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The complete nucleotide sequence of *Alternanthera mosaic virus* infecting *Portulaca grandiflora* represents a new strain distinct from phlox isolates

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Abstract A southeastern European isolate of *Alternanthera mosaic virus* (AltMV-MU) of the genus *Potexvirus* (family *Flexiviridae*) was purified from the ornamental plant *Portulaca grandiflora*. The complete nucleotide sequence (6606 nucleotides) of AltMV-MU genomic RNA was defined. The AltMV-MU genome is different from those of all isolates described earlier and is most closely related to genomes of partly sequenced portulaca isolates AltMV-Po (America) and AltMV-It (Italy). Phylogenetic analysis supports the view that AltMV-MU belongs to a new "portulaca" genotype distinguishable from the "phlox" genotype.

Keywords Potexvirus · *Portulaca grandiflora* · Trailing portulaca · Phylogenetic analysis · Strain

In the past decade, potexviruses infecting the ornamentals creeping phlox (*Phlox stolonifera*) and trailing portulaca (*Portulaca grandiflora*) have been described [3–6]. One of these viruses was purified from *Alternanthera pungenes* (*Amaranthaceae*) in Australia and was named "*Alternanthera mosaic virus*" (AltMV) [4]. A few years later, another potexvirus (originally called phlox potexvirus) was identified in the United States as an AltMV isolate (AltMV-PA), and the complete nucleotide sequence of the AltMV-PA genome was determined [5]. Serological analysis showed that this virus was closely related to the *Papaya mosaic virus* (PapMV) [11], but the sequencing of genomic RNA revealed a significant difference between the new virus and PapMV at the nucleotide and amino acid levels. Four biologically active full-length cDNA clones of another phlox isolate AltMV-SP (from *Phlox stoloniphera* cv. Sherwood purple) were recently described [7]. All clones were infectious in *Nicotiana benthamiana* with symptoms differing from those of each other and those of AltMV-SP. Two clones were fully sequenced; both sequences differ from the partial 3′-terminal AltMV-SP sequence previously published [6]. Potexvirus from symptomless portulaca plants (AltMV-Po) was isolated and characterized by the same laboratory; the nucleotide sequence of the region including two genes from the so-called triple gene block (TGB2, TGB3), the coat protein (CP) gene, and the 3′-non-translated region (3′-NTR) was reported [5, 6]. Another partial 3′-terminal sequence including the CP gene and 3′-NTR of the portulaca isolate of *Alternanthera mosaic virus* (AltMV-It) came from Italy in 2004 [3]. But the complete sequence of the portulaca strain of AltMV was not available until now. The full nucleotide sequence of a new isolate AltMV (AltMV-MU) from Europe is presented here.

The initial infected material was received from the German Collection of Microorganisms and Cell Cultures, Braunschweig. This isolate is serologically related to PapMV and was, therefore, misdiagnosed there as a strain of PapMV. The exact region of provenance remains unknown, but the approximate origin was described as South-Eastern Europe. We propagated this virus in *P. grandiflora*, defined its host range and performed a sequence analysis.

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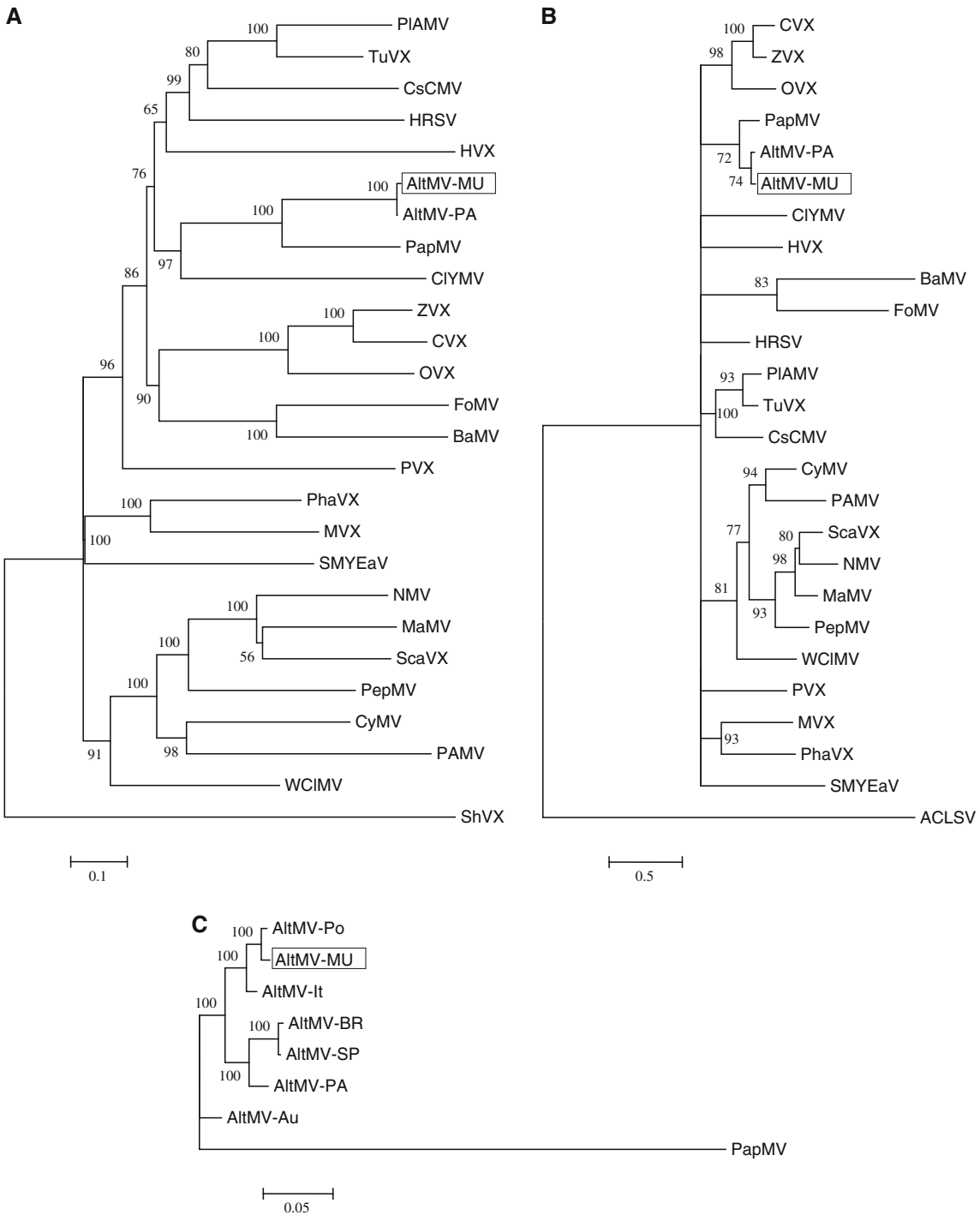

Virus was purified from systemically infected leaves using the method described for PVX isolation [2] with some modifications. The infected material was homogenized in 50 mM Tris–HCl, 10 mM EDTA buffer pH 8.0. The viral cDNA was synthesized, cloned, and sequenced in two steps. First, some constructs were obtained by random priming followed by a conventional reverse transcription reaction in order to clone double-stranded cDNA and obtain the initial sequence. Two sequences overlapped each other and represented an RNA-dependent RNA-polymerase gene (nucleotides 2107–3654). It became clear that the sequence is original and is closely related to the *Alternanthera mosaic virus* (AltMV-PA from Pennsylvania, United States) infecting creeping phlox (*Phlox stolonifera*) [5]. Four additional sets of RT-PCR clones obtained with specific primers covering nucleotides 223–5563 were

◀ **Fig. 1** Phylogenetic analysis of aligned amino acid sequences of **a** the RNA-dependent RNA-polymerase (RdRp) and **b** the CP genes of different potexviruses after bootstrapping for 1000 replicates: *Alternanthera mosaic virus* (phlox isolate), AltMV-PA, Accession number AY863024; *Bamboo mosaic virus*, BaMV, D26017; *Cactus virus X*, CVX, AF308158; *Cassava common mosaic virus*, CsCMV, U23414; *Clover yellow mosaic virus,* ClYMV, D29630; *Cymbidium mosaic virus*, CyMV, U62963; *Foxtail mosaic virus*, FoMV, M62730; *Hosta virus X*, HVX, AJ620114; *Hydrangea ringspot virus*, HRSV, AY707100; *Malva mosaic virus*, MaMV, DQ660333; *Mint virus X*, MVX, AY789138; *Narcissus mosaic virus*, NMV, D13747; *Opuntia virus X*, OVX, AY366209; *Papaya mosaic virus*, PapMV, D13957; *Pepino mosaic virus,* PepMV, AF484251; *Phaius virus X*, PhaVX, AB353071; *Plantago asiatica mosaic virus*, PlAMV, Z21647; *Potato aucuba mosaic virus*, PAMV, S73580; *Potato virus X*, PVX, X05198; *Scallion virus X*, ScaVX, AJ316085; *Strawberry mild yellow edge virus*, SMYEaV, D12517; *Tulip virus X*, TuVX, AB066288; *White clover mosaic virus*, WClMV, X06728; *Zygocactus virus X*, ZVX, AY366208. AltMV-MU indicates portulaca isolate of *Alternanthera mosaic virus*, FJ822136. RdRp gene of *Shallot Virus X* (ShVX, M97264) and CP gene of *Apple chlorotic leaf spot virus* (ACLSV, M58152) were taken as outgroup sequences. **c** Phylogenetic tree constructed to compare CP nucleotide sequences of different phlox and portulaca isolates of *Alternanthera mosaic virus*. AltMV-BR (AY850928) and AltMV-SP (AY850931) indicate Maryland phlox isolates, AltMV-PA—Pennsylvania phlox isolate (AY863024). AltMV-Po (AY850930) and AltMV-It (AY566288) show sequences of portulaca isolates from the United States and Italy. AltMV-Au designates Australian isolate (AF080448). The CP gene of *Papaya mosaic virus* (PapMV, D13957) was used as the phylogenetic outgroup. Analysis was performed by the ClustalW 1.83 and PHYLIP 3.67 packages with visualization by the MEGA 4.0.1 program [12]. Trees were constructed by a neighbor-joining algorithm, except the tree in **c**, which was constructed by maximum likelihood algorithm. Nodes with less than 50% bootstrap support were collapsed to polytomies

analyzed. The 5′-terminal (nt 1–307) and 3′-terminal (nt 5479–6606) sequences were cloned, respectively, using the 5′-RACE method and the oligo(dT) primer. As a rule, two independent clones representing each part of the genome were sequenced. In case of need, a third clone was looked at to resolve discrepancies at specific positions and the RT-PCR product was directly sequenced to decrease the possible influence of PCR mistakes. Thus, each questionable nucleotide in the final sequence was determined and confirmed at least twice. Comparison with several other 3′-terminal sequences showed that the new isolate was more similar to (98.8% of identity) but still different from the partial sequence recently reported for AltMV-Po from portulaca [6] (American isolate, Maryland).

The complete AltMV-MU genome has been deposited in GenBank under accession number FJ822136. It consists of 6606 nucleotides (nt). The index of similarity at the nucleotide level to the complete sequence of AltMV-PA is 94.0%. The 5′-non-translated region (NTR) is 94 nt long, 3′-NTR (124 nt excluding the poly(A) tail) is 2 nt shorter than 3’-NTR of AltMV-PA and has the same length as AltMV-Po. Computer analysis revealed five major open reading frames (ORF) that are very much like other known potexviruses [13]. The 5′-proximal ORF (nt 95–4720) codes for a polypeptide of 1541 amino acids (aa) with a predicted molecular weight of 174 kDa. Phylogenetic analysis demonstrates that it is closely related to the RNA-dependent RNA-polymerases of PapMV [11] and AltMV-PA [5] (see Fig. 1a). The putative triple gene block (TGB) responsible for cell-to-cell and systemic virus movement [10] is encoded by ORF2 (TGB1 protein, 232 aa, 26 kDa, nt 4704–5402), ORF3 (TGB2, 110 aa, 12 kDa, nt 5356–5688) and ORF4 (TGB3, 63 aa, 7 kDa, nt 5624–5815). The 3′-proximal ORF5 is located downstream of ORF4 without overlapping (nt 5859–6482). Apparently, ORF5 codes for the viral CP (207aa, 22 kDa).

A graphic phylogenetic tree of potexvirus CPs was constructed (see Fig. 1b). The alignment of the CP amino acid sequences of PapMV and several isolates of AltMV shows that the CP of portulaca isolates (AltMV-MU, AltMV-Po, and AltMV-It) are almost identical to each other at the amino acid level (data not shown). The only differences are located at the positions 106 (methionine to isoleucine) and 185 (serine to phenylalanine). The amino acid sequences of AltMV-MU and the main phlox isolate AltMV-PA are more distinguishable; 12 changes were revealed, mostly in the N-terminal half of the protein. The index of similarity between AltMV-MU and PapMV CPs is 75.7% as calculated by the Lipman–Pearson method [8]. An additional phylogenetic tree calculated between seven known AltMV CPs and PapMV showed that the CP genes belonging to portulaca isolates and the CP genes from several phlox isolates might be separated into two main subgroups (see Fig. 1c).

Table 1 Index of similarity between AltMV-MU, two other isolates of *Alternanthera mosaic virus* and *Papaya mosaic virus* (PapMV) calculated by the Martinez-NW method [9] of the MegAlign 7.1.0 program [1] from the Lasergene 7 package

Genes and regions	AltMV-PA	AltMV-Po	PapMV
5′-NTR	96.8	Unknown	60.4
Replicase	93.5	Unknown	51.6
TGB1	94.4	Unknown	40.1
TGB2	96.7	99.1	57.3
TGB3	95.3	99.0	27.1[a]
CP	95.0	99.0	69.9
3′-NTR	98.4	98.4	68.2

The AltMV-MU sequence was taken as a matrix for comparison (100%); the numbers show the percentage of identity. *AltMV-PA*—the phlox isolate, *AltMV-Po*—portulaca isolate from the United States. [a] Similarity was calculated by the Wilbur–Lipman method [14] because the main method did not indicate sufficient similarity for an alignment

The results of analyzing the nucleotide sequence similarities between AltMV-MU, AltMV-PA, AltMV-Po, and PapMV are presented in Table 1. It can be seen that AltMV-MU is closely related to partly sequenced portulaca strains AltMV-Po (American) and AltMV-It (Italian, data not shown). The phylogenetic trees of the potexvirus RdRp and CP amino acid sequences show that the *Papaya mosaic virus* remains the closest potexvirus to AltMV at both the amino acid (Fig. 1a, b) and the nucleotide levels (51.9% of identity to AltMV-MU). But the degrees of similarity between AltMV-MU and PapMV for individual genes vary significantly from 27.1% (TGB3) to nearly 70% (CP and 3′-NTR). Our comparisons indicate that *Alternanthera mosaic virus* represents a group of potexvirus species distinct from PapMV.

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