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Annotated Sequence Record

Partial sequencing of the genomic RNA of Araujia mosaic virus and comparison of the coat protein sequence with those of other potyviruses

B. Massey[1], X. Cui[2], E. Hiebert[3], M. S. Elliott[3], N. Waipara[1], L. Hayes[2], R. Charudattan[3]

[1] Landcare Research, Auckland Mail Centre, Auckland, New Zealand
[2] Landcare Research, Lincoln, New Zealand
[3] Department of Plant Pathology, University of Florida, Gainesville, FL, USA

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Introduction

Araujia mosaic virus (ArjMV, genus *Potyvirus*, family *Potyviridae*) was first reported in *Araujia angustifolia* and *Morrenia odorata* from Argentina [8]. It is transmitted by mechanical inoculation and through transmission by *Aphis nerii*, *A. spiraecola*, and *Myzus persicae*. ArjMV has a highly restricted host range, manually infecting only six genera in the *Asclepiadaceae* (*Araujia*, *Cynanchum*, *Hoya*, *Matelea*, *Morrenia*, and *Sarcostemma*) [9]. ArjMV capsid protein (CP) reacts with antisera to CPs of several potyviruses including dasheen mosaic virus (DsMV), soybean mosaic virus (SMV), tobacco etch virus (TEV), tobacco vein mottling virus (TVMV), watermelon mosaic virus (WMV; previously known as WMV 2), and papaya ringspot virus type W (PRSV-W; previously known as WMV 1). Except for the viral protein sizes [14], no other information is available on the molecular properties of ArjMV.

Correspondence: Dr. Xianlan Cui, Landcare Research, PO Box 40, Lincoln 7640, New Zealand
e-mail: cuix@landcareresearch.co.nz

Moth plant (*Araujia sericifera*) is a troublesome environmental weed in northern regions of New Zealand. Due to its rapid spread, its ability to cause substantial damage to natural ecosystems, and the increasing cost of conventional control methods, a biological control programme was initiated in New Zealand. A survey in South America found that ArjMV was widespread throughout most of the plant's range and that populations of *Aphis nerii*, a known vector of *Araujia* viruses [23], were frequently observed on plants exhibiting mosaic symptoms [24]. Because ArjMV has a highly restricted host range, Landcare Research has been exploring the potential for using ArjMV for the biocontrol of the moth plant in New Zealand, and to assess its specificity, it is being tested on a large range of plants that are of interest to New Zealand. In this study, we report for the first time the 3′ terminal region of the genomic RNA of ArjMV.

Provenance of the virus material

Diseased specimens of *Araujia angustifolia* and *Morrenia odorata* were transported in 1976 from Argentina to Gainesville, Florida, where, under

quarantine, they were processed for inoculation and electron microscope examinations. The causative agent (ArjMV) was tentatively described as a new member of the potyvirus group on the basis of particle morphology, aphid vector transmission, and induction of characteristic cylindrical inclusions in infected tissues [9]. It was then further characterized as a distinct member of potyvirus group based on reactivity of in vitro-translated ArjMV proteins with antisera against other potyviruses' proteins [14].

In this study, total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) from *M. odorata* leaves harvested 2 weeks after the plants were manually inoculated with ArjMV strain ARG1973 in a biosafety level 2 quarantine greenhouse. First-strand cDNAs were synthesized using SuperScript reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and M4T (5′-GTT TTC CCA GTC ACGAC (T)15-3′) as the initial primer [10]. Primers 5′-GGN AAY AAY AGY GGN CAR CC-3′ (N = A, G, C, or T; Y = T or C; R = A or G) and 5′-GTT TTC CCA GTC ACG AC-3′ [10] and *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) were used to amplify a ~1.7-kb fragment from the cDNA of ArjMV RNA. The PCR product was purified from agarose gel using a QIAEX II gel extraction kit (QIAGEN, Chatsworth, CA, USA) and cloned into pCR®-XL-TOPO® vector (TOPO XL PCR Cloning Kit; Invitrogen, Carlsbad, CA, USA). One Shot TOP10 chemically competent cells were transformed with the plasmid containing the cloned fragment, according to the manufacturer's procedures. Two separated colonies were grown overnight in culture medium and the DNA extracted for sequencing using M13 forward and reverse primers. The resultant sequences were analyzed using EditSeq software of the Lasergene Software Suite (Windows 4.05, DNA star, Madison, WI, USA). The nucleotide and amino acid sequence alignments were performed with MegAlign of the Lasergene Software Suite, and a phylogenetic tree was constructed using PAUP* 4.0 (Version b10), Phylogenetic Analysis Using Parsimony (and Other Methods) [22]. Parsimony analysis was performed with the tree-bisection-reconnection (TBR) branch-swapping algorithm. The initial tree was obtained by neighbor-joining. One thousand bootstrap replicates were run for the protein tree using a heuristic search and retaining groups with a frequency of greater than 50%.

Sequence properties

The nucleotide and amino acid sequences of the partial NIb protein and the complete coat protein (CP) and the nucleotide sequence of the 3′ untranslated region (UTR) of ArjMV were deposited in GenBank (Accession no. EF710625). These continuous regions consist of a single open reading frame of 1413 nucleotides followed by an UTR of 291 nucleotides and a poly-A tail. The partial NIb gene codes for 207 amino acids of the carboxy terminal region of the NIb protein, whereas the full-length CP gene codes for a protein of 264 amino acids that is similar to the molecular weight of CP as estimated by gel analysis [14]. The cleavage site between NIb and CP was predicted to be located between amino acids Q and A as determined by sequence comparisons with other potyviruses. The NIb/CP cleavage site has been shown to be located at the junction of amino acids Q/A, Q/S, or Q/G in other potyviruses [1].

The nucleotide and amino acid sequences of ArjMV were aligned with the corresponding regions of other potyviruses accessed from the GenBank database (Table 1). As proposed by Shukla and Ward [19], a 38–71% (average 54%) sequence identity between two viruses indicates that they belong to distinct species, while a 90–99% (average 95%) sequence identity indicates that they are related strains. The 80% CP amino acid identity was proposed to be a key criterion for species demarcation [2, 7]. The CP of ArjMV exhibits 58–69.7% amino acid identities to those of the other potyviruses (Table 1), indicating that ArjMV is a member of the genus *Potyvirus* of the family *Potyviridae*. The relatively low (59.8–70.8%) nucleotide sequence identities of ArjMV CP to those of the other potyviruses (Table 1) add further confidence to the above classification according to Adams et al. [2].

The phylogenetic tree of the CP amino acid sequences of potyviruses (Fig. 1) shows that ArjMV

Table 1. Nucleotide and amino acid identities of the capsid protein and nucleotide homology of the 3′ UTR region of ArjMV with those of other potyviruses

	ArjMV CP amino acid	ArjMV CP nucleotide	ArjMV 3′UTR nucleotide	Accession number
1. Araujia mosaic virus (ArjMV)	–	–	–	–
2. Bidens mosaic virus (BiMV)	64.9	66.2	42.6	AY960150
3. Chilli veinal mottle virus (ChiVMV)	68.3	65.8	36.4	NC_005778
4. Colombian datura virus (CDV)	68.6	62.8	30.4	AJ437482
5. Dasheen mosaic virus (DsMV)	65.7	63.3	28.8	AF048981
6. Henbane mosaic virus (HMV)	68.7	64.2	28.0	AM184113
7. Japanese yam mosaic virus (JYMV)	65.7	68.6	25.9	AB016500
8. Narcissus late season yellows virus (NLSYV)	67.7	66.7	29.4	AJ493579
9. Narcissus yellow stripe virus (NYSV)	67.3	66.4	24.9	AM158908
10. Omphalodes virus Y (OVY)	66.4	70.8	30.1	AY974328
11. Papaya leaf distortion mosaic virus (PLDMV)	64.9	65.0	24.7	AB088221
12. Papaya ringspot virus type W (PRSV-W)	64.0	62.5	33.9	NC_006262
13. Plum pox virus (PPV)	68.3	64.3	36.3	AY912058
14. Pepper severe mosaic virus (PeSMV)	64.9	64.0	47.9	NC_008393
15. Pepper veinal mottle virus (PVMV)	69.7	68.5	not reported	not reported
16. Potato virus A (PVA)	67.0	66.5	33.2	AJ131400
17. Potato virus Y (PVY)	65.7	64.2	43.8	AF463399
18. Ranunculus mild mosaic virus (RanMMV)	69.4	67.3	27.6	EF445546
19. Scallion mosaic virus (ScaMV)	67.5	64.9	27.7	NC_003399
20. Soybean mosaic virus (SMV)	63.6	63.0	33.2	S42280
21. Sunflower mosaic virus (SuMV)	68.2	64.9	38.1	AF465545
22. Tamarillo mosaic virus (TamMV)	67.0	66.2	33.2	AY995213
23. Tobacco etch virus (TEV)	66.9	64.1	32.9	L38714
24. Tulip mosaic virus (TulMV)	68.4	66.7	35.8	X63630
25. Turnip mosaic virus (TuMV)	64.0	63.0	28.1	AB252141
26. Tobacco vein banding mosaic virus (TVBMV)	66.0	64.7	29.8	L28816
27. Tobacco vein mottling virus (TVMV)	58.0	59.8	32.6	NC_001768
28. Watermelon mosaic virus (WMV)	63.6	62.4	33.8	D13913

is unique within the genus *Potyvirus* and not grouped into any cluster with other potyviruses in the same genus. Even though previous studies have shown that the ArjMV capsid protein (CP) reacts with antisera against CPs of DsMV, SMV, TEV, TVMV, PRSV-W, and WMV [14], only DsMV, SMV, PRSV-W and WMV belong to the same cluster in the tree. The 3′UTR of ArjMV shared only 24.7–47.9% nucleotide homology with those of other potyviruses (Table 1), well below the 71.9% nucleotide criterion for a particular potyvirus species [2], confirming once more that ArjMV is a unique potyvirus. The length and sequence of the 3′UTR of potyviruses is highly conserved between strains of the same family (83–99%), whereas this region is divergent for distinct potyviruses (39–53%) [12].

It has been well documented that the potyvirus coat protein is involved in aphid transmission, cell-to-cell movement, encapsidation of the viral RNA, and the regulation of viral RNA amplification. The N terminus of the potyvirus CP is known to be located on the surface of the subunit [3, 6, 20]. In this N-terminal region, the DAG box [4, 5, 13, 18] has been shown to be involved in determining aphid transmissibility [11, 15, 16, 21]. The DAG motif was found between amino acids 12 and 14 from the proposed start of the ArjMV CP in this study. The presence of this motif was expected as populations of *Aphis nerii* were frequently observed on plants exhibiting mosaic symptoms [24]. The DAG motif and the surrounding context in the N terminus of TVMV affect aphid transmissibility [15]. Loss and decrease in aphid transmissibility correlate

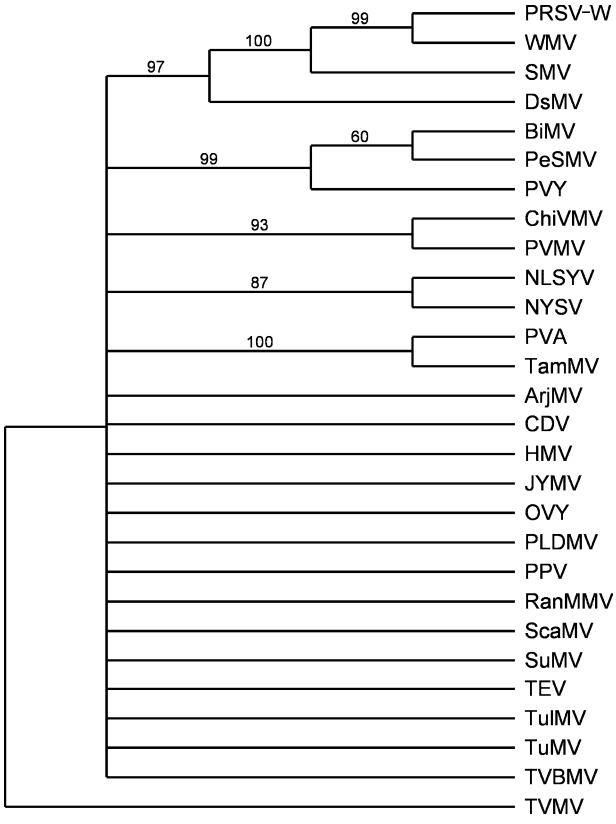

Fig. 1. Maximum parsimony tree obtained from the potyvirus capsid proteins. Bootstrap values >50% are displayed above branch nodes. The tree was rooted with the TVMV capsid protein sequence. Definitions of virus abbreviations and GenBank accession numbers are in Table 1

with loss or mutation in the DAGX motif [16, 21]. Mutations in the core region of the potyvirus CPs impaired or abolished cell-to-cell movement [17]. All the mutations in the DAG motif of the CP abolished aphid transmissibility of peanut stripe virus but did not affect virus infectivity [11].

The alignments of ArjMV CP nucleotide and amino acid sequences and the 3′UTR nucleotide sequence with those of other potyviruses in this study show for the first time that ArjMV is a unique potyvirus, differing from published potyviruses at the molecular level. All of the mutations in the CP DAG motif of peanut stripe virus potentially abolish aphid transmissibility but not virus infectivity [11]. It therefore may be possible to make such mutations in the DAG motif of ArjMV that potentially eliminate the aphid transmissibility of the virus to make it more species-specific. Determination of the host specificity is currently being carried out in the Florida quarantine facility in the USA, profiling a range of host plants as a means to assess the potential of this virus for use as a biological control agent for the moth plant in New Zealand.

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