss of transmissibility because of specific propagation of the non-aphid-transmissible variant. This variant might have been present in the original KMV-infected *Kalanchoë* plant, or it could be the result of a single point mutation (GAA to GGA). This possibility is now under investigation.

From the data presented in this paper, we conclude that the potyvirus infecting *K. blossfeldiana* is probably a hitherto undescribed potyvirus. We propose the name *Kalanchoë* mosaic potyvirus (KMV).

So far, identification of KMV has been performed by sap transmission or by grafting healthy *K. blossfeldiana* 'Attraction' to infected cuttings (30). These methods, however, are labor intensive, and the symptom expression requires from 3 wk to 2 mo. For these reasons, an alternative routine testing method is greatly needed. Since the ELISA test shows no cross-reaction to healthy *K. blossfeldiana* and has high specificity and sensitivity, the method is recommended for routine testing.

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