A multipartite single-stranded negative-sense RNA virus is the putative agent of fig mosaic disease

Toufic Elbeaino,1 Michele Digiaro,1 Abdulkader Alabdullah,1 Angelo De Stradis,2 Angelantonio Minafra,2 Nicole Mielke,3 Maria Antonietta Castellano2 and Giovanni P. Martelli2

Correspondence
Toufic Elbeaino
elbeaino@iamb.it

1Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy
2Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy
3Department of Molecular Phytopathology and Genetics, University of Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany

Several dsRNA bands (approx. 0.6–7 kbp in size) were recovered from tissues of mosaic-diseased fig seedlings which contained the enveloped round structures known as double membrane bodies (DMBs). BLAST analysis of a 4353 and a 1120 nt sequence from the two largest RNA segments showed homology with the polymerase and the putative glycoprotein precursor genes of negative-sense single-stranded RNA viruses of the family Bunyaviridae. Negative- and positive-sense riboprobes designed from both RNA segments hybridized to two bands of approximately 7 and 2.3 kbp in Northern blots of dsRNAs. Thus, these segments were identified as putative RNA-1 and RNA-2 of a novel virus for which the name fig mosaic virus (FMV) is proposed. Identity levels of predicted amino acids of the protein encoded by FMV RNA-1 with those of species of the family Bunyaviridae and European mountain ash ringspot-associated virus (EMARaV) were 28 and 54 %, respectively. RNA-2 showed 38 % identity at the amino acid level only with EMARaV. RNA-1 segment contained five conserved motifs (A–E) and an endonucleolytic centre of comparable genes of L RNA of bunyaviruses and EMARaV RNA-1. In a phylogenetic tree constructed with RdRp sequences, EMARaV grouped with FMV in a clade distinct from those of all bunyavirus genera. The consistent association of DMBs with mosaic symptoms and the results of molecular investigations strongly indicate that DMBs are particles of FMV, the aetiological agent of fig mosaic disease.

INTRODUCTION

Fig (Ficus carica) mosaic disease (FMD), the only currently known infectious disorder of this species, has an extremely variable symptomatology (Condit & Horne, 1933; Martelli et al., 1993). FMD is graft- but not seed-transmissible (Condit & Horne, 1933; Blodgett & Gomec, 1967) and, in nature, is spread by the eriophyid mite Aceria ficus with a semipersistent modality (Flock & Wallace, 1955; Proeseler, 1972; Slykhuis, 1973). Although a number of different isometric and filamentous viruses have been found in infected trees (Quacquarelli, 1971; Grbelja & Eric, 1983; Namba, 1983; Doi, 1989; Serrano et al., 2004; Martelli et al., 1993; Elbeaino et al., 2006, 2007; Castellano et al., 2007), none of them was identified as the possible causal agent of the disease. Thus, the aetiology of FMD is still undetermined.

Electron microscope observations of thin-sectioned tissues of symptomatic fig leaves have shown the presence of peculiar membrane-bound, round to ovoid electron-dense structures 90–200 nm in diameter in the cytoplasm of parenchyma cells, known as double membrane bodies (DMBs; Bradfute et al., 1970).

DMBs have an envelope consisting of a unit membrane approximately 12 nm thick, which is apparently acquired from the endoplasmic reticulum (Martelli et al., 1993; Appiano et al., 1995), contain proteinaceous material and fine fibrils, and are often next to aggregates of convoluted electron-dense filamentous elements that contain carbohydrates and are partly digested by pronase (Martelli et al., 1993; Appiano & Conti, 1993). DMBs are insensitive to tetracycline (Martelli et al., 1993).

DMBs are a consistent feature of diseased figs, regardless of the variety and the country of origin of infected samples (Plavsic & Milicic, 1980; Martelli et al., 1993; Appiano et al. 1995; Castellano et al., 2007). Because of this and of their

The GenBank/EMBL/DDBJ accession nos of FMV RNA-1 and FMV RNA-2 determined in this study are AM941711 and FM864225, respectively.
cytochemical and ultrastructural properties, DMBs were suggested to represent possible particles of an uncharacterized virus (Martelli et al., 1993; Ahn et al., 1996).

As reported in the present paper, multiple dsRNAs were extracted from symptomatic fig seedlings and used for the partial molecular characterization of what seems to be a novel virus with a multipartite, single-stranded, negative-sense RNA genome, herein referred to as fig mosaic virus (FMV).

**METHODS**

**Plant material.** In summer 2007, 15 symptomless fig seedlings at the six leaf stage were placed in a glasshouse box at 28–30 °C, which also contained potted symptomless rooted cuttings from a mosaic-infected fig tree infested naturally by A. ficus. Within a couple of weeks, the leaves of eight seedlings began to show chlorotic ringspots which increased in size, coalesced, and evolved into a mosaic pattern (Fig. 1). These seedlings, but not the symptomless ones, hosted small populations of the mite.

**Electron microscopy.** Pieces of leaf tissue from symptomatic and symptomless seedlings and symptomatic fig rooted cuttings were processed according to standard procedures (Martelli & Russo, 1984), i.e. fixation for 2 h at 4 °C in 4 % glutaraldehyde in 0.05 M phosphate buffer, post-fixation in 1 % osmium tetroxide, staining overnight in 2 % aqueous uranyl acetate, dehydration in graded ethanol dilutions and embedding in TAAB low viscosity resin. Thin sections were stained with lead citrate and viewed with a Philips Morgagni 268 electron microscope.

**Extraction of total nucleic acids (TNAs).** TNAs were extracted from 100 mg leaf tissue according to Foissac et al. (2001), i.e. grinding in liquid nitrogen and homogenization in 1 ml extraction buffer (6 M guanidine isothiocyanate, 0.2 M sodium acetate, 1 M potassium acetate, 0.025 mM EDTA, 2.5 % PVP-40), addition of 6 M sodium iodide and 0.15 M sodium sulphite, 150 μl ethanol and 25 μl silica particles suspension (1 g ml⁻³ pH 2.0). After stripping by heat treatment in sterile water (70 °C for 3 min) and centrifugation for 3 min at 16000 g, TNAs were recovered and stored at –20 °C until use.

**Extraction of double-stranded RNA.** Leaf or young root tissues (15–30 g) from symptomatic and symptomless seedlings were used to recover dsRNAs by phenol/chloroform extraction and chromatography through cellulose columns in the presence of 17 % ethanol, according to a modified protocol of Dodds (1993). Tissue samples were frozen in liquid nitrogen, ground to a fine powder and homogenized in extraction buffer consisting of 60 ml 1 x STE (0.1M Tris/HC1, 0.2 M NaCl, 2 mM EDTA, pH 7.0), 1.6 ml 2b-mercaptoethanol, 30 ml SDS 10 % and 50 ml phenol/chloroform (1:1 v/v), respectively, followed by vigorous shaking for 30 min. After centrifugation at 15300 g for 20 min, 1:5 volume of 96 % ethanol and 3 g CFI11 cellulose (Sigma) were added to the supernatant and the mixture was again shaken for 30 min. The cellulose was pelleted by centrifugation (10 min at 5500 g) and washed three times in 40 ml washing buffer (1 x STE with 17 % ethanol). The cellulose was eluted stepwise with 13 ml elution buffer (1 x STE). Cellulose CC41 (0.5 g) and 1:5 volume of 96 % ethanol were then added to the eluted solution, washed twice and eluted again with 2 ml elution buffer in two aliquots of 1 ml each. DsRNA was then precipitated with 1:10 volume of 3 M sodium acetate, pH 5.2, and 2.5 volume ethanol at –20 °C overnight, and resuspended in double-distilled and DEPC-treated water. DNA and single-stranded RNA were sequentially enzyme-digested as described by Saldarelli et al. (1994). A dsRNA quantity roughly equivalent to 5 g of tissues was electrophoresed in 5 % TAE polyacrylamide gel and revealed by silver staining.

**Strategies for reverse transcription, cloning and sequencing.** Two hundred and fifty nanograms of nuclease-treated dsRNA in 9 ml of water were denatured by boiling at 95 °C for 10 min, chilled on ice and incubated at room temperature for 10 min in the presence of 1 μg random hexamers and 1 μl methyl mercuric hydroxide (100 mM; Saldarelli et al., 1994). The methyl mercuric hydroxide was inactivated by adding 1 μl 2b-mercaptoethanol 0.5 M. Denatured dsRNA were reverse transcribed with the Moloney murine leukemia virus reverse transcriptase enzyme for 1 h at 39 °C according to the manufacturer’s instruction (Invitrogen), in a final volume of 20 μl. Degenerate oligo primed PCR (DOP-PCR), RT-PCR and rapid amplification of cDNA ends PCR (RACE-PCR) were used for the amplification and cloning of FMV sequences.

For DOP-PCR, performed on random-primed cDNA, the primer DOP6 (5′-CGACTCAGGNNNNNNTTCAGG-3′) gave a consistent pattern of PCR amplicons. In this protocol, 50 μl PCR mixture contained 4 μl reverse transcription reaction, 0.5 mM each dNTP, 2 μM DOP6 primer, 1 x PCR buffer (Roche) and 0.5 μl Taq DNA polymerase (5 U μl⁻¹; Roche). PCR was carried out in a Perkin-Elmer 7600 thermal cycler as described by Rott & Jelkmann (2001).

Following a preliminary alignment of some sequenced viral clones, sets of specific primers were used for closing sequence gaps. PCR was performed on a random-primed reverse-transcribed dsRNA template as described by Rott & Jelkmann (2001), but at an annealing temperature of 58 °C. Sequences of some of the viral clones showed high homology with the polymerase gene coded by L RNA of members of the family Bunyaviridae (De Haan et al., 1991) and RNA-1 of the bunyavirus-like European mountain ash ringspot-associated virus (EMARaV) (Mielke & Muehlbach, 2007). Therefore, amino acid sequences of the largest RNAs of EMARaV (GenBank accession no. AM563040), tomato spotted wilt virus (TSVV, D10066), groundnut bud necrosis virus (GBNV, AF025358) and watermelon silver mottle virus (WSMoV, NC_003832) were aligned and two degenerate primers were designed on the conserved polymerase motifs A (BUN1-s: 5′-TCNAAARTGTCNGC-3′) and C (BUN3-a: 5′-ATCATCGAGTGNACAT-3′). PCR conditions under which these primers were used were 2.5 mM MgCl₂ (final concentration), 0.5 mM dNTPs, 2 μM each primer, 0.3 μl Taq polymerase (5 U μl⁻¹) in a final volume of 25 μl. PCR was carried out at 94 °C for 5 min,
followed by 35 cycles at 94 °C for 30 s, 45 °C for 1 min and 72 °C for 30 s. Final extension was at 72 °C for 7 min.

For 3’ RACE-PCR, a poly(A) tail was added at the 3’ terminus of total dsRNAs preparations using the poly(A) polymerase enzyme (Promega). A complementary oligo(dT) primer was used to reverse transcribe the termini of the tailed dsRNAs. For amplifying the viral 5’ genome terminus, 5 μl cDNA were used in PCR with an antisense-specific primer (E5-a: 5’-AACACTGTTTTTGCCGATGG-3’) designed on a DOP-PCR clone denoted E5 (see Fig. 4), together with the oligo(dT) primer. PCR consisted of 35 cycles at 94 °C for 30 s, 52 °C for 30 s, 72 °C for 2 min and final extension at 72 °C for 7 min. A similar strategy was used to amplify the 5’ terminus of the second virus genome segment obtained by DOP-PCR (clone F1, see Fig. 4), using the antisense-specific primer F1-a (5’-CAGAACCATCAACTGAAGT-3’).

3’ RACE-PCR was used to further extend the sequence of the putative RNA-1 beyond motif C (see Fig. 4). In this case, the denatured dsRNAs were reverse-transcribed with a sense-specific primer (MotC-s: 5’-TGACCCCATATCTCCATCTCG-3’), designed on the sequence close to the end of motif C. The primer P1 (5’-GATCCATAGTTCAGACGCGC-3’) was ligated to the 3’ end of the synthesized cDNA using the terminal deoxynucleotidyl transferase enzyme (TdT; Promega). P2 primer, complementary to P1 (Coutts & Livieratos, 2003, modified), was used together with primer MotC-s for amplification. PCR conditions consisted of 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min and final extension at 72 °C for 7 min.

All PCR products were directly cloned into the pGEM-T Easy vector (Promega) according to the manufacturer’s instructions and used to transform Escherichia coli DH5α competent cells. Selected clones were subjected to automated sequencing (Pirum) and sequences were assembled with the Strider 1.1 program (Marck, 1988). Alignment for nucleotide and amino acid homology was done with the CLUSTAL_X program (Thompson et al., 1997). Homology with known proteins from the Protein Information Resources (PIR, release 47.0) was determined using the FASTA (Pearson & Lipman, 1988) and BLAST (Altschul et al., 1990) programs. Phylogenetic trees were constructed and bootstrap analysis made with the NEIGHBOR, SEQBOOT, PROTDIST and CONSENSE programs of the PHYLIP package (Felsenstein, 1989).

**Molecular detection of FMV in tissue extracts.** TNA extracts from four symptomatic and two symptomless seedlings which had been observed under the electron microscope for the presence of DMBs were assayed by RT-PCR and molecular hybridization.

Primers E5 s (5’-CGTAGAAAATTGGAATGAAA-3’) and E5-a (5’-AACACTGTTTTTGCCGATGG-3’), which amplify a 302 bp DNA fragment from viral RNA-1, were used for virus detection. Randomly primed cDNA (from either TNA or dsRNA) was added to 2.5 μl Taq polymerase buffer 10 x (Promega) containing a final concentration of 1 mM MgCl2, 0.2 mM dNTPs, 0.2 μM each specific primer and 0.2 μl Taq polymerase (5 U μl−1) in a final volume of 25 μl. PCR consisted of an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. Final extension was at 72 °C for 7 min. Amplification products were visualized in 6% polyacrylamide slab gels run in TBE buffer.

Hybridization was performed on Northern blots of dsRNA extracts and dot blots of TNAs, again from symptomatic and symptomless seedlings. For Northern blots, approximately 50 ng dsRNA were boiled for 5 min, chilled on ice, separated in a denaturing 1% agarose/formaldehyde gel, and transferred onto a Hybond-N+ nylon membrane (GE Healthcare, USA) as described by Chomczynski (1992). TNA extracts (2 μg) were denatured at 65 °C for 5 min in a 50 mM NaOH solution before spotting onto nylon membranes. Hybridization was performed overnight at 56 °C, with digoxigenin-labelled positive- and negative-sense RNA probes, designed on the sequences of viral clones E5 and F1, as described by Astruc et al. (1996). Blots were washed twice for 15 min each in 0.1 x saline sodium citrate (SSC) and 0.1% SDS at 65 °C. Hybridized RNA bands and spots were visualized using the DIG chemiluminescent detection protocol (Roche), following the manufacturer’s instructions. Films were exposed for 20–30 min, and resulting images were digitized.

**RESULTS**

**Electron microscopy**

Leaves of the naturally infected glasshouse-grown rooted cuttings and of all symptomatic seedlings exposed to A. ficus infestation contained DMBs (Fig. 2a, b). In contrast, no such structures were found in the leaves of symptomless seedlings (not shown). This not only confirmed the alleged ability of A. ficus to transmit fig mosaic disease, but was taken as evidence that DMBs are mite-transmissible.

**dsRNAs extraction and analysis**

dsRNA extraction from symptomatic leaf tissues was unsatisfactory, probably because of the high content of contaminants (polyphenols and polysaccharides) in the preparations. The procedure, however, gave better results when starting materials were young succulent root tissues from symptomless seedlings. From this material different dsRNA bands, with size ranging from 0.6 kbp to approximately 7 kbp, were obtained (Fig. 3). A consistent pattern of four major bands was observed in repeated extractions from different mosaic-diseased seedlings and from greenhouse-grown mosaic-infected rooted cuttings. Additional faint bands, with a size smaller than that of the main RNAs, were also observed. The nature of these bands, i.e. whether they represent FMV subgenomic RNAs or RNAs of a contaminant latent virus, was not ascertained. The point remains that hybridization assays with probes designed on FMV RNA-1 and RNA-2 sequences gave a

![Fig. 2. Groups of DMBs, i.e. FMV particles, in the cytoplasm of cells from a naturally infected fig (a) and a seedling exposed to A. ficus (b). Arrows point to the aggregates of convoluted electron-dense filamentous elements that usually accompany DMBs. CW, Cell wall; Ch, chloroplast. Bar, 200 nm.](http://vir.sgmjournals.org)
positive signal only with the largest dsRNAs (see Fig. 6). No dsRNA bands were found in preparations from symptomless seedlings which, as shown by electron microscope observations of thin-sectioned leaf tissues, did not contain DMBs.

Cloning and analysis of virus sequences

Denatured reverse-transcribed dsRNA subjected to amplification by DOP-PCR yielded amplicons from 300 to 600 bp in size. Of 35 clones sequenced, only six (E1, E3, E5, E7, E11 and F1) proved to be of viral origin (Fig. 4), as they shared homology with those of bunyavirus sequences following BLAST analysis. In particular, all E clones aligned with sequences encoded by bunyavirus L RNA and EMARaV RNA-1. Thus it was possible to expand the sequence by closing the gaps between the sequenced clones by specific RT-PCR.

Degenerate primers BUN1-s and BUN3-a amplified a 384 bp product from FMV RNA-1, which comprises three conserved motifs, A, B and C, of the RdRp gene (Fig. 4, fragment 'Deg'). RACE-PCR toward the 5’ and 3’ termini of RNA-1, using the virus-specific primers E5-a and MotC-s, amplified the 5’ terminus, yielding a product of 910 bp, and an amplicon of 550 bp beyond motif C, respectively (Fig. 4).

In summary, the strategy encompassing the use of DOP-PCR, RACE-PCR and RT-PCR yielded a 4353 nt sequence (GenBank accession no. AM941711) in the polymerase gene at the 5’ terminus of FMV RNA-1 (Fig. 4).

Comparison of the predicted amino acid sequence of FMV RNA-1 with comparable sequences of different members of the family Bunyaviridae revealed the presence of five motifs (A–E), which represent a highly conserved region of the RdRp gene (Fig. 5). Various levels of identity at the amino acid level were found, which never exceeded 28%. However, 54% identity was found with the sequence of the RdRp gene of EMARaV RNA-1. According to computer-assisted analysis and comparable amino acids located after the Motif E of EMARaV and members of family Bunyaviridae, an additional highly conserved domain of bunyaviral RdRp, named F (Fig. 4), was also identified (Aquino et al., 2003; Duijsings et al., 2001).
The 5′ RACE extension of clone F1 (395 bp) produced an additional PCR fragment 725 bp in size which yielded an overall stretch of 1120 bp at the 5′ end of the putative viral RNA-2 (GenBank accession no. FM864225). The expression product of this genomic region was recognized by a BLAST search as the glycoprotein precursor of bunyaviruses showing 38% amino acid identity with the comparable region of EMARaV (Mielke & Muehlbach, 2007).

Alignment of the 5′ proximal sequence of RNA-1 and RNA-2 with those of EMARaV and various bunyaviruses showed a conserved stretch of 15 nt (5′-AGUAGUGUUCUCCTC-3′) homologous to the 5′ terminus of RNA-1 of EMARaV and to the terminal 11 nt of L RNAs of representatives of the genera Orthobunyavirus (5′-AGUAGUGUgCU-3′) and Hantavirus (5′-AGUAGUaUgCU-3′). No such similarity was found with the comparable terminal sequences of L RNA of members of the genera Tospovirus, Nairovirus, Phlebovirus and Tenuivirus.

Probe E5 (positions 852–1154 of RNA-1) hybridized to the largest dsRNA band (approx. 7 kbp) (Fig. 6a), thus confirming that it is the replicative form of FMV RNA-1. Likewise, the positive Northern blot hybridization with the F1 probe provided evidence that the smaller dsRNA is the replicative form of FMV RNA-2 (Fig. 6b).

In a phylogenetic tree constructed with polymerase sequences of members of the family Bunyaviridae and EMARaV, FMV clustered with EMARaV in a separate clade close to representatives of the genera Tospovirus, Hantavirus and Orthobunyavirus (Fig. 7).

**Fig. 5.** Amino acid alignment between conserved RdRp motifs A–E of FMV (AM941711), EMARaV (AY653040), members of five genera belonging to the family Bunyaviridae and two members of the unassigned genus Tenuivirus. I, Genus Tospovirus: TSWV, tomatospotted wilt virus (GenBank accession no. D10086); WSMoV, watermelon silver mottle virus (NC003832); GBNV, groundnut bud necrosis virus (AF025538). II, Genus Orthobunyavirus: LACV, La Crosse virus (GenBank accession no. U12396); BUNV, Bunyamwera virus (X14383); OROV, Oropouche virus (AF484424). III, Genus Hantavirus: DOBV, Dobrava-Belgrade virus (GenBank accession no. AJ140619); HTNV, Hantaan virus (X55901); PUUV, Puuma virus (Z66548). IV, Genus Phlebovirus: RVFV, Rift Valley fever virus (GenBank accession no. X56464); ToSV, Toscana virus (X68414); UUKV, Uukuniemi virus (D10759). V, Genus Tenuivirus: RSV, rice stripe virus (GenBank accession no. AY186787); RGSV, rice grassy stunt virus (AF509470). VI, Genus Nairovirus: DUGV, Dugbe virus (GenBank accession no. U15018).

**FMV detection in fig seedlings**

In dot spot hybridization assays, the digoxigenin LABELLED riboprobes synthesized from the positive strand of FMV RNA-1 sequence hybridized TNA extracted from symptomatic DMB-containing fig seedlings (Fig. 8a). No hybridization signal was obtained with extracts from symptomless DMB-free seedlings. The negative strand riboprobe gave a very weak signal resulting from the
hybridization of this probe to mRNA in the infected cells (Figs 8, 2b and 3b). These finding were taken as further evidence of the strict relationships between DMBs and FMD, and as a strong indication that, most likely, FMV RNA-1 accumulates in infected tissues as negative strand. Similar results were obtained when the F1 positive- and negative-sense riboprobes were used for dot spot hybridization of TNA (not shown).

The E5 primers used for RT-PCR on nucleic acid extract templates from infected fig seedlings proved efficient and reliable as a detection tool.

**DISCUSSION**

In the present investigation there was a complete agreement between the results of RT-PCR, molecular hybridization tests, dsRNA analysis and electron microscope observations of symptomatic and symptomless fig seedlings. Only symptomatic plants were PCR-positive, gave a hybridization signal, and contained dsRNAs and DMBs. This can be retained as evidence that, as previously suggested (Martelli et al., 1993; Ahn et al., 1996), DMBs are indeed the particles of an enveloped virus eliciting FMD, for which the name FMV seems appropriate. In this context, it is worth mentioning that dsRNAs, with sizes ranging from 0.6 to 6.6 kb, were recovered in Portugal and Turkey from mosaic-diseased figs (Nolasco & de Sequeira, 1991; Açikgöös & Döken, 2003), which further supports our conclusions.

Comparative analysis of the available sequence of FMV RNA-1 and RNA-2 with those of members of the family Bunyaviridae showed that it has a low level of similarity with all these viruses (23–28 % identity at the amino acid level). A higher correlation (54 % identity) was found with...
EMARaV. Likewise, RNA-2 showed identity only with EMARaV, though at lower level (approx. 38%). Notwithstanding this similarity, FMV and EMARaV are clearly distinct viruses. In fact, EMARaV resembles tospoviruses in the shape and size of the particles and cytopathology of infected cells (Ebrahim-Nesbat & Izadpanah, 1992), but differs enough molecularly from them, to be assigned to a putative new genus denoted *Eamaravirus* (Benthack *et al.*, 2005; Mielke & Muehlbach, 2007).

FMV is much closer to a range of different viruses infecting herbaceous and woody hosts, all of which are transmitted by eriophyid mites and have the same type of virions and comparable cytopathological features (Bradfute *et al.*, 1970; Kim & Martin, 1978; Gergerich *et al.*, 1983; Gergerich & Kim, 1983; Ahn *et al.*, 1996; Kumar *et al.*, 2002, 2003; Skare *et al.*, 2006).

Two of these viruses, pigeonpea sterility mosaic virus (PPSMV) and maize red stripe mosaic virus (Skare *et al.*, 2006), renamed by the same authors wheat mosaic virus (WMV), differ biologically from FMV for they can be transmitted artificially either by sap inoculation (PPSMV) or vascular puncture (WMV). However, like FMV, both these viruses have a multipartite, negative-sense, single-stranded RNA genome. In particular, five to seven RNA molecules with size ranging from approximately 1.1 to 6.8 kb were recovered from PPSMV particles (Kumar *et al.*, 2003), whereas WMV has three RNA molecules approximately 8 kb (RNA-1), 2–2.5 kb (RNA-m) and 1.4 kb (RNA-s) in size (Skare *et al.*, 2006). Sequences of a single small genome fragment from each of these viruses are reported in GenBank, i.e. 764 nt from RNA-5 of PPSMV (accession no. AJ439561) and 1329 nt from the nucleocapsid gene of WMV (accession no. DQ324466).

Although neither of these sequences showed any similarity with FMV genome fragments, we have sequenced, the point remains that, because of the similarity in genome type and in the epidemiological, morphological and ultrastructural features, FMV, PPSMV and WMV seem to be coherent members of a virus group which, as recently proposed (Kumar *et al.*, 2003; Skare *et al.*, 2006), warrants classification as a novel genus of plant viruses in the family Bunyaviridae.

**REFERENCES**


