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Short communication

Nandina mosaic virus is an isolate of *Plantago asiatica mosaic virus*

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Abstract

The complete nucleotide sequence of the genome of Nandina mosaic virus (NaMV), which has tentatively been assigned to the genus *Potexvirus*, is reported. The sequence is 6066 nt in length, excluding the poly(A) tail, and contains ORFs coding for proteins of 155, 25, 12, 13, and 21 kDa (ORFs 1–4 and the CP), respectively. The genomic organization of the virus and the signature motifs in the putative protein products are similar to the data reported for potexviruses for which complete sequences are known. Phylogenetic comparisons indicated that NaMV is most closely related to *Plantago asiatica mosaic virus* (PlAMV). Pairwise comparisons of the sequence data for these two viruses indicate that, based on criteria recently proposed for genera within the family *Flexiviridae*, NaMV and PlAMV should be considered to be strains/isolates of the same viral species. Both NaMV and PlAMV were first reported in 1976 but, as PlAMV was sequenced first, this name should take precedence with the name NaMV being relegated to a synonym.

Viruses of the genus *Potexvirus* have flexuous filaments 470–580 nm in length and 13 nm in diameter. Virions comprise a coat protein with capsid subunits (M_r 18–27 $\times 10^3$) and a single linear molecule of positive sense ssRNA of between 5.9 and 7.0 kb in length (M_r 2.10–2.39 $\times 10^6$). The genomes of potexviruses typically contain 5 open reading frames (ORF). The largest ORF codes for a protein of 150 kDa which contains methyl transferase-like signatures, helicase-like signatures, and polymerase signatures (Candresse et al., 1990) and is considered to be the viral replicase. ORFs 2–4 represent a triple gene block of proteins that are believed to be involved in viral movement. ORF 5 codes for the coat protein (CP). Over 25 distinct potexviruses have been reported with a further 19 viruses being tentatively assigned to the genus (van Regenmortel et al., 2000). Potexviruses have no known vectors but infect many plant species across a wide range of plant families. Individual species tend to have very narrow natural host ranges (Koenig and Lesemann, 1978; Francki et al., 1985). The name Nandina mosaic virus was first applied to a potexvirus isolated from *Nandina domestica* cv Harbor Dwarf (Zettler et al., 1980). The virus did not display serological relationships with six other potexviruses but showed an homologous serological reaction with another potexvirus isolated from nandina growing in California (Santos-pulgar, 1978). Neither the study which originally used this name (Zettler et al., 1980) nor the previous studies of a potexvirus isolated from nandina (Moreno et al., 1976; Santos-pulgar, 1978) provided unequivocal evidence that this virus was a unique species of potexvirus. To the present time NaMV has been considered to be a ''tentative species'' within the genus *Potexvirus*.

Plantago asiatica mosaic virus (PlAMV) was first described by Kostin and Volkov (1976) where it was reported to have spread in the central Asian region of the former USSR. The sequence of the

complete genome of the virus was described by Solovyev et al. (1994). As part of a project to determine the viruses that are present in the asexually propagated dwarf nandina cultivar 'Firepower', we have cloned and sequenced a potexvirus that we isolated from this cultivar and which gave an homologous reaction against antiserum prepared to NaMV by Zettler et al. (1980). Phylogenetic comparisons indicate that the virus should be considered to be a strain/isolate of the definitive potexvirus PlAMV.

Sap inoculation from the dwarf nandina cultivar 'Firepower' to *Chenopodium quinoa* Wild. using 0.03 M sodium phosphate buffer containing 0.002 M 2-mercaptoethanol produced a systemic mottle. Inoculation from these plants to *Gomphrena globosa* produced necrotic local lesions. Isolation from a single local lesion to *Spinacea oleracea* and *Nicotiana benthamiana* produced systemic infection in both species. Sap produced from the systemically infected *N. benthamiana* was inoculated to *G. globosa* and a single local lesion selected and inoculated to *N. benthamiana*. Systemic infection developed and this isolate (PLH1) was purified and sequenced. The virus was purified using the method described by Zettler et al. (1980) and the concentration of the purified preparation was estimated spectophotometrically using an extinction coefficient at 260 nm of 3.08 mg ml^{-1} cm^{-1} (Koenig and Lesemann, 1978). The molecular mass (M_r) of the viral coat protein was estimated using SDS-PAGE electrophoresis with low-range molecular weight standards, 14,400–97,400 (Bio-Rad, Hercules, CA). Viral RNA was extracted using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA). The RNA preparation was analyzed in a denaturing gel using the RNA of *Tobacco mosaic virus* (M_r 2.05×10^6) and the 16S (M_r 0.55×10^6) and 23S (M_r 1.07×10^6) ribosomal RNAs from *Escherichia coli* as standards. An initial cDNA library was synthesized using Superscript II reverse transcriptase and oligo-dT according to manufacturer's instructions (Invitrogen, Carlsbad, CA). The remaining sequence was obtained by synthesizing additional cDNAs with primers complementary to areas of sequence in the initial clones and to sequence that was determined later. All cDNAs were ligated into *p*Bluescript. The 5′ terminus of the genomic molecule was determined by 5′ RACE (Frohman et al., 1988). All clones and their subclones were sequenced completely in both directions using oligonucleotide primers (KS, M13 forward, M13 reverse, and p79) (De Bellis et al., 1992) plus other primers synthesized as the sequence was determined. Sequencing reactions were analyzed in an ABI 377 sequencer (Applied Biosystems, Inc., Foster, CA). Alignments of sequences were completed using CLUSTAL X and dendrograms were plotted using NJPLOT (Jeanmougin et al., 1998). Paired comparisons were completed using the BCM Search Launcher website (http://searchlauncher.bcm.tmc.edu/seq-search/alignment.html) at Baylor College of Medicine, Texas, USA.

A single purification from *N. benthamiana* yielded 0.42 mg of virus/g of leaf tissue. SDS-PAGE of the purified virus showed a single protein with an

Table 1. A comparison of NaMV and PlAMV

NAMV			PlAMV		
Open reading Frame	Location within genome	Size of putative translation product (kDa)	% Identity	Location within genome	Size of putative translation product (kDa)
5′ UTR			89		
1	86–4192	155	82	86–4243	155.6
2	4194–4886	24.8	86	4245–4937	24.7
3	4852–5184	11.8	85	4903–5235	11.7
4	4988–5353	12.5	69	5039–5404	12.7
CP	5325–5948	21.7	91	5376–5999	21.8
3′ UTR			84 (91)		

The location of ORFs within the respective genomes and the sizes of the putative translation product for which the individual ORF codes are shown. The % identity between 5′ and 3′ UTRs is calculated using the nucleotide sequences. The % identity of the 3′ UTR is calculated based on the complete sequence for the 3′ UTR of both NaMV and PlAMV (84%) and on the complete sequence for NaMV compared against the sequence of PlAMV minus a repeated CTTTT motif present in PlAMV (91%). The % identity of the ORF is calculated using the amino acid sequences of the full length putative translation products.

M_r of 21,500. Denaturing gels showed a single RNA with a molecular mass similar to that of TMV. The complete sequence of the RNA is 6066 nt in length (GenBank Accession AY800279) and contains five open reading frames (ORFs) (Table 1). The putative CP – ORF 5 has an estimated M_r of 21,778. A methyl transferase-like signature, a helicase-like signature, and a polymerase signature (Candresse et al., 1990) are present in the putative product of ORF 1.

Phylogenetic analyses of the putative products of all the ORFs and signature motifs contained within the product of ORF 1 show the virus to be very closely related to PlAMV (Figure 1 and Table 1). The 3′ UTR regions of both viruses exhibit a high degree of identity with the exception of a repeated CTTTT motif present in PlAMV (Figure 2) Examination of the 3′ UTR regions of other potexviruses for which more than a single complete genomic sequence is available (*Pepino*

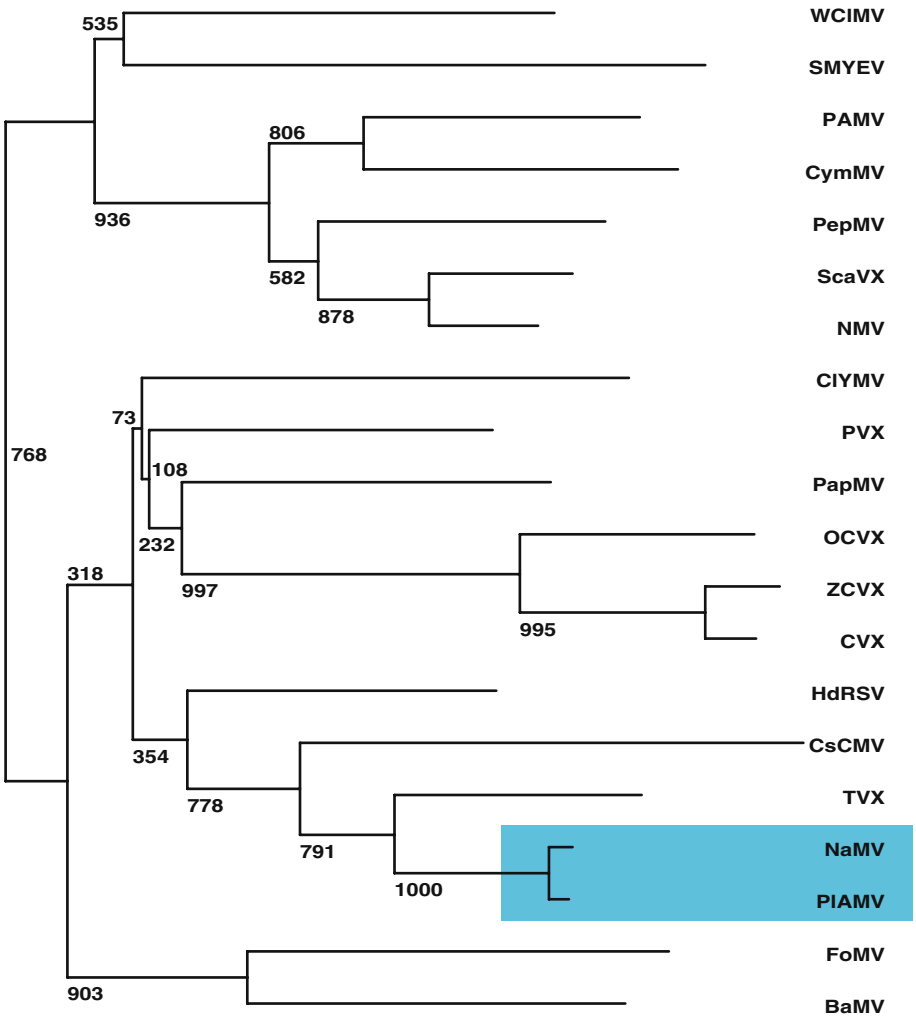

Figure 1. Unrooted phylogenetic tree for amino acid sequence of the polymerase signature of NaMV, *Bamboo mosaic virus* (BaMV; D26017), *Cactus Virus X* (CVX; AF308158), *Cassava common mosaic virus* (CsCMV; U23414), *Clover yellow mosaic virus* (ClYMV; D29630), *Cymbidium mosaic virus* (CymMV; U62963), *Foxtail mosaic virus* (FoMV; M62730), *Hydrangea ringspot virus* (HdRSV; AY 707100), *Narcissus mosaic virus* (NMV; D13747), *Opuntia virus X* (OCVX; AY366209), *Papaya mosaic virus* (PapMV; D13975), *Pepino mosaic virus* (PepMV; NC_004067), *Plantago asiatica mosaic virus* (PlAMV; Z21647), *Potato aucuba mosaic virus* (PAMV; S73580), *Potato virus X* (PVX; D00344), *Scallion virus X* (ScaVX; AJ316085), *Strawberry mild yellow edge virus* (SMYEV; D12517), *Tulip virus X* (TVX; AB066288), *White clover mosaic virus* (WClMV; X06728), and *Zygocactus virus X* (ZCVX; AY366207). The trees were bootstrapped using 1000 replications. The cluster containing NaMV and PlaMV is indicated by a shaded box.

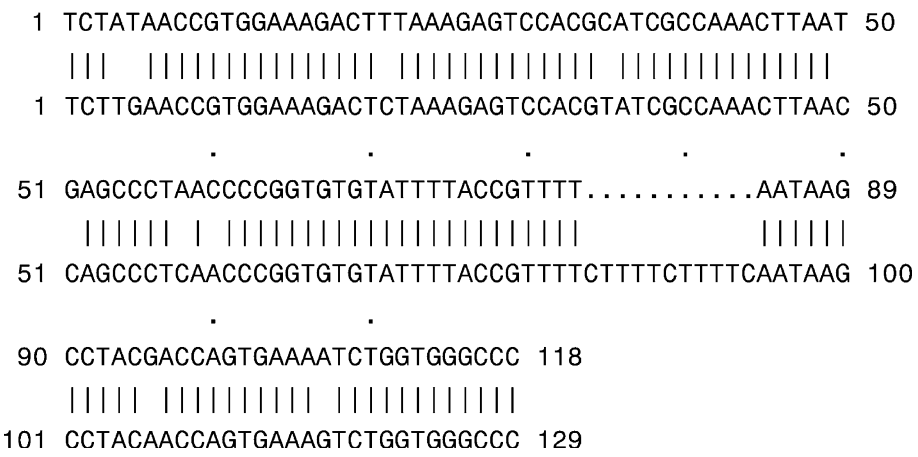

Figure 2. An alignment of the 3′ UTRs of NaMV (top) and PlAMV (bottom). The two 3′ UTRs share 84% identity. However, if the repeated CTTTT motif present in PlAMV is ignored the percentage identity increases to (91%).

mosaic virus – PepMV and *Potato virus X* – PVX) shows almost complete identity among sequences (2 nucleotide changes in the 64 nucleotides of the 3′ UTR of PepMV and 1 nucleotide change in the 72 nucleotides of the 3′ UTR of PVX). Thus isolates of the same virus would appear to have almost identical 3′ UTRs. The variation between the 3′ UTRs of NaMV and PlAMV is greater than that seen in PepMV and PVX and this would argue against NaMV and PlAMV being isolates of the same virus. However the extent of the conservation at the protein level would suggest that they are indeed members of the same species. This being true leads to the speculation that the repeated CTTTT motif in PlAMV may be a cloning/sequencing artifact.

Four viruses have been reported to infect nandina: *Cucumber mosaic virus* – CMV (Brierley and Smith, 1960; Barnett and Baxter, 1973), NaMV (Zettler et al., 1980), *Nandina stem pitting virus* – NSPV (Ahmed et al., 1983), and *Tobacco ringspot virus* – TRSV (Stouffer, 1965) with NaMV being the only potexvirus described. Members of the genus *Plantago* are infected by a number of different viruses (Fauquet and Mayo, 1999) including two other potexviruses: *Plantago severe mottle virus* (PlSMoV) and *Plantain virus X* (PlVX). We were unable to examine the relationship between these viruses and NaMV at the molecular level as the viruses were neither available to us nor are sequences under these names present in GenBank. However, based on distinct differences in host range and serological reactivity it is unlikely that either PlSMoV (a strain of the potexvirus *Papaya mosaic virus* – PapMV – Gracia et al., 1983) or PlVX is related to either PlAMV or NaMV. PlSMoV infects *G. globosa* but is a member of a cluster of serologically related potexviruses including PapMV (Gracia et al., 1983). Phylogenetic comparison (Figure 1) shows that PapMV is clearly distinct from either PlAMV or NaMV. PlVX is not serologically related to other potexviruses (Milne, 1988) and does not infect *G. globosa* (Hammond and Hull, 1983). The absence of a relationship between PlAMV and other viruses that infect members of the genus *Plantago* is reiterated in the description of the sequence for PlAMV (Solovyev et al., 1994).

The closeness of the relationship between NaMV and PlAMV at the molecular level is such that NaMV must be considered to be a strain/isolate of PlAMV and the name NaMV should be considered to be a synonym for PlAMV. The core region of the CP of strains of individual potexviruses can show between 72 and 90% homology (van Regenmortel et al., 2000). Percentage identities of between 62 and 76% among the amino acid (aa) sequences of the CPs of 3 potexviruses isolated from cactus distinguished them as three different species (Koenig et al., 2004). Strains of the same potexvirus displayed between 82 and 98% identity in the aa sequences of the CP (Yamaji et al., 2001). In establishing molecular criteria for the family *Flexiviridae* greater than 90% identity among the aa of the coat protein is shown for strains of potexviruses (Adams et al., 2004). The

level of identity (91%) between the complete aa sequences of the CP of NaMV and PlAMV and relatively little variation between the 3′ UTRs of the two viruses would offer support for them being at least strains of the same virus with observed variations in the sequence being attributable to existence in markedly different host species. Ironically, *Plantago asiatica* is found growing in the region of genetic origin for Nandina (eastern Russia/Asia) and this raises the question of which of the two hosts was the original natural host of the virus.

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