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Molecular evidence that the whitefly-transmitted sweetpotato mild mottle virus belongs to a distinct genus of the *Potyviridae*

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Summary. Complementary DNA representing 2 108 nucleotides at the 3' end of the genomic RNA of the whitefly-transmitted sweetpotato mild mottle virus (SPMMV) was cloned after PCR. Sequence analysis revealed an open reading frame of 1 797 nucleotides which codes for a protein of 599 amino acids, followed by a 3' non-coding region of 311 nucleotides. Alignment of the deduced amino acid sequence with corresponding sequences of other members of the *Potyviridae* demonstrated that part of the presumptive RNA-dependent RNA polymerase and the coat protein coding regions of SPMMV are found at the 3' end of its genome, in that order. Alignment of the amino acid sequence of the core of SPMMV coat protein with those of selected members of the *Potyviridae* showed limited identity, thus demonstrating – with phylogenetic analysis – that SPMMV belongs to a distinct genus of the family *Potyviridae*.

Introduction

The taxonomic family *Potyviridae*, which is the largest family of plant viruses, has been divided into three recognised genera (*Potyvirus*, *Rymovirus*, and *Bymovirus*) on the basis of coat protein amino acid and genomic RNA sequences [31]. Potyviruses, rymoviruses and bymoviruses are transmitted, respectively, by aphids, eriophyid mites and the fungus *Polymyxa graminis* [31]. The *Potyviridae* genome consists of a single-stranded positive-sense RNA. Potyviruses and rymoviruses have a single RNA molecule of approximately 10000 nucleo-tides, while the genome of bymoviruses consists of two molecules of RNA [25].

The *Potyviridae* also contains some unassigned viruses, among which the whiteflytransmitted sweetpotato mild mottle virus (SPMMV) was tentatively classified in a fourth possible genus, Ipomovirus [2, 31]. However, this genus would require validation based on coat protein sequence data.

SPMMV was isolated in East Africa from sweetpotatoes showing leaf mottling, veinal chlorosis, dwarfing and poor growth [12]. SPMMV has not been characterised so far, but the morphologies of virions and cytoplasmic inclusions are similar to those of potyviruses [19]. The virus particle is filamentous and contains one coat protein of 37.7 kDa [12], which shares epitopes with those of other potyviruses [29].

The PCR DNA amplification procedure using degenerate primers has been shown to be a powerful approach for obtaining virus sequence information in the potyvirus genus [4, 5, 17, 21, 23]. To unequivocally assign SPMMV to the family *Potyviridae*, we used cDNA amplification by PCR to obtain information on part of its genome organisation and sequence. The 3' terminus of the SPMMV genome was analysed and its coat protein core amino acid sequence was aligned with those of selected members of the *Potyviridae*.

Materials and methods

RNA extraction, cDNA synthesis and amplification with degenerate primers

SPMMV was received from Dr. Salazar (International Centre of Potato, Lima, Peru) and propagated in *Nicotiana benthamiana*. Total RNA was extracted from symptomatic leaves by the method of Chirgwin et al. [3]. Purification of polyA-containing RNA was performed with Oligotex-dT (Qiagen). Oligo(dT)-primed single-stranded cDNA was synthesised using the Gibco/BRL Superscript Preamplification System. Amplification of oligo(dT)-primed cDNA with Pot8 and Pot9 degenerate primers (Fig. 1) was performed for 35 cycles (Biometra cycler): template denaturation at 94 °C for 30 sec, primer annealing at 37 °C (cycles 1–5) or 50 °C (cycles 6–35) for 1 min and DNA synthesis at 72 °C for 1 min. After electrophoresis, the amplified fragment was directionally cloned into pBluescript [28].

cDNA synthesis and nested amplification of cDNA 3' end

Oligo (dT_{17}) -adapter [9] primed single-stranded cDNA was synthesised from total RNA. Nested amplification of the cDNA was performed with 0.1 µg each of adapter primer [8] and specific primer (MVCP1 in the primary amplification, MVCP2 in the secondary nested amplification, Fig. 1). After denaturation for 5 min at 94 °C, primer annealing for 1 min at 50 °C and elongation for 10 min at 72 °C, the following thermal cycling scheme was used: template denaturation at 94 °C for 30 sec annealing at 50 °C (cycles 1–5) or 55 °C (cycles 6–35) for 1 min and elongation at 72 °C for 2 min. The second nested amplification was performed on 1 µl from a 1000-fold dilution of the first PCR reaction. The amplified fragment was cloned into pCRII using the TA-Cloning Kit from Invitrogen.

Sequence analysis of the amplified fragments

The nucleotide sequence of each amplified fragment was obtained from three independent clones either by cleavage with restriction enzymes or by specific primers derived from the sequence. Double-stranded DNA sequencing by the dideoxy chain termination method was performed using T7 DNA polymerase (Pharmacia) according to the manufacturer's instructions.

Multiple sequence alignments were done using ClustalV [10, 11] which produced a dendrogram of sequence relationship and calculated a phylogenetic neighbor-joining tree. Groupings were considered significantly supported when they were found in at least 95% of 1000 bootstrap replicates. The following members of the *Potyviridae* were used for sequence comparisons: barley yellow mosaic virus (BaYMV) [15], plum pox virus (PPV) [18], potato virus Y (PVY) [26], tobacco etch virus (TEV) [1], tobacco vein mottling virus (TVMV) [6] and wheat streak mosaic virus (WSMV) [20].

Results

Design of the degenerate PCR primers

The amino acid sequences of the polyproteins of BaYMV, Johnsongrass mosaic virus (JGMV) [9], papaya ringspot virus (PRSV) [34], PPV, pea seed-borne mosaic virus (PSbMV) [14], PVY, soybean mosaic virus (SbMV) [13], TEV, turnip mosaic virus (TuMV) [22], TVMV and the amino acid sequence of the C-terminal part of the polyprotein of WSMV were compared. Two conserved regions were selected in the RNA-dependent RNA polymerase. One of these regions, corresponding to the Pot9 primer, contained the GDD-box involved in the RNA polymerase function of postive strand RNA viruses [7, 16, 24]. Degenerate primers Pot8 (a 23-mer oligonucleotide with a 32-fold degeneracy)



Fig. 1. Genetic map of the 3' terminal part of SPMMV genome showing the relative positions of the two degenerate primers Pot8 (equivalent to G⁷⁶²⁵ to A⁷⁶³⁸ in PVY) and Pot9 (equivalent to A⁸⁰⁶³ to C⁸⁰⁷⁴ in PVY) and the two specific primers MVCP1 (Fig. 2, position 372 to 391) and MVCP2 (Fig. 2, position 391 to 411). Lower case letters represent nucleotides not derived from the viral sequences. *Nib* Nuclear inclusion b protein cistron, *CP* coat protein cistron, *3'-NCR* 3' non-coding region

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1 N Y F Y K C H L Q G P W T V G I N K F N 1 AATTATTTCTATAAGTGTCATTTGCAAGGACCATGGACAGTTGGGATCAATAAATTCAAT G W N K L A N Y F N H D W V F I D C D 61 CGAGGGTGGAATAAGTTGGCGAACTATTTCAATCATGATTGGGTTTTCATTGACTGTGAT G S R F D S S I P P I M F N A V C M L R 41 121 GGGAGTCGGTTTGACAGTTCCATACCCCCAATTATGTTTAATGCAGTTTGCATGTTAAGA V F G D L D P D E N Q T L S N L Y T E S 181 TCAGTTTTTGGTGATTTGGATCCAGATGAAAATCAGACGTTGAGCAATCTTTACACTGAG I V N T P I L T I E G N I I R K F R 81 G N 241 ATTGTGAACACGCCAATACTCACCATTGAAGGGAATATCATCCGGAAATTTAGAGGCAAT 101 N <u>S G Q P S T V V D N T</u> L I L M I A M E 301 AACAGTGGGCAGCCTTCAACAGTTGTTGATAACACGTTGATTCTGATGATTGCTATGGAG IAKVFVTRPDIKYVCNG А 361 TACGCGATAGCTAAGGTTTTTGTCACACGCCCTGACATCAAGTATGTCTGTAATGGGGGAC 141 D L L I N C P R S T A N A I S E H F K D 421 GACCTCTTAATCAATTGTCCAAGGAGCACTGCAAACGCCATTAGTGAGCACTTCAAAGAC V F A D L S L N Y D F D H V C D K I T D 161 481 GTATTCGCAGACTTAAGCTTGAACTATGATTTTGATCATGTTTGTGATAAAATCACAGAT V D FMSHSFMWLDTEOM 1.81 Y Т Ρ ĸ 541 GTTGATTTTATGAGTCATAGCTTCATGTGGCTAGATACTGAACAGATGTATATACCAAAG L D K E R I V A I L E W E R S D E Q F 201 P 601 TTGGACAAGGAGCGAATTGTGGCAATTTTGGAATGGGAAAGGAGTGATGAGCÄATTCAGA T R S A L N A A Y I E S F G Y E D L M T 661 ACAAGGAGCGCCCTGAATGCTGCTTATATTGAGAGTTTTGGATACGAAGATCTAATGACT K F A H F W A K K H G L N D V Т E 241 E T. M 721 GAGATCGAGAAATTCGCTCACTTCTGGGCTAAAAAGCATGGTCTCAATGATGTCCTAATG 261 E R E K V R S L Y V D E N F D A S R F E 781 GAGCGGGAAAAGGTTAGAAGCTTGTACGTCGATGAAAATTTTGATGCGTCACGCTTTGAG 281 K F Y P E S F S P F D V Y V E P H A S T 841 AAGTTCTATCCGGAAAGCTTTTCGCCATTTGACGTTTATGTTGAACCACATGCATCAACA S K T I E E L Q Q E M E D L D A D T T т 301 901 TCCAAAACAATCGAAGAACTGCÃGCÃAGAAATGGAGGATTTGGACGCAGACAACAATC 321 T V V Q R E T Q K A G I R D Q I E А L R 961 ACTGTGGTTCÄGAGGGAAACACÄGAAGGCAGGAATAAGAGATCÄAATTGAGGCACTTAGG ΡΕΑ т 37 Ρ O L O PD V T \cap R P \cap 1021 GCACÃGCÃAATTGTGAGGCCTCCTGAGGCACÃACTACÃGCCTGACGTAACTCCTGCGCÃA 361 I V T F E P P R V T G F G A L W I P R 1081 ATTGTTACGTTTGAACCACCGAGAGTCACTGGATTTGGCGCTCTATGGATTCCGCGCCAA R N Y M T P S Y I E K I K A Y V P 0 381 Η S 1141 CAAAGGAACTACATGACGCCATCTTACATCGAAAAGATAAAGGCTTATGTTCCACACTCA 401 N L I E S G L A S E A Q L T S W F ENT 1201 AACTTGATTGAATCCGGACTAGCTAGTGAAGCTCÄATTGACTAGTTGGTTCGAGAACACG C R D Y Q V S M D V F M S T I L P A 421 1261 TGCAGAGATTATCÃAGTCAGTATGGATGTTTTCATGAGTACAATATTGCCAGCATGGATT INGTS ERTNE VN C т 0 н т W R 441 1321 GTCAATTGCATAATCAATGGAACGTCTCAGGAGCGCACTAATGAGCATACTTGGAGAGCT I M A N M E D Q E V L Y Y P I K P 1381 GTGATTATGGCAAATATGGAAGAAGATCAAGAAGTGCTTTATTATCCCCATCAAACCCATAATT

481 1441	. I N A Q P T L R Q V M R H F G E Q A V ATTAATGCTCAACCAACTTTGAGGCAGGTGATGCGCCACTTTGGCGAGCAAGCCGI	A TGCC
501 1501	QYMNSLQVGKPFTVKGAVT CAATACATGAATAGCCTTCAAGTTGGCAAACCTTTCACAGTGAAAGGTGCCGTGAC	A TGCT
521 1561	. G Y A N V Q D A W L G I D F L R D T M GGGTATGCTAATGTTCAGGATGCTTGGCTAGGTATTGACTTTCTTCGAGACACGAT	i k 'Gaag
541 1621	L T T K Q M E V K H Q I I A A N V T R TTAACAACAAAGCAGATGGAAGTCAAACACCAAATCATCGCAGCGAACGTCACAAG	R GCGG
561 1681	KIRVFALAAPGDGDELDTE AAAATCCGTGTTTTTGCTCTTGCAGCACCGGGAGATGGCGATGAATTAGACACGGA	R AAGG
581 1741	H V V D D V A R G R H S L R G A Q L D CATGTTGTCGATGACGTAGCTGAGAGGCCGTCACAGTCTGAGAGGAGGCTCAACTCGA	TTAA
1801	ATGAGCATGTTTATCTTTACTTTCAACTGCGTGTTTTATTTCACTTACGTTTTATG	CTTT
1861	GTGTTTGTGTGTGTGGGCACTTGAACCAGGTACAGCTGGCAAGTGTTTCGGCATGG	TGTG
1921	GTTAGACAATTGGTTTGCACCGGTAGTCTAAGAAGCGCTATGTATCACGTGGTTGG	TTAA
1981	TTCATGGTTTATGTGGGTTAATCAAGAAGCGTTTATCACCCAAAAGGGTACCAAAA	AATG
2041	GTTGCGTCATTCATGGCGTAATCAGTTTTTGGAGTTTAGTTAG	TACC
2101	TAAAAGCC (A)	

Fig. 2. Nucleotide sequence of the 3' 2108 nucleotides of SPMMV genomic RNA. The predicted amino acid sequence is shown above the nucleotide sequence. The consensus motifs (T/S)GXXXTXXXN(T/S) and GDD characteristic for RNA-dependent RNA polymerases of positive strand RNA viruses are boxed (EMBL accession no. Z48058)

and Pot9 (a 21-mer with a 32-fold degeneracy) were synthesised (Fig. 1). To increase the stability of the primer-template duplex after the first cycles of amplification and to facilitate later cloning of the PCR products, sequences containing EcoRI or BamHI restriction sites were added at the 5' end of each primer, respectively.

Amplification of part of the putative RNA-dependent RNA polymerase of SPMMV

PCR amplification was performed on $polyA^+$ RNA isolated from SPMMVinfected *Nicotiana benthamiana*, using degenerate primers Pot8 and Pot9. A 440 bp fragment was amplified and cloned in pBluescript. Sequence analysis (Fig. 2, nucleotides 1 to 411) revealed the presence of the consensus motif (T/S)GXXXTXXN(T/S) at position 102–112 of the deduced amino acid sequence. This motif is characteristic of RNA-dependent RNA polymerases of positive-strand RNA viruses [7, 16, 24]. The amino acid sequence deduced from the 440 bp amplified fragment was 43.3% identical to the BaYMV sequence, and 48.9% to 51.9% identical to the sequences of four potyviruses (PPV, PVY, TEV and TVMV).

Amplification of the 3' terminal region of SPMMV genome

The RACE (Rapid Amplification of cDNA Ends) method [8] was used to amplify a cDNA fragment corresponding to the 3' terminal region of the SPMMV genome. Two nested specific primers, MVCP1 and MVCP2 (Fig. 1), were designed from the nucleotide sequence of the 440 bp amplified fragment. Amplification performed with the two nested specific primers and the adapted primer yielded a 1700 bp fragment; this PCR product was cloned in the pCRII vector.

Sequence analysis of the 3' terminal part of SPMMV genome

The 1 700 bp PCR product nucleotide sequence (Fig. 2, position 412 to 2108) was combined with that previously determined from the 440 bp fragment amplified with Pot8 and Pot9 degenerate primers. The complete nucleotide sequence of the 3' terminal part of SPMMV genome is presented in Fig. 2. Computer analysis revealed one large open reading frame (ORF), open at the 5' end and extending for 1797 nucleotides. The ORF has the capacity to encode 599 amino acids. It terminates in a UAA stop codon and is followed by an untranslated region of 311 nucleotides and a poly (A) tail.

The deduced SPMMV amino acid sequence was aligned with the corresponding sequences of four potyviruses (PPV, PVY, TEV and TVMV), one rymovirus (WSMV) and one bymovirus (BaYMV). This alignment indicated that the putative RNA-dependent RNA polymerase and coat protein coding regions of SPMMV are found consecutively at the 3' end of the genome, as for other *Potyviridae* (Fig. 3). However, the cleavage site between these two proteins was not determined.

Sequence comparison between the coat protein cores of SPMMV and other members of the Potyviridae

Pairwise percent sequence identities between the coat protein cores of SPMMV (equivalent to Q_{353} - R_{580}) and selected members of the *Potyviridae* are shown in Table 1. The sequence identities between SPMMV and selected potyviruses ranged from 25.7% to 29.9%. The coat protein core sequence from BaYMV displayed 17.6% identity with SPMMV. The coat protein core sequence identity between SPMMV and the rymovirus WSMV was 30.7%. The dendrogram of sequence relationship and the phylogenetic tree generated from the alignment of the coat protein core of SPMMV and those of selected members of the *Potyviridae* are shown in Fig. 4. Grouping of selected polyviruses was supported at 95% of the bootstrap replicates (results not shown).

Discussion

Available sequence data for the family *Potyviridae* permitted the design of two degenerate primers from conserved regions in the RNA-dependent RNA polymerase. The use of these primers and subsequent application of the RACE procedure [9] led to the cloning of 2 108 nucleotides from the 3' terminus of the whitefly-transmitted SPMMV genome. The amino acid sequence deduced from SPMMV cDNA was aligned with the corresponding sequences of selected members of the *Potyviridae* and revealed that the nucleotide sequence corresponded to the C-terminal region of a polyprotein including part of the putative

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PPV	2548	TPDQTXVKKKGMHEGQESTVVDNTLMVILAMTXSLLKL GYHPDTHDCECRYFVNGDDLVLAV
PVY	2573	PPDGTTVKKERGNWSGOPSTVVDNSLMVVLAMHYALIKE CVEFEEIDSTCVFFVMGDDELTAV
TEV	2575	TPDGTTTKEHKGNNSGODSTUNDETMUTTANI, TCEKC - GIN KRETU- VYUNGODSTTAT
m 177 M 177	2520	
TVMV	2520	TPDSTIVKAR KSARSAOPSTIVBALLMVVLARI XALSKLGVDINSQEDVCKFFANGDBEITAI
SPMMV	88	TIEGNILREFRONDSGOPSTVVDNTLILDHIAMEYA I AKVFVTRPDI - KYVCNGDDLLINC
WSMV	1	SKMERLCHSEEVITVANQAQLLTTEMCHIEANHECRIEANHECHIEANHEE-LEEPHENE
BAYMV	1909	LANGMVEKENVGTOR - OPSTYVENTEVENTAFLYBY IHKTGDRELALLNERFIFVCNODDNKFAT
0017	7611	UD X V P C T V D X = 1 A F U F C A X (X W W F X F F F V V F F T V X M Y W A X W Y Y A A X W Y Y A X W Y Y A X Y Y A X Y Y A X Y Y A X Y Y Y A X Y Y Y Y
EEV	2011	HARDSTIDE DELESCHERWITEATATENALELWARDARG - VLIDDATTEALEPERTVSTDA
BAX	2636	NPEKESILDR - MSQH#SDBGLNYD#SSRTRRKEELWFMSBRG LLIEGMYVPELEEBREVSILQ
TEV	2634	HPDKAERLER - MAESAGESGSKXEFACTTRDKTQLWMMAHRA LERDGMTTMKSEEEMAXVSILA
TVMV	2583	SPELEHVLDG - FOOHFSDEGEWYDFSSRTRDKKELWFMSBRA ESKDGILTPKIEPERIVSTIE
SPMMV	147	PRSTANATSEHEREVERADLSINVDENHUCDETEDUDEWSHSENWEDTEONVTEETUSTIE
WCWN	6.2	DEVELOVELOVELOVER FILME FREX VDC TREM VDC TREM TO THE TO THE TREM TO THE TREM TO THE TREM TO THE TREM
HOLLV	0.5	DIRDADI 10012ADIAREA BARISTULAIRSIELE INDATIA - ARNSHI 17RUARERIVALIE
BAYMV	1973	SPOFDEEFGHD#SPELVERGDT#EFODITSDICENPY#SLTMVKTPFGVGFSLPV#REI#TMQ
PPV	2673	「親D教装N葉PIHRLE演IC我SMV装AW袋菜KE録LR業業RX続YS被VLEQ美PYNALS核D供KAPY-IAET
PVY	2698	RORADLPEHRLEAICARMIESWEESELTHOERREYS WILLOOOPFATIAOREKAPY - TASM
TRV	2696	NDSSFEDSHELFSTCSSMTESWEEDELUFETENEVS
DT73/77	2010	
IVMV	2045	RDARARFAARLEALCASALRAWSTIDELQARARFIKATLEVEPIRSLAEQGAPI-LSEV
SPMMV	212	*ERDIROFRINSALNARYIESFGYEDIMIEIEKFAHFMAKKHGLNDVLMERE
WSMV	126	NOXXKXPKAIQXXIIXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
BAYMV	2036	WSKKGGVLHSYLAGISAIYZSENTPKEFKSEYAYLLWLTEEHEAEILAAMTOSSTALPIP
DD 17	2722	λ). UVXXXMM TO λ (D_{-} , R) TO UV D_{-} , λ SUMD SUDD (STRUCTURAL DED NOT SUDARTIDAL OF A UNITAL D
v	2752	ALKAN I EI BADE I EI EKILLE AFI DEFADOGEN VVN OADEREDEE VDAGRES VVI AF
PVY	2757	ALEKAEMDRAVDE EELRAETEMMVALDDEF ELDEYEVHHQAND TIDAGESSKKDAR
TEV	2755	ALKFAXTSQHGTN - AEIREYLKVLYD - YRIPTTENLYF QSGTVDAGADAGKKKD
TVMV	2704	ALERGETSQIATD NELTDYEKEILA - NNEFLRETVRF OSOTVDAGKDKARDOK
SPMMV	264	KVRSLEVOENFD ASE FEKEEPESFSPFBUVVEPHASESKTIER, OORMEBLDEDTT.
WSMU	187	PTD LGEFIVECGEOVCUVESSEAMDAXI.AAANSGEGSAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
DAVMIT	2006	
DAIMV	2090	SMLDV & KLH
PPV	2792	AATSPILQPPPVIQPAPRTTASMLNPIFTPATTQPATKPVSQVPGPQLQTFGTYGNEDASPSNSN
PVY	2813	PEQGSIGVNP
TEV	2807	OKDD-KVA
TWW	2756	
	N / U U	
CDWWT	220	
SPMMV	320	
SPMMV WSMV	320 249	GSGSGAAGGSGSGAAQTQSNNVSWAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGRÅ
SPMMV WSMV BAYMV	320 249 2139	GSGSGAAGGSGSGAAQTQSNNVSWAGLDTGGAKTOGGSGSKGTGGSFTSNPVRTGGRA DADRRR
SPMMV WSMV BAYMV	320 249 2139	ITYVQET KAZIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGAQTQSNNVSWAGLDTGGAKTGGGSSSKGTGGSFTSNPVRTGGR DADRRR
SPMMV WSMV BAYMV PPV	320 249 2139 2857	GSGSGAAGGSGSGAAQTQSNNVSWAGLDTGGAKTGGGSSSKGTGGSFTSNPVRTGGR DADRRR
SPMMV WSMV BAYMV PPV PVY	320 249 2139 2857 2823	GSGSGAAGGSGSGAAQTQSNNVSVMAGLDTGGAKTGQGSGSKGTGGSFTSNPVRTGGRÄ DADRRR
SPMMV WSMV BAYMV PPV PVY	320 249 2139 2857 2823 2814	ITVVQETVKQDQIEALRAQQIVRPPEA GSGSGAAGGSGSGSAQTQSNNVSWAGLDTGGAKTGGSSSKGTGGSFTSNPVRTGGR DADRRRKVEADEVEÄAEVKKAADAALKPVN-LTATRTPTEDDG ALVNTNRDRUWDAGSIGTTVFELKAMTS - KISL*KVKGKAIMN- LNHLAHKSFAQVDLSNTR NKGKDKNAGTSGTHTVFEIKAITA - KKNMFESKGAIVLH - LEHLLEFAEQQIDESNTR NKGKDKNAGTSGTHTVFEIKAITA - KKNMFESKGAIVLH - LEHLLEFAEQQIDESNTR
SPMMV WSMV BAYMV PPV PVY TEV	320 249 2139 2857 2823 2814	ITVVQBET@KAĞIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGAAQTQSNNVSWMAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGRÄ DADRRRKVEADRVEÄARVKKAADAALKPVN-LTATRTPTEDDG ALVNTNRDRBWDAGSIGTTVERLKAMTS - KISLKVKGKAIMN- LNHLAHYSFAQVDLSNTR NKGKDKDWNAGTSGTHVFEIKAITA - KMRMMESKGATVLH - LEHLLEXAEQQIDISNTR EQASKDRBWNAGTSGTFSVFINAMAT - KLQYFMRGEVVVN - LNHLLGYKEQQIDISNA
SPMMV WSMV BAYMV PPV PVY TEV TVMV	320 249 2139 2857 2823 2814 2764	ITVVQETVAGIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGGAQTQSNNVSVMAGLDTGGAKTGGGSSSKGTGGSFTSNPVRTGGRA DADRRRKVEADEVEARVKKAADAALKPVN.ITATRTPTEDDG ALVNTNRDRDWDAGSIGTFTVERLKAMTSKISLKVKGKAIMNLNHLAHKSFAQVDLSNTR NKGKDKDWAGTSGTHTVFIKAITAKNRM ESKGATVLHLEHLLEXAFQQIDISNTR SQASKDRDNAGTSGTFVFINAMATKQYFMRGEVVNN.LINLLGXFQQIDISNAR IDRTKDKDWNTGTSGTFSIFELKKAMNKLVKGGSSVVNLDHLLTXKFAQEFVVNTR
SPMMV WSMV BAYMV PPV PVY TEV TVV SPMMV	320 249 2139 2857 2823 2814 2764 351	GSGSGAAGGSGSGSAQTQSNNVSWAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGR ADDRR
SPMMV WSMV BAYMV PPV PVY TEV TEV TVMV SPMMV WSMV	320 249 2139 2857 2823 2814 2764 351 308	GSGSGAAGGSGSGAAQTQSNNVSWAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGRÄ DADRRR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV	320 249 2139 2857 2823 2814 2764 351 308 2181	ITVVQETOKAĞIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGSAQTQSNNVSVMAGLDTGGAKTGĞSSSKGTGGSFTSNPVRTGGRÄ DADRRKVEADEVEÄARVKAADAALKPVN-ITATRTPTEDDĞ ALVNTNRDRIWDAGSIGTFTVFELKAMTS - KISL¥KVKGKAIMN-INHLAHYSPAQVDLSNTR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV	320 249 2139 2857 2823 2814 2764 351 308 2181	ITVVQPETQKAĞIRDQIEALKAQQIVRPPEA GSGSGAAGGSGSGSAQTQSNNVSVMAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGR DADRRKVEADRVEÄARVKKAADAALKPVN-LTATRTPTEDDG ALVNTNRDRUVDAGSIGTTVFRLKAMTS - KISLFKVKGKAIMN-LNHLAHXSFAQVDLSNTR NKGKDKÜNAGTSGTHTVFRIKAITA - KMMI SKGATVLH - LKHLLEXAFQQIDLSNTR -EQASKDRÜNAGTSGTFSVRINAMAT - KLQVFRMGEVVVN - LNHLLGXKFQQIDLSNTR -IDRTKDKDVNAGTSGTFSVRINAMAT - KLQVFRMGEVVVN - LNHLLGXKFQQIDLSNAR CLQ
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV	320 249 2139 2857 2823 2814 2764 351 308 2181 2918	ITVQET KORNER GSGSGAAGGSGSGSAAQTQSNNVSVMAGLDTGGAKTGÖGSSSKGTGGSFTSNPVRTGGRÄ DADRR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV PPV	320 249 2139 2857 2823 2814 2764 351 308 2181 2918	ITVVQBETOKAĞIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGSAQTQSNNVSVMAGLDTGGAKTGÖGSSSKGTGGSFTSNPVRTGGRÄ DADRRKVEADEVEAARVKAADAALKPVN-ITATRTPTEDDG ALVNTNRDRUWDAGSIGTFTVFELKANTSKISL*KVKGKAIMNLNHLAHYSFAQVDLSNTR NKGKDKÖNAGTSGTHTVFELKAITAKMRMFESKGATVLHLEHLLE¥AEQQIDISNTR EQASKDRÜNAGTSGTFSVFEINAMATKLQVFMRGEVVVN-LINHLLG¥KEQQIDISNTR IDTKKDKÖNTGTSGTFSVFEINAMATKLQVFMRGEVVVN-LUHLLG¥KEQQIDISNTR DASKDRÜNAGTSGTFSVFEINAMATKLQVFMRGEVVVN-LUHLLG¥KEQQIDISNTR DOTKDKÖNTGTSGTFSVFEINAMATKLQVFMRGEVVVN-LUHLLE¥KEQEVVNT COPDVTFAQEVVTFEPEVTGFGALWIEBQORNYMTFSYIKKKAYVEHSNIZESGL TDVCHQTEGLVFPAFKITT-KAIVMERTVÄDKIKKEMXNNMIKTQERTELIDNRY KIKTPSGARIFSSÄADGNWSVFATKQVNÄGLTIKTELNKLKSVFKSVMÄHNDSV
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV PPV PVY	320 249 2139 2857 2823 2814 2764 308 2181 2918 2881	GSGSGAAGGSGSGSAQTQSNNVSWAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGR ALVNTNRDRUVDAGSIGTTVFRLKAMTSKISL*KVKGKAIMNLNHLAHXSFAQVDLSNTR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV BAYMV BAYMV PPV PVY TEV	320 249 2139 2857 2823 2814 2764 351 308 2181 2918 2881 2873	ITVVQETVVQETOKAŠIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGAAQTQSNNVSVMAGLDTGGAKTGŽGSŠSKGTGGSFTŠNPVRTGGRÄ DADRRKVEADEVEÄARVKKAADAALKPVN.ITATRTPTEDDĞ ALVNTNRDRIWDAGSIGTFTVFELKANTSKISLKKVKGKAIMNLNHLAHSPAQVDLSNTR NKGKDKDNAGTSGTHTVFELKAITAKNMFESKGATVLHLEHLLEXARQQIDISNTR DASKDRDNAGTSGTFSVFEINAMATKLSVFMRGEVVVNLNHLLGXKEQQIDISNTR DASKDRDNAGTSGTFSVFEINAMATKLVYFMRGEVVVNLNHLLGXKEQQIDISNTR DRTKDKDVNTGTSGTFSVFEINAMATKLVYFMRGEVVVNLNHLLGXKEQQIDISNTR DRTKDKDVNTGTSGTFSIFELKKAAMNKLKVGGSSVVNLDHLLTKFAQEFVVNT QQPDVTFAQVVTEFPEVSGFGAWIEQCMNYMTFSYIKIKAVEHSNIFESGL TDVQLQTFGLVFPAFKITT.KAIVMEKTVEDKIKFMINNMIKQQRTELIDNRY KIKTPSGARIPSSADGNWSVFATKQVNGLTKKILNKLKSVFKSVMKHNNSV KIKTPSGARIPSSADGNWSVFATKQVNGLTKKILNKLKSVFKSVMKHNDGETQV
SPMMV WSMV BAYMV PPV PVY TEV TEV SPMMV WSMV BAYMV PPV PVY FVY TEV TVMV	320 249 2139 2857 2823 2814 2764 350 2181 2918 2881 2918 28873 2823	GSGSGAAGGSGSGSAQTQSNNVSVMAGLDTGGAKTGQGSSKGTGGSFTSNPVRTGGR ALVNTNRDRUDAGSIGTTVVHLKAMTSKISL*KVKGKAIMNLNHLAHSFAQVDLSNTR NKGKDKDNAGTSGTHTVFIKAITAKMRMIESKGATVLHLHLLEFAEQQIDISNTR NKGKDKDNAGTSGTHTVFIKAITAKMRMIESKGATVLHLHLLEFAEQQIDISNTR DASKDRDNAGTSGTSVFINAMATKLQYFMRGEVVVNLNHLLGKKPQIDISNTR DASKDRDNAGTSGTSVFINAMATKLQYFMRGEVVVNLNHLLGKKPQIDISNTR DBTKKDKDVNAGTSGTSVFINAMATKLQYFMRGEVVVNLNHLLGKKPQIDISNTR DBTKKDKDVNAGTSGTSVFINAMATKLQYFMRGEVVVNLNHLLGKKPQIDISNTR DBTKKDKDVNAGTSGTSVFINAMATKLQYFMRGEVVVNLNHLLGKKPQIDISNTR DBTKKDKDVNAGTSGTSIFELKKAAMNMKLKVGGSSVVNLDHLLTKKAQEFVVNT CLQPDVTFAQTVFFEPFKVTGFGALWIFTQQKNYMTFYIKKIKAYVFHSMIFSGL TDVQUOTEGLVKPAKITT-KAIYMKTVKDKIKEMINNMIKYQERTELIDNKY KIKTPSGARIPSSAADGNWSVFATKQVNAGLTIKTTLNKLKSVPKSVMHNDGETQV ACCSFTDTYXGVKEBYDTDDE-MSIXINGLMVWCIENOTSPNINGMWVMNDGETQV THEGFAAHQAVMTAXGWNEQ-MKILINGFMWWCIENOTSPNINGWVMNDGEDQV
SPMMV WSMV BAYMV PPV PVY TEV SPMV WSMV BAYMV PVY TEV TVMV SPMWV	320 249 2139 2857 2823 2814 2764 351 308 2181 2918 2881 2918 2881 2873 2823 408	ITVQETVQET KANTRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGGAQTQSNVSVMAGLDTGGAKTGÖGSSKGTGGSFTSNPVRTGGRA DADRRGTGGSFTSNPVRTGGRA DADRRKVEADEVEARVKKAADAALKPVN.ITATRTPTEDDĞ ALVNTNRDRUDAGSIGTTVERLKANTSKISIKKVKGKAIMNLNHLAHKSFAQVDLSNTR DKGKDRUNAGTSGTHTVFIKAITAKMRM BSKGATVLHLKHLLEXAFQQIDISNTR DKGKDRUNAGTSGTFSVFRINAMATKQVFAMRGEVVN.LLHLLEXAFQQIDISNTR DRTKDKDVNTGTSGTFSIFELKKAMNMKLFKVGGSSVVNLDHLLTKFAQEFVVNT COPUTKQVVTEPPRVTGFGGWIFAQCNNMTFYIKKIKAVFHSNIESGL TDVQROTEGLVFPPRVTGFGGINFKVVDKIKFEMINNMIKYQERTEIDNRY KKTPSGARIFSSADGNWSVFATKQVNGLTKIFIKKLKSVFKSVMHNNSV
SPMMV WSMV BAYMV PPV PVY TEV TEV TVMV SPMMV BAYMV PVY TEV TVMV SPMMV SPMV	320 249 2139 2857 2823 2814 2764 351 308 2181 2918 2881 2873 2823 4062	ITVO
SPMMV WSMV BAYMV PVY PVY TEV TVMV BAYMV PVY TEV TEV TEV TEV SPMMV WSMV WSMV	320 249 2139 2857 2823 2857 2823 2814 308 2181 2918 2881 2881 2881 2823 2823 2823 2823 28	ITVVQET CAAGE AND
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV PPV TEV TVMV SPMMV SPMV BAYMV	320 249 2139 2857 2823 2814 2764 351 308 2181 2873 2881 2873 2823 408 362 2235	ITVQ ET GASGGSSGSGAQTQSNVSVMAGLDTGGAKTGGSSSK GTGGSFTSNPVTTGGRÄ DADRRR
SPMMV WSMV BAYMV PPV PVY TEV TEV SPMMV SPMV PVY TEV TVMV SPMMV WSMV BAYMV	320 249 2139 2857 2823 2857 2823 2764 351 308 2181 2918 2881 2918 2887 2823 408 2823 408 2235	ITVQ ET GKAGIR
SPMMV WSMV BAYMV PVY TEV TVMV SPMV BAYMV PVY TEV TVV TVV SPMMV WSMV BAYNV PPY	320 249 2139 2857 2853 2814 2764 3508 2181 2918 2881 2873 2828 408 362 2235 2975	ITVQET WQET WASTRDQIEARAQQIVRPPEA GSGSGAAGGSGSGGAQTQSNVSYMAGLDTGGAKTGGSSSKGTGGSFTSNPVRTGGRA DADRR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV PPV TEV TVMV SPMV BAYMV PPV PVY	320 249 2139 2857 2852 2814 2764 351 308 2918 2823 2823 408 2873 2823 408 2235 2975 2938	LTT VQ ET OKASIR DQIEARAQQIVR PPEA GSGSGAAGGSGSGAAQTQSNNVSVMAGLDTGGAKTGGSSSKGTGGSFTSNPVRTGGRÄ DADRR
SPMMV WSMV BAYMV PVY TEV TVMV SPMMV BAYMV PVY TEV TVMV SPMMV WSMV BAYMV PPV PVY TEV	320 249 2139 2857 2823 2857 2823 2764 351 2821 2821 2821 2821 2821 2823 2823 282	ITVQ ET WQ ET WASTE. DQIEARAQQIVRPPEA GSGSGAAGGSGSGGAQTQSNNVSVMAGLDTGGAKTGÖGSSKGTGGSFTSNPVRTGGRA DADRR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV BAYMV BAYMV PPV PVY TEV PPV PVY TEV TVMV	320 249 2139 2857 2823 2857 2823 2764 351 3081 2918 2873 2823 408 362 2235 2938 2938 2938 2938	LITVQBET OKASIRDQIEARAQQIVRPPEA GSGSGAAGGSGSGAAQTQSNNVSVMAGLDTGGAKTGGGSSSKGTGGSFTSNPVRTGGRÄ DADRRKGKDKDAGSIGTTVFELKANTSKISLKVKGKAIMA-LNHLAHSFAQVDLSNTR KGKDKDNAGTSGTHTVFELKANTSKISLKVKGKAIMA-LNHLAHSFAQVDLSNTR NKGKDKDNAGTSGTHTVFELKANTSKISLKVKGKAIMALNHLAHSFAQVDLSNTR NKGKDKDNAGTSGTHTVFELKANTSKISLKVKGKAIMALNHLAHSFAQVDLSNTR DASKDRDNAGTSGTSVFEINAMATKIQYFMRGGVVNLNHLAHSFAQVDLSNTR DASKDRDNAGTSGTFSVFEINAMATKIQYFMRGGVVNLNHLAHSFAQVDLSNTR DASKDRDNAGTSGTFSVFEINAMATKIQYFMRGGVVNLNHLAHSFAQUFDVNTR QLQPDVTFQQVXFEPFRVGGGGLWIFACGSSVVNLDHLLTKFAQEFVVNTR QLQPDVTFQQVXFEPFRVGGGGAWIFACQNWTFSYIEKIKAVVHSNLESGL TDVQLQTFGLVFPAFKITT-KAIYMFKTVXDKIKFEMINNMIKYQRTELIDNRY KIKTPSGARIPSSADGNWSVFATKQVNGLTKKILNKLKSVFKSVMKHNSV *PQSCFQTWYEQVKEDYDTDDE-MSIINGLMWCIENGTSPNINGWVMMDGETQV *THSGFKAMHTNVMAELELNEQ-MKILINGFMWCIENGTSPNINGWVMMDGEDQV *THSGFKAMHTNVMAELELNEQ.MKILINGFMWCIENGTSPNISGWVMMDGEDQV *THSGFKAMHTNVMAELELNEQ.MKILINGFMWCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVWTMMDGDQV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVXTMAMDGDPVV *THSGFKAMTTCRDVQVSMDVFMSTILFAWINCIENGTSPNISGVXTMA
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV BAYMV SPMV SPMV SPMV WSMV BAYMV PPV FVY TEV TVMV SPMV SPMV SPMV	320 249 2139 2857 2823 2857 2823 2813 308 2181 2918 2823 408 2823 408 2235 2823 2823 2823 2823 2823 2823 282	GSGSGAAGGSGSGAAQTQSNNVSVMAGLDTGGAKTGQGSSSKGTGGSFTSNPVRTGGRÄ DADRRR
SPMMV WSMV BAYMV PVY TEV TVMV SPMV BAYMV PVY TEV TVMV SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV	320 249 2139 2857 2853 2814 2764 3516 2181 2873 2823 408 2918 2873 2823 2938 2938 2938 2938 2938 2938 293	ITVQBET OKAB IRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGGAQTQSNNVSVMAGLDTGGAKTGÖGSSSGTGGSFTSNPVTTGGRA DADRRKVEADEVEARVKAADAALKPVN-LTATRTPTEDDG ALVNTNRDRBWDAGSIGTTVERLKAMTSKISLKKVKGKAIMN-LNHLAHKSFAQVDLSNTR NKGKDKDWAGTSGTHTVFEIKAITAKNRM BSKGATVLH-LEHLLEXAFQQIDISNTR DKGKDRDWAGTSGTHTVFEIKAITAKNRM BSKGATVLH.LEHLLEXAFQQIDISNTR DRTKDKDWNGTSGTFSIFELKKAMKIQY MRGGVVVN.LNHLGKKEQUDISNA DRTKDKDWNTGTSGTFSIFELKKAMNNKLFKVGGSSVVN.LDHLLTKFAQEFVVNT GQPUTKQUVTEPPRVTGFGGLWIFAQQNNTFIYIKKIKAVUHSNLESGL TDVQBQTEGLV.FFPRVTGFGGLWIFAQQNNTFIYIKKIKAVUHSNLESGL TDVQBQTEGLV.FFPRVTGFGGNNFV DKIKFEMINNMIKYQERTEIDNRY KIKTPSGARIFSSAADGNWSVFATKQVNGLTKKIELNKLKSVFKSVMHNNSV *PQSCFQTWYKGVKEDYDTDDF.MSIINGLMWCIENGTSPNIN.GWVMNDGETQV #THEGFAAHQAVMAXDIGETE.HPTVMDGLMWCIENGTSPNIN.GWVMNDGEDQV #THSGFKAWHTNVMAELELNEEQ.MKILINGFMIWCIENGTSPNIN.GWVMNDGEDQV #THSGFKAWHTNVMAELELNEEQ.MKILINGFMIWCIENGTSPNIN.GWVMNDGBQV #THSGFKAWHTNVMAELELNEEQ.MKILINGFMIWCIENGTSPNIN.GWVMNDGBQV #THSGFKAWHTNVMAELELNEEQ.MKILINGFMIWCIENGTSPNIN.GWVMNDGBQV #THSGFKAWTTCRYQVSSMUFMITILFAMINGILINGSOGTNEHTMEANIMANDEDQVII #TTEOINTHIKEASEGLDTEDVFINTLIPGWVHCIINTTPPNRALGTWRVNNAGKDNEQQI #LESEKAWTDAVTSLGITTBEAWIDALIFFIGWCCNNGTSDKHAENQV.MQDSKGAVTEM #HPIXFLLDHAKFTFRIVARSDVAEA.CVEKRNYEMAYMPRYGIQRNLTDYSLARYAFDYEV #THEOINTHIKEASEGLDTEDVFINTLEFGWVHCIINTTPPNRALGTWRVNNAGKDNEQQI #LESEKAWTDAVTSLGITTBEAWIDALIFFIGWCCNNGTSDKHAENQV.MQDIKAPFYYEV #ILENLLUHAKFTFRIVARSDVAEA.SIEMNRERYMPRYGIQRNLTDYSLARYAFDYEV #ILENLLUHAKFTFRIVARSDVAEA.SIEMNRERYMPRYGIQRNLTDYSLARYAFDYEV #ILENNVHANNSISQIINKHFSNLEE.SIEMNRERYMPRYGIQRNITDMSLSRYAFDYEV #ILENNVHANNSISQIINKHFSNLEE.SIEMNSLQVWEFTVKGAVTASYANVQDAWLGIDELRD
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMMV BAYMV BAYMV BAYMV PPV TEV TVMV SPMMV WSMV SPMV WSMV	320 249 2139 2857 2823 2857 2823 2823 2764 351 308 2918 2873 2828 2873 2828 2873 2823 408 2235 2975 2938 2930 2880 2830 2873 2823 2975 2938 2930 2857 2938 2930 2857 2938 2930 2857 2938 2930 2857 2823 2857 2823 2857 2823 2857 2823 2857 2823 2857 2823 2857 2823 2857 2823 2857 2823 2857 2823 2857 2857 2823 2857 2857 2857 2857 2857 2857 2857 2857	GSGSGAAGGSGSGAAQTQSNNVSVMAGLDTGGAKTGÜGSSSCGTGGSFTSNPVTTGGRÄ DADRRR
SPMMV WSMV BAYMV PVY TEV TVMV SPMV BAYMV PVY TEV TVMV SPMMV WSMV BAYMV BAYMV BAYMV	320 249 2139 2857 2823 2857 2823 2764 351 2873 2823 408 2918 2881 2873 2823 408 2235 2975 29380 2880 473 2298	LTVVQBET ØKA BIRDQIEALRAQQIVRPPEA GSGSGAAGGSGSGSAAQTQSNNVSVMAGLDTGGAKTGÖGSSSKGTGGSFTSNPVRTGGRA DADRR
SPMMV WSMV BAYMV PPV PVY TEV TVMV SPMV BAYMV PVY TEV SPMV BAYMV PPV PVY TEV TVMV SPMMV WSMV BAYMV	320 249 2139 2857 2823 2857 2823 2764 351 308 2918 2873 2823 408 2918 2873 2823 408 2235 2938 2938 2938 2938 2938 2938 2938 2938	LITVQ ET GKASIR DQIEARAQQIVRPPEA GSGSGAAGGSGSGGAQTQSNNVSVMAGLDTGGAKTGGSSSK GTGGSFTSNPVTTGGRÄ DADRR KVEADEVEÄARVKKAADAALKPVN- LTATRTPTEDDG ALVNTNRDRIVDAGSIGTTVFELKANTS - KISLKVKGKAIMN- LNHLAHSFAQVDLSNTR NKGKDKUNAGTSGTHTVFELKANTS - KISLKVKGKAIMN- LNHLAHSFAQVDLSNTR SQASKDRUNAGTSGTHTVFELKANTS - KISLKVKGKAIMN- LNHLAHSFAQVDLSNTR SQASKDRUNAGTSGTHTVFELKANTS - KISLKVKGKAIMN- LNHLAHSFAQVDLSNTR SQASKDRUNAGTSGTFSVFEINAMAT - KIQYFANGGVVVN- LNHLLGXKEQIDISNTR SQASKDRUNAGTSGTFSVFEINAMAT - KIQYFANGGVVVN- LNHLLGXKEQIDISNTR SQASKDRUNAGTSGTFSVFEINAMAT - KIQYFANGGVVN- LNHLLGXKEQIDISNTR SQASKDRUNAGTSGTFSVFEINAMAT - KIQYFANGGVVN- LNHLLGXKEQIDISNTR SQASKDRUNAGTSGTFSVFEINAMAT - KIQYFANGTGVNTFSYIEKIKAYVEHSNLESGL TDV OLOTEGUV- FPAFXITT-KA - IYMFKTVXDKIKFEMINNMIKYQERTEIIDNRY KIKTPSGARIPSSADGNWSVFATKQVNGLTKKILNKLKSVFKSVMKH NDSV VTCSSFDTYEAVKMAYDIGTDE - MEIINGLMVWCIENGTSPNIN - GMWVM NDGETQV ATGSSFDTYEAVKMAYDIGTDE - MEIINGLMVWCIENGTSPNIN - GMWVM NDGETQV THSGFKANHTNMABLELNEQ - MKILINGFMVWCIENGTSPNIN - GMWVM NDGEDQV THSGFKANHTNMABLELNEQ - MKILINGFMVWCIENGTSPNIS - GWVM NDGEDQV THSGFKANTTCRUVQSMUPHYTIFANVUNCIENGTSPNIS - GWVM NDGBDQV SSAQUTSSFFXNTCRUVQSMUPHYTIFANVUNCIENGTSPNIS - GWVM NDGBDQV SSAQUTSSFFXNTCRUVQSMUPHYTIFANVUNCIENGTSPNIS - GWVM
SPMMV WSMV BAYMV PVY TEV TVMV BAYMV BAYMV PVY TEV TVMV SPMMV WSMV BAYMV PVY TEV TVMV SPMMV BAYMV PPV PVY TEV TVMV SPMV	320 249 2139 2857 2857 2823 2857 2823 2018 2181 2881 2881 2881 2887 308 2181 2887 362 2938 362 2938 2930 2880 4737 2298 3039	LITYVQ ET GKAGIR
SPMMV WSMV BAYMV PPV PVY TEV TVMV BAYMV BAYMV PPV TEV TVMV SPMMV WSMV BAYMV PPV PVY TEV TEV TVMV SPMMV BAYMV PPV PVY PVY	320 249 2139 2857 2823 2857 2823 2814 2764 3551 2918 22918 22918 22918 22938 22938 2938 2938 2938 2938 2938 2	LTVVQBET ØKASIRDQIEARAQQIVRPPEA GSGSGAAGGSGSGSAQTQSNNVSVMAGLDTGGAKTGÖGSSSGTGGSFTSNPVTTGGRÄ DADRRCGTGSFTSNPVTTGGRÄ DADRRKVEADEVEAARVKKAADAALKPVN.LTATRTPTEDDÖ ALVNTNRDRBWDAGSIGTTVFELKANTSKISLKKVKGKAIMNLNHLAHSSAQVDLSNTR NKGKDRWNAGTSGTHTVFELKANTSKISLKKVKGKAIMNLNHLAHSSAQVDLSNTR NKGKDRWNAGTSGTHTVFELKANTSKISLKKVKGKAIMNLNHLAHSSAQVDLSNTR NKGKDRWNAGTSGTFSVFEINAMATKIQYEMRGEVVUN.LIHLLEXAFQQIDISNTR DRTKDKDWNGTSGTFSIFELKKAAMNMKLEKVGGSSVVNLDHLLTKFAQEFVVNTR QCPUTKQUVTEFFRVTGFGGLWIFAQQNVMTFYIKKIKAVVHSNLESGL TDVQUQTEGLVFFFRVTGFGGLWIFAQQNVMTFYIKKIKAVVHSNLESGL TDVQUQTEGLVFFFRVTGFGGLWIFAQQNVMTFYIKKIKAVVHSNLESGL TDVQUQTEGLVFFFRVTGFGGLWIFAQQNVMTFYIKKIKAVVHSNLESGL TDVQUQTEGLVKFVMGLGTKIKIENKLKSVFKSVMHNSV *PQSCFQTWYKGVKEDYDTDDF.MEIIINGLMVWCIENGTSPNINGWVMMDGETQV ATGSFTDTYKAVRMAYDIGETE.HFTVMDGLMVWCIENGTSPNINGWVMMDGETQV *THEGFAAMHQAVMTAXGWNEQ.MKILINGFMIWCIENGTSPNINGWVMMDGEQV *THSGKAWTTNMAELELNEQ.MKILINGFMIWCIENGTSPNINGWVMMDGEQV *THSGKAWTTCRXQVSSMVFWITILFANINGLINGFORMEVOLUNTSPNINGWVMMDGEQV *THSGKAWTTCRXQVSSMVFWITILFANINGLINGFORMEVOLUNTSPNISGUVTMMDGEQV *THSGKAWTTCRXQVSSMVFWITILFANINGLINGFORMEVOLUNTSPNISGUVTMMDGEQV *THSGKAWTTCRXQVSSMVFWITILFANINGLINGFORMEVOLUNTSPNISGUVTMMDGEQV *THSGKAWTTCRXQVSSMVFWITILFANINGLINGFORMEVOLUNTSPNIS.GUVTMMDGEQV *THSGKAWTTCRXQVSSMVFWITILFANINGLINGFORMEVONNAGKDNEQQ *THSGKAWTTRACKSSKAVTSSCHNATSTRACKSVYNPRYGIQRNLTDYSLARYAFDYEV STILLDHAKFTFRIVARSDVAEA.CVEKRNVERAYMPRYGIQRNLTDYSLARYAFDYEV STILKFILVENKFTFRIVARSDVAEA.SIEMNRESVYNPRYGIQRNLTDYSLARYAFDYEV STILKFILVENKFTFRIVARSDVAEA.SIEMNRESVYNPRYGIQRNLTDSLSRAFDYEV STILKFILVENKFTFRIVARSDVAEA.SIEMNRESVYNPRYGIQRNLTDYSLARYAFDYEV STILKFILVENKFTFRIVARSDVAEA.SIEMNRESVYNPRYGIQRNLTDSLSRAFDYVL EHP INSPLACTINGTAHFSDVAEA.SIEMNESUVFFYNKGAVTAGYANVQDAWLGIDFLRD EHP INSPLACTINGTHAFSDLEE.SIEMNESUVFFYNKGAVTAGYANVQDAWLGIDFLRD EHP INSPLACTINGTHKFSNLEE.SIEMNESVYTPYKGAVTAGYANVQDAWLGIDFLRD EHP INSPLACTINGTHKFSNLEE.SIEMN
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Fig. 3. Multiple alignment of the C-terminal 511 amino acids of SPMMV polyprotein with the corresponding protein sequences of BaYMV, PPV, PVY, TEV, TYMV and WSMV. Residues identical to SPMMV sequence are shadowed. Sequences were aligned using ClustalV

Table 1. Pairwise percent amino acid sequence identities between the core of the coat proteins of SPMMV and other selected members of the *Potyviridae*

	2	3	4	5	6	7
1 SPMMV 2 PPV 3 PVY 4 TEV 5 TVMV 6 WSMV	29.9	28.5 68.5	29.9 66.2 68.1	25.7 58.3 59.3 63.0	30.7 28.0 26.2 27.1 28.5	17.6 21.5 22.6 21.5 23.1 16.6
7 BaYMV						

Pairwise percent sequence identities calculated using ClustalV



Fig. 4. Taxonomic relationship between the core of the coat protein of SPMMV and those of selected members of the *Potyviridae*. a Sequence relationship dendrogram produced using ClustalV. b Phylogenetic neighbor-joining tree produced using ClustalV

RNA polymerase and all of the presumptive coat protein. The site of cleavage between these two proteins, generally V-x-x-Q/(A, S, G or V) common to all members of the *Potyviridae* [31], could not be determined. The RNA is polyadenylated and probably has a single large open reading frame typical of the *Potyviridae*.

Amino acid sequence analysis of the coat proteins of members of the *Potyviridae* has been used extensively for establishing the taxonomic status of viruses in this family [27, 30–33]. The conserved core of the coat protein defined four levels of sequence identity [31]. Among these, the lowest correlates with vector specificity and corresponds to genera [31]. The dendrogram of sequence relationship and the phylogenetic tree generated from the alignment of the coat protein core amino acid sequences demonstrated that SPMMV does not belong to any recognised genus in the *Potyviridae*. Coat protein core amino acid sequence identity between SPMMV and four typical potyviruses was 25.7–29.9%, in contrast to the 55–75% range of identities observed between distinct potyviruses [31]. Amino acid sequence identity with the bymovirus was even lower (17.6%). SPMMV seems somewhat closer to WSMV (30.7% coat protein core sequence identity). However, grouping of both viruses is not supported by bootstrapping.

In conclusion, sequence analyses revealed strong similarities between SPMMV and members of the *Potyviridae* in terms of structure and organisation of the genome and homologies in the putative RNA polymerase. However, limited coat protein core sequence identity with potyviruses, bymoviruses and rymoviruses validates the assignment of SPMMV to a distinct genus of the *Potyviridae*. This largest plant virus family would thus comprise four genera based on vector specificity and coat protein sequence data.

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