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Soybean Dwarf Luteovirus Contains the Third Variant Genome Type in the Luteovirus Group

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Complementary DNAs covering the entire RNA genome of soybean dwarf luteovirus (SDV) were cloned and sequenced. Computer analysis of the 5861 nucleotide sequence revealed five major open reading frames (ORFs) possessing conservation of sequence and organisation with known luteovirus sequences. Comparative analyses of the genome structure show that SDV shares sequence homology and features of gene organisation with barley yellow dwarf virus (PAV isolate) in the 5′ half of the genome, yet is more closely related to potato leafroll virus in its 3′ coding regions. In addition, SDV differs from other known luteoviruses in possessing an exceptionally long 3′ terminal sequence with no apparent coding capacity. We conclude from these data that the SDV genome represents a third variant genome type in the luteovirus group. © 1994 Academic Press, Inc.

INTRODUCTION

Soybean dwarf luteovirus (SDV) is a phloem-limited, aphid transmitted pathogen of over 50 leguminous plant species (Damsteegt *et al.*, 1990). Although its damaging effects have not been fully evaluated, it is regarded as a potentially serious threat to agriculture in the United States and can cause high yield losses in soybean crops in Japan (Smith *et al.*, 1991, and references therein). Additionally, SDV may constrain pastoral production by infection of subterranean clover, hence the Australasian synonym subterranean clover red leaf virus (SCRLV; Ashby and Johnstone, 1985).

Luteoviruses have isometric capsids containing a single-stranded positive-sense RNA genome. The genomic RNA is linked to a VPg (virally encoded genome-linked protein) and lacks a polyadenylate sequence at the 3′ end (Mayo *et al.*, 1982). The nucleotide sequences of several luteovirus genomes have been reported (Miller *et al.*, 1988a; Veidt *et al.*, 1988; van der Wilk *et al.*, 1989; Vincent *et al.*, 1991; Ueng *et al.*, 1992). These data indicate that the luteoviruses fall into two subgroups based on genome organisation (Figure 1A). In particular, variation exists primarily in the 5′ end of the genome where the genes (ORFs 1 and 2) thought to encode the viral components of the RNA replicase reside (Habili and Symons, 1989). Of the luteoviruses sequenced to date, the putative replicase genes bear homology to those of either the carmoviruses (barley yellow dwarf virus (BYDV)-PAV subgroup, including BYDV-PAV and -MAV) or the sobemoviruses (potato leafroll virus (PLRV) subgroup, including PLRV, beet western yellows virus (BWYV), and BYDV-RPV) (Martin *et al.*, 1990; Vincent *et al.*, 1991; Ueng *et al.*, 1992). Further distinctions between the groups are the presence of a unique open reading frame (ORF0) at the 5′ end of the PLRV-like luteoviruses and a small 3′ ORF (ORF6) present only in the BYDV-PAV subgroup. Serological relationships between viral coat proteins affirm this division of the luteoviruses (Martin *et al.*, 1990).

We now present the full 5861 nucleotide sequence of the SDV genome. Analysis of the gene structure indicates that SDV has a unique genome structure and as such is the third variant genome in the luteovirus group. The data provide further evidence for the role of genomic rearrangement in the evolution of the luteoviruses.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. L24049.

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MATERIALS AND METHODS

Maintenance of strains and virus purification

SDV isolate Tas-1 (Helms *et al.*, 1983) was maintained in subterranean clover (*Trifolium subterraneum* cv. Mount Barker). For large virus preparations, virus was transmitted to pea (*Pisum sativum* cv. sugar snap) by viruliferous *Aulacorthum solani* aphids. Infected material was harvested 3 weeks postinfection and virus purified by the method of Waterhouse and Helms (1984).

Cloning and sequencing of the SDV Tas-1 genome

SDV genomic RNA was extracted from purified virions and cDNA clones synthesised as described previ-

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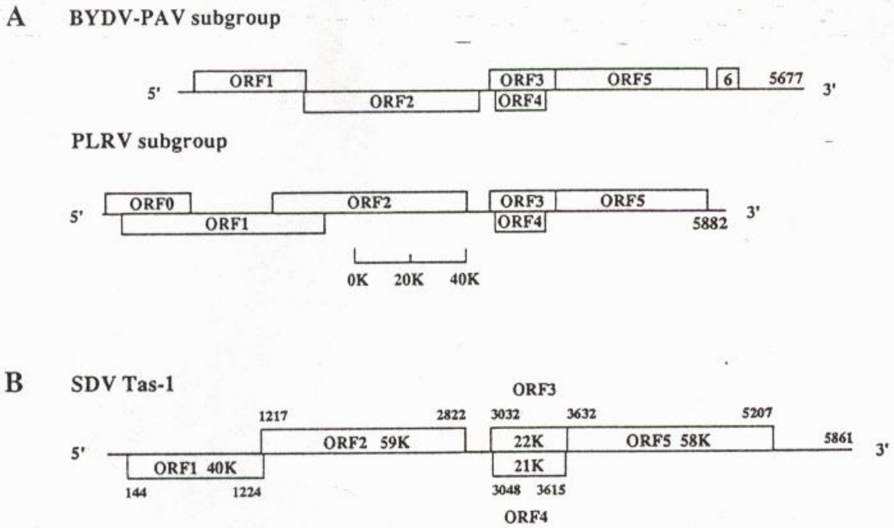

FIG. 1. Luteovirus genome organization. (A) Genome organization of representatives of two luteoviral subgroups. The virus genomes depicted are those of the Victorian isolate of BYDV-PAV and the Dutch isolate of PLRV (Miller *et al.*, 1988a; van der Wilk *et al.*, 1989). Open boxes represent open reading frames (ORFs). The scale refers to protein size after computer translation of each ORF. Numbers at the 3′ end of the genome denote the size of the respective RNAs in nucleotides. ORF0 has an unknown function; ORFs 1 and 2 contain helicase and polymerase motifs respectively (Habili and Symons, 1989); ORF3 encodes the viral coat protein; ORF4 possibly encodes the VPg; ORF5 possibly encodes the aphid transmission factor; ORF6 has an unknown function (Martin *et al.*, 1990). Only ORFs 3, 4 and 5 are homologous between the subgroups. The ORF numbering scheme is that of Martin *et al.* (1990). (B) Genome organisation of SDV Tas-1. Open reading frames (ORFs) were detected using the computer program DNA Strider version 1.1. The nucleotide positions of initiation and termination of the five major ORFs are shown, as are the sizes of proteins obtained by computer translation of the ORFs . The number at the 3′ end of the genome refers to the size of the SDV RNA in nucleotides. ORFs above the line are in the +1 reading frame; those below the line are in the -1 reading frame. No ORFs capable of encoding a protein of greater than 2.8K were found in the sequence 3′ of ORF5.

ously (Miller *et al.*, 1988a). cDNA clones were subcloned into M13mp18 and mp19 vectors and single-stranded DNA isolated, which was sequenced by the dideoxy method of Sanger *et al.* (1980) using kits supplied by Bresatec Ltd. (Adelaide, South Australia). A map of clones used to derive the full sequence of SDV Tas-1 is presented in Fig. 2.

Cloning of central region. A 1.2-Kb DNA fragment covering the central region not represented in the initial cDNA cloning experiment was amplified using standard reverse transcriptase/PCR conditions. Briefly, reverse transcription using AMV reverse transcriptase (Promega, Madison, WI), was primed from positive-strand viral RNA using the oligodeoxynucleotide primer SDV 3089 (5′-CTCTCGTAGGGCAGCAAGAC-3′, complementary to residues 3070–3089 of the SDV genome) in a reaction volume of 20 μl. One microliter of the cDNA product was amplified using *Taq* polymerase (Bresatec, Adelaide) and enzymically phosphorylated primers in a PCR reaction employing primer SDV 1853 (5′-ATAGCCAATAAATGGTCCAA-3′, homologous to residues 1853–1872 of the SDV genome) as the second-strand primer. Thermal cycling was performed for 30 cycles of 94° for 1 min, 50° for 1 min, and 72° for 1 min 30 sec in a FTS-1c Thermal Sequencer (Corbett Research, Sydney, New South Wales, Australia). The major PCR product of ~1.2 Kb was resolved by agarose gel electrophoresis and the band of interest purified using Geneclean (Bio101, La Jolla, CA), then blunt-end cloned into the *Sma*1 site of M13mp18 to create pSD01. This clone was restricted with *Sac*1 to release a 900 nucleotide fragment which was cloned into the *Sac*I site of pGEM1 (Promega) to give pSD11. Double-stranded sequencing was completed using synthetic oligodeoxynucleotides and the T7 Sequencing Kit (Pharmacia P-L Biochemicals, Milwaukee, WI).

RACE cloning of the 5′ end of the genomic RNA. This was performed largely as described by Frohmann (1990). First-strand cDNA was synthesised from the oligodeoxynucleotide SDV 621 (5′-CCTCCTTCTTCT-GAATGA-3′, complementary to residues 604–621 of the SDV genome) and purified from the primer and reaction components using a Qiagen column (TIP-5; DIAGEN, Düsseldorf, Germany). The cDNA was tailed with dATP using terminal deoxynucleotidyl transferase (Promega), then heated at 70° for 15 min to denature the enzyme. The reaction was diluted to 200 μl with TE buffer (10 m*M* Tris–HCl, pH 8.0, 1 m*M* EDTA), and 1 μl of this solution was used in the PCR. PCR amplification of the cDNA employed primer SDV 282 (5′-GTGCAG-CAAACACGCCTTGGAG-3′; complementary to residues 261–282) as the specific primer and the adaptor-primer A-RACE (5′-GACTCGAGATCGA$[T]_{17}$-3′; a gift of Dr. P. Rathjen). The reaction was conducted over 45 cycles composed of 94° for 5 sec, 55° for 5 sec, and 72° for 30 sec using Vent DNA polymerase (New England Biolabs, Beverly, MA) in a capillary Thermal Sequencer (Corbett Research). The major reaction product of 300 nucleotides was resolved on a 2% agarose minigel in TBE running buffer (90 m*M* Tris–borate, pH 8.3, 2 m*M* EDTA) and cloned into the *Sma*I site of pBluescript SK$^+$ (Stratagene, La Jolla, CA) to create pSD5RACE. Double-stranded sequencing of the cloned cDNA was performed as described above.

RACE cloning of the 3′ end of the genomic RNA. Total RNA was isolated from SDV Tas-1-infected pea essentially as described by Dunsmuir *et al.* (1988). One microgram of the RNA was treated with poly(A) polymerase (Bresatec, Adelaide), then reverse-transcribed using AMV reverse transcriptase (Promega) using A-RACE as the primer. PCR was performed on the first-strand cDNA using primers A-RACE and SDV 5178 (5′-GGGCATATATCGATGGTTTA-3′; homologous to residues 5178–5197) and Vent DNA polymerase (New England Biolabs). The reaction consisted of 40 cycles of 94° for 5 sec, 51° for 5 sec and 72° for 45 sec and was carried out on a capillary Thermal Sequencer (Corbett Research). Reaction products were cloned into pBluescript (Stratagene) and sequenced double-stranded, as described above.

Cloning of the 3′ end of the SDV AP-1 genome

Total RNA was extracted from a whole young subterranean clover plant infected with SDV isolate AP-1 (a kind gift of Dr. G. Johnstone, Department of Primary Industries, Tasmania, Australia) according to the method of Maes and Messens (1992). One hundred nanograms of the RNA was reverse-transcribed by AMV reverse transcriptase (Promega) using SDV3TERM (5′-GGGGCAGGTGGACACAAAG-3′; complementary to residues 5843–5861 of the SDV Tas-1 genome) as the first strand primer in a reaction volume of 20-μl. Part of the cDNA (5%) was amplified by Vent polymerase (New England Biolabs) in a 20-μl reaction employing SDV 5178 as the second strand primer. The reaction was conducted over 40 cycles of 94° for 5 sec, 49° for 5 sec, and 72° for 30 sec and was performed on a capillary Thermal Sequencer (Corbett Research). The major reaction product of 680 nucleotides was gel purified and cloned into pBluescript (Stratagene) as described above. The insert was subcloned prior to double-stranded sequencing.

Computer analysis of sequences

Sequences were assembled with the package of Staden (1980) and analysis performed using the UWGCG programs (Devereux *et al.*, 1984) or DNA Strider version 1.1. Default values of 3.00 for gap weight and 0.10 for gap length weight were used with the UWGCG program GAP in amino acid sequence comparisons.

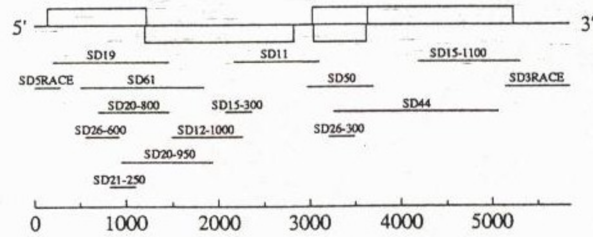

FIG. 2. cDNA clones used to derive the sequence of SDV Tas-1. All clones used to derive the nucleotide sequence presented in Fig. 3 are shown. The scale indicates the size of the clones in nucleotides. The upper part of the figure is a schematic representation of the organization of the SDV genome (ORFs are boxed).

RESULTS

Sequencing of SDV RNA

The sequence of SDV isolate Tas-1 (Fig. 3) was derived from 15 indepedent clones (Fig. 2). Both strands of all clones were sequenced, and 81% of the genome was determined from more than one independently isolated cDNA clone. Multiple clones obtained from the 5′ and 3′ RACE reactions were sequenced in order to determine the precise termini of the viral genome. Of these, variation was seen predominately as the presence or absence of the terminal nucleotide (data not shown). Further confirmation of the termini is provided by the close homology of the 5′ end to that of BYDV-PAV (Fig. 4; Miller *et al.*, 1988a; Ueng *et al.*, 1992), and the presence of the 5′-CCC-3′ motif at the 3′ end that also occurs at the 3′ genomic terminus of a number of viruses possessing a carmovirus-like polymerase ORF; carnation mottle virus (CarMV; Guilley *et al.*, 1985); BYDV-PAV (Miller *et al.*, 1988a); turnip crinkle virus (Carrington *et al.*, 1989); the RNA associated with BWYV strain ST9 (Chin *et al.*, 1993); and pea enation mosaic virus RNA 2 (Demler *et al.*, 1993). Despite these arguments, we cannot formally exclude that the 5′ terminal nucleotide is a U, or that the 3′ terminal nucleotide is an A, because of the RACE strategy used to obtain these results.

Genome organization of SDV

The 5861 nucleotide sequence of SDV Tas-1 RNA, together with the deduced amino acid sequences of the ORFs, is presented in Fig. 3. The positive-sense strand specifies 5 major ORFs arranged in two groups (Figure 1B). ORF1 begins after a leader sequence of 143 nucleotides and encodes a protein of M_r 40K. ORF1 overlaps ORF2 by 7 nucleotides. The coding sequence of ORF2 between successive in-frame stop codons specifies a protein product of M_r 59K. By analogy to BYDV-PAV, ORF2 is likely to be expressed as a ~99K frameshift product of the first and second reading frames. This is consistent with the presence of a sequence 5′-AGGUUUU-3′ at the overlap of ORFs 1 and 2 which strongly resembles the potential "shifty" heptanucleotide 5′-GGGUUUU-3′ proposed to mediate frameshifting in BYDV-PAV (Brault and Miller, 1992). ORFs 1 and 2 contain helicase and polymerase motifs respectively (Habili and Symons, 1989), and probably constitute the viral components of the replicase enzyme. The organisation of this part of the genome closely resembles that of the BYDV-PAV subgroup of luteoviruses (see below).

A non-coding intergenic sequence of 210 nucleotides separates ORFs 1 and 2 from the second block of coding sequence. The characteristic arrangement of the 3′ block of three genes common to all luteoviruses (ORFs 3, 4, and 5; Martin *et al.*, 1990) is conserved in SDV. ORF3, which encodes the 22K coat protein, is the first ORF to initiate after the non-coding sequence. Confirmation of this ORF as the coat protein gene (as is the case for all other luteoviruses sequenced to date) has been obtained by sequencing of tryptic fragments of the purified coat protein monomer (data not shown; see Miller *et al.*, 1988b). ORF4, which is completely contained within the coat protein gene, extends for 567 nucleotides thus encoding a protein of M_r 21K. It is believed that this gene encodes the VPg in the luteovirus group (Martin *et al.*, 1990).

ORF5 is contiguous and in-frame with ORF3, but separated by a UAG (amber) stop codon. The reading frame specifies a protein product of M_r 48K when calculated from the first methionine residue, although it is likely that ORF5 is expressed as a readthrough protein from ORF3, as demonstrated for other members of the luteovirus group (Tacke *et al.*, 1990; Bahner *et al.*, 1990; Dinesh-Kumar *et al.*, 1992). Further evidence for this mode of expression is provided by the sequence context flanking the ORF3 stop codon, 5′-AAA*UAG*-GUAGA-3′, which is identical in all luteoviruses (Dinesh-Kumar *et al.*, 1992). Such an expression strategy would give a protein product of M_r ~80K. A large 3′ untranslated region (UTR) of 654 nucleotides follows the UAG stop codon of ORF5.

Nucleotide analysis of the terminal 680 nucleotides of SDV isolate AP-1

The 3′ untranslated region of SDV Tas-1 (654 nucleotides) is longer than expected given the lack of coding potential in this region. Therefore, we sequenced the 3′ end of a second isolate, SDV AP-1, in order to confirm the non-coding nature of the SDV sequence 3′ of ORF5. Thirty-seven nucleotide changes relative to the sequence of SDV Tas-1 were found, including five deletions and one insertion (Fig. 3). Translation of the sequence in three reading frames revealed only one ORF of appreciable size, extending from bases 5654 to 5788 of the SDV Tas-1 genome and capable of encoding a protein of M_r 5K. However, this ORF is not conserved in SDV Tas-1. Furthermore, the ORF shares no significant homology at the nucleotide or amino acid level with ORF6 of BYDV-PAV or BYDV-MAV. Therefore, for lack of any evidence to the contrary, we conclude that this ORF is not expressed and that the 3′ terminal ~650 nucleotides of SDV do not contain any coding sequence.

Comparisons with other luteoviruses

The 5′ half of the SDV Tas-1 genome (comprising the 5′ UTR, and ORFs 1 and 2) resembles that of BYDV-PAV (Fig. 1). First, the length of the 5′ leader sequence is 143 nt versus 141 nt for the Victorian isolate of BYDV-PAV (Miller *et al.*, 1988a). There is extensive similarity between the first ~42 nucleotides of the SDV and BYDV-PAV genomes (33 out of 42 nucleotides after the addition of gaps; Fig. 4). Furthermore, the first two reading frames of SDV and BYDV-PAV show strong homology. Alignment of the amino acid sequences of ORF1 of SDV Tas-1 and BYDV-PAV (Victorian isolate; Miller *et al.*, 1988a) reveals that 32.0% of the residues are identical after the addition of gaps. Sequence comparisons of ORF2, which contains the GDD amino acid motif common to all plant viral putative RNA polymerases, are presented in Table 1. These data show that SDV ORF2 is highly homologous to BYDV-PAV ORF2 (~61% identity after addition of gaps). Additionally, SDV ORF2 shows significant homology (34.4%) to the polymerase gene of CarMV (as do members of the BYDV-PAV subgroup), but not to that of PLRV (15.6%) or southern bean mosaic virus (SBMV; 21.4%). SDV therefore contains a carmovirus-like polymerase gene, rather than the sobemovirus-like protein of the PLRV subgroup (van der Wilk *et al.*, 1989).

Contrary to the above results, SDV shows greater homology to the PLRV than the BYDV-PAV subgroup over the region comprising the intergenic UTR and the 3′ coding block (ORFs 3, 4, and 5). Specifically, the intergenic region of SDV (210 nt) is longer than that of BYDV-PAV (116 nt) and of similar size to PLRV (200 nt). Amino acid sequence comparisons between luteoviral coat protein genes (ORF3) are presented in Table 2. These data clearly establish the close relationship between SDV and the PLRV subgroup at this point in the genome. Similar results were obtained for ORFs 4 and 5 (not given). A notable feature of the SDV genome is the size of ORF4, which would encode a protein of 21K

FIG. 3. The nucleotide sequence of SDV Tas-1 and translations of the major ORFs. Numbers refer to nucleotide positions in the genome. Amino acid sequences of the 5 major ORFs are given as single-letter abbreviations. The number of each ORF is indicated on the left hand side of the diagram. Nucleotides variant in the sequence of SDV isolate AP-1 are represented in lowercase letters at the 3′ end of the genome; Δ refers to a deletion, + indicates an insertion. Sequences used to derive the primers SDV3TERM and SDV 5178 are underlined.

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1    AGUAAAGUUGACACCUUUACAGAAGUGGUCUUACUUGUAAGAGUUAACUCAUCAAGAGUUAAUAUAAGAUCCACCUCCCG   80
          10                  30                  50                  70

                                                                         M  F  N  F  D  S—
81   GCACCUUCGUAUCGUGUUUGAGGUAUCUCUAGUGUUUGGUUGUUUAAAUACUAGCUAAUAUUCAUGUUUAAUUUCGAUAG   160
          90                  110                 130                 150

ORF1  L  V  S  A  T  A  K  V  V  K  D  F  I  H  F  C  Y  N  R  A  R  H  V  Y  Y  A  L
161  UUUAGUGUCGGCCACCGCCAAGGUGGUCAAAGAUUUUAUUCAUUUUUGUUAUAAUAGGGCGAGGCACGUAUAUUAUGCCC   240
          170                 190                 210                 230

ORF1   K  R  W  L  W  E  L  Q  G  V  F  A  A  H  D  A  F  V  D  M  C  Y  D  A  M  Y
241  UCAAACGCUGGCUUUGGGAACUCCAAGGCGUGUUUGCUGCACAUGAUGCCUUUGUGGACAUGUGCUACGACGCCAUGUAU   320
          250                 270                 290                 310

ORF1 G  V  E  E  F  E  W  E  L  Q  K  Q  F  S  S  A  E  H  D  V  L  I  A  K  H  E  F
321  GGCGUCGAGGAGUUUGAGUGGGAGUUGCAAAAGCAAUUCUCCAGUGCCGAACAUGAUGUGCUCAUCGCCAAGCACGAAUU   400
          330                 350                 370                 390

ORF1  E  R  L  L  K  D  G  A  P  L  R  T  W  P  Q  P  C  A  P  L  G  S  F  R  S  S  D
401  UGAGCGCCUAUUGAAAGAUGGCGCCCCAUUGAGGACAUGGCCACAACCAUGUGCUCCUUUGGGUAGUUUCCGGUCGUCUG   480
          410                 430                 450                 470

ORF1   D  F  Q  E  A  A  R  E  V  K  T  V  L  D  G  P  E  P  S  L  I  K  G  S  G  D
481  ACGACUUCCAAGAAGCUGCCAGGGAAGUGAAAACUGUCCUUGAUGGACCUGAACCCUCCUUGAUUAAAGGGUCAGGAGAU   560
          490                 510                 530                 550

ORF1 Y  S  L  D  N  P  N  R  I  E  K  F  I  N  L  I  Q  K  K  E  V  L  S  A  T  E  R
561  UACUCACUUGACAAUCCUAACCGGAUUGAAAAGUUUAUCAACCUCAUUCAGAAGAAGGAGGUACUUUCCGCCACCGAGCG   640
          570                 590                 610                 630

ORF1  M  I  K  H  A  Y  E  E  H  I  G  E  A  P  F  G  K  W  F  N  T  L  P  S  R  M  D
641  AAUGAUCAAACAUGCUUAUGAAGAGCAUAUCGGUGAGGCACCAUUCGGAAAGUGGUUCAACACUUUGCCCUCUCGUAUGG   720
          650                 670                 690                 710

ORF1   Y  I  K  R  A  A  S  K  R  A  K  A  A  K  R  S  N  S  I  R  Q  M  V  E  E  V
721  ACUACAUCAAGAGGGCCGCUUCAAAGAGAGCCAAGGCGGCUAAAAGAUCCAACUCUAUCCGCCAAAUGGUAGAAGAGGUA   800
          730                 750                 770                 790

ORF1 N  V  I  P  D  F  I  S  I  C  D  V  V  Q  V  D  T  G  E  K  L  P  P  K  K  D  K
801  AAUGUCAUUCCUGACUUUAUCUCAAUAUGUGAUGUUGUCCAGGUGGACACAGGUGAGAAACUCCCCCCAAAGAAAGACAA   880
          810                 830                 850                 870

ORF1  D  G  E  P  M  E  P  E  P  K  L  K  M  V  R  R  V  R  F  E  H  Y  G  D  A  R  K
881  AGAUGGGGAGCCAAUGGAACCUGAACCCAAACUAAAAAUGGUGAGAAGGGUUAGGUUCGAGCAUUAUGGUGAUGCUCGUA   960
          890                 910                 930                 950

ORF1   Y  I  R  Q  H  I  R  N  N  N  M  R  L  T  D  G  S  D  V  S  H  A  T  I  N  R
961  AGUACAUAAGACAGCAUAUUCGCAACAACAACAUGCGUCUUACUGACGGCUCUGAUGUUAGUCAUGCUACCAUCAACCGC   1040
          970                 990                 1010                1030

ORF1 Y  A  L  K  F  C  E  D  L  E  L  D  M  T  S  T  C  Y  A  G  D  Y  A  M  T  M  V
1041 UAUGCUCUUAAGUUUUGCGAAGACUUGGAGCUUGAUAUGACCAGUACUUGCUAUGCUGGUGAUUAUGCUAUGACAAUGGU   1120
          1050                1070                1090                1110

ORF1  P  I  P  L  K  N  D  I  E  R  A  K  I  V  H  S  P  A  A  R  Q  I  R  Q  E  L  G
1121 GCCCAUUCCCCUCAAGAAUGACAUUGAAAGGGCGAAGAUUGUCCAUUCUCCUGCAGCCAGGCAAAUCAGGCAGGAGUUGG   1200
          1130                1150                1170                1190

ORF2                G  F  L  E  G  L  C  S  D  S  G  F  E  S  P  F  S  I  L  G  L  P
ORF1  V  L  N  A  E  V  F
1201 GCGUUCUCAACGCUGAGGUUUUUUAGAGGGGCUCUGCUCCGACUCUGGUUUUGAAUCCCCGUUUUCUAUUUUGGGGUUAC   1280
          1210                1230                1250                1270

ORF2  E  I  V  V  R  S  G  A  A  P  R  K  S  R  S  V  I  S  F  L  S  Q  F  T  L  G
1281 CAGAGAUCGUGGUUCGCAGUGGAGCUGCACCUAGGAAGAGUCGUAGUGUAAUUAGUUUUUUAUCGCAGUUUACCUUAGGU   1360
          1290                1310                1330                1350

ORF2 L  D  Y  Q  C  P  N  P  S  L  H  N  A  L  V  A  V  E  R  R  V  F  T  V  G  K  G
1361 CUAGAUUAUCAAUGCCCAAAUCCCAGUUUACACAAUGCAUUGGUGGCGGUUGAACGACGUGUGUUCACCGUUGGGAAAGG   1440
          1370                1390                1410                1430

ORF2  N  E  V  V  L  P  Y  K  N  K  P  G  I  F  S  N  L  D  Y  F  R  D  S  I  V  N  K
1441 AAAUGAGGUAGUGCUACCUUACAAGAACAAACCAGGAAUAUUUUCCAAUCUUGAUUACUUUAGAGACUCAAUUGUCAACA   1520
          1450                1470                1490                1510

ORF2   V  G  C  P  R  T  H  T  P  E  E  L  A  A  T  Y  H  S  G  K  R  S  L  Y  N  A
1521 AAGUUGGCUGUCCGAGGACCCACACUCCUGAGGAACUUGCUGCAACGUACCACUCUGGAAAGAGAAGUUUGUAUAAUGCU   1600
          1530                1550                1570                1590

ORF2 A  V  Q  S  L  K  K  K  A  V  E  R  S  D  A  N  V  T  A  F  L  K  M  E  K  H  L
1601 GCAGUUCAAAGCCUCAAAAAGAAGGCAGUCGAAAGGAGUGAUGCCAAUGUGACAGCUUUUCUCAAGAUGGAAAAACAUUU   1680
          1610                1630                1650                1670

ORF2  M  S  K  K  I  A  P  R  L  I  C  P  R  N  K  R  Y  N  V  E  L  G  R  R  L  K  F
1681 AAUGAGUAAGAAGAUAGCACCCAGGUUGAUAUGUCCCCGCAACAAACGGUAUAAUGUUGAAUUGGGACGUCGAUUGAAGU   1760
          1690                1710                1730                1750

ORF2   N  E  K  K  F  M  H  A  I  D  S  T  F  D  S  P  T  V  L  S  G  Y  D  S  F  R
1761 UCAAUGAGAAGAAAUUUAUGCAUGCAAUCGACUCAACCUUUGAUUCCCCAACUGUUCUUAGUGGAUAUGACAGUUUCAGA   1840
          1770                1790                1810                1830

ORF2 V  G  K  I  I  A  N  K  W  S  K  F  K  R  P  V  A  I  G  V  D  A  S  R  F  D  Q
1841 GUUGGGAAGAUAAUAGCCAAUAAAUGGUCCAAAUUCAAGAGACCAGUUGCAAUAGGUGUUGAUGCGAGCAGAUUUGAUCA   1920
          1850                1870                1890                1910

ORF2  H  V  G  V  E  A  L  Q  W  E  H  S  I  Y  N  G  A  F  K  D  P  I  L  K  E  L  L
1921 ACAUGUGGGGGUAGAAGCACUCCAAUGGGAGCACUCAAUUUACAACGGUGCAUUCAAAGAUCCCAUUCUUAAGGAGUUGC   2000
          1930                1950                1970                1990
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ORF2  H W Q T E N R I M L F V E D K I L K F K V K G H R M
2001  UACACUGGCAAACAGAGAAUAGAAUUAUGCUGUUUGUUGAAGAUAAAAUCCUCAAGUUCAAGGUCAAAGGACAUAGAAUG  2080

ORF2  S G D I N T S S G N K L I M C G M M H Y Y F K T L G V
2081  UCCGGCGACAUUAACACCUCUUCUGGCAACAAAUUAAUCAUGUGUGGUAUGAUGCACUACUACUUCAAAACUCUUGGAGU  2160

ORF2  K A E L C N N G D D C V I I C E R K D E N K F Q H M H
2161  CAAAGCCGAGCUCUGCAAUAACGGCGAUGAUUGUGUUAUCAUAUGCGAGCGGAAAGAUGAGAACAAAUUCCAACACAUGC  2240

ORF2  S W F K D Y G F D M Q I E T P V Y K I G Q I E F C Q
2241  ACAGCUGGUUUAAAGACUAUGGGUUUGACAUGCAGAUUGAGACUCCUGUCUACAAGAUUGGACAGAUAGAGUUUUGUCAA  2320

ORF2  S K P V K I N G Y Y R M V R K P E S I S K D A H S L I
2321  AGUAAACCAGUUAAAAUUAAUGGCUAUUAUAGGAUGGUGCGUAAACCAGAGAGCAUAUCCAAAGACGCCCAUUCCCUCAU  2400

ORF2  S M A S A E D V K T F M S A T A Q C G M I L N S G V P
2401  UUCGAUGGCAUCAGCUGAAGAUGUCAAAACUUUCAUGAGUGCAACCGCCCAGUGCGGUAUGAUCUUGAAUAGUGGUGUAC  2480

ORF2  V L D A F H K C L F K A S G Y K K V S Q E A I E R I
2481  CUGUUCUUGAUGCUUUUCACAAAUGCCUAUUUAAAGCAUCGGGGUAUAAGAAAGUAUCGCAAGAAGCUAUCGAAAGGAUA  2560

ORF2  V S F G T Q D K L G L R K E R V E E P I T M D N R L S
2561  GUCUCUUUUGGAACACAGGACAAACUCGGGCUCAGAAAAGAGCGAGUCGAAGAACCAAUAACAAUGGAUAACAGGUUGAG  2640

ORF2  Y W E S S G V D P Q T Q V L V E R Y F D N L T V H I E
2641  UUAUUGGGAGUCCAGUGGGGUUGACCCUCAGACACAGGUCCUUGUCGAACGAUAUUUUGACAAUCUGACGGUCCAUAUCG  2720

ORF2  P R G V K R L T P L L D K T L L S I A S V A R K S V
2721  AACCCCGCGGUGUAAAGAGAUUGACGCCUUUACUAGAUAAAACUUUGCUUUCAAUUGCGAGUGUAGCACGAAAAUCUGUG  2800

ORF2  S L P I L S K
2801  UCGCUACCAAUACUUUCAAAAUAGAAGCACUCAUAGUAACCAUAGUAAUAAUAUUAGUAGUAGUAAUUAGUUUAUUAGAU  2880

2881  UACGCUAGUUCGUACAGAUAAAUUGCAACGGAUUAUAAAUUCCUAUCAGGUUUUGCUUCUGGAUUUAUUUCCAGCAUCCC  2960

ORF3                                                                       M V A
2961  UGUAUCAAUAUUGGCAAUUUAUUUUGUUUACCUAAAAAUUUCAAAGAAUCUGCGCGAGAUAAUCAACGAGUAUGGUCGCG  3040

ORF4     M S Q Y N D D A L V G Q Q D A L Q E F S S W L F Q
ORF3  V S N V A I Q R R R S R R A A R R A P R V Q L M A V P
3041  GUUAGCAAUGUCGCAAUACAACGACGACGCUCUCGUAGGGCAGCAAGACGCGCUCCAAGAGUUCAGCUCAUGGCUGUUCC  3120

ORF4  R P P A D H N A E D D N D D E G E I I E E E A L F P
ORF3  T A T S R P Q R R G R Q R R R R N N R G G S F V S G
3121  AACGGCCACCAGCCGACCACAACGCAGAGGACGACAACGACGACGAAGGAGAAAUAAUAGAGGAGGAAGCUUUGUUUCCG  3200

ORF4  E D Q A R L T H S C F Q R T A S M V V P R E V S L S G
ORF3  G S G K A H T F V F S K D G I N G S S K G S I T F G
3201  GAGGAUCAGGCAAGGCUCACACAUUCGUGUUUUCAAAGGACGGCAUCAAUGGUAGUUCCAAGGGAAGUAUCACUUUCGGG  3280

ORF4  R L Y Q N A S H S L M E Y S R P T M N I R S R V S Y Y
ORF3  P S L S E C K P F S D G I L K A Y H E Y K I T S V L L
3281  CCGUCUUUAUCAGAAUGCAAGCCAUUCUCUGAUGGAAUACUCAAGGCCUACCAUGAAUAUAAGAUCACGAGUGUCUUAUU  3360

ORF4  S S S P R P L P P R Q V P S L M N L T H T A S T P K
ORF3  Q F I T E A S S T S S G S I A Y E L D P H C K Y S E I
3361  ACAGUUCAUCACCGAGGCCUCUUCCACCUCGUCAGGUUCCAUCGCUUAUGAACUUGACCCACACUGCAAGUACUCCGAAA  3440

ORF4  F N R Y S I N S V S Q R A V R N V S Q P E L S M A S N
ORF3  Q S L L N K F S I T K S G S K R F P T R A I N G L E
3441  UUCAAUCGCUACUCAAUAAAUUCAGUAUCACAAAGAGCGGUUCGAAACGUUUCCCAACCAGAGCUAUCAAUGGCCUCGAA  3520

ORF4  G M I P V R I N S R S T I K G T E S P R S Q A P S R S
ORF3  W H D T S E D Q F K I H Y K G N G E S K I A G S F K I
3521  UGGCAUGAUACCAGUGAGGAUCAAUUCAAGAUCCACUAUAAAGGGAACGGAGAGUCCAAGAUCGCAGGCUCCUUCAAGAU  3600

ORF4  R S M S
ORF3  S I N V L T Q N A K * V D G E P G P K P G P D P A P Q  ORF5
3601  CUCGAUCAAUGUCCUAACGCAGAAUGCUAAAUAGGUAGACGGAGAACCGGGUCCGAAACCAGGUCCAGACCCAGCACCCC  3680

ORF5  P T P T P K P T P A K H E R F I A Y T G T L S T L I
3681  AACCAACACCAACACCUAAACCAACGCCAGCCAAACACGAGAGGUUUAUCGCGUACACCGGUACGUUGUCUACACUCAUU  3760

ORF5  S A R Q S S D S I S L Y S I R N Q R I R Y I E D E N S
3761  AGUGCUAGGCAGUCUUCUGAUAGCAUCUCCUUAUAUUCCAUUCGGAAUCAGAGGAUCAGAUACAUUGAAGAUGAAAACUC  3840
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FIG. 3—*Continued*

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ORF5  S  W  T  N  I  D  A  K  W  Y  S  Q  N  S  V  E  A  I  P  M  F  V  Y  P  V  P  E
3841  CAGUUGGACAAACAUAGAUGCAAAAUGGUACUCACAGAACUCUGUUGAGGCUAUUCCAAUGUUUGUUUAUCCAGUACCUG  3920
          3850                3870                3890                3910

ORF5   G  T  W  S  I  E  I  S  C  E  G  Y  Q  A  A  S  S  T  S  D  P  H  R  G  K  C
3921  AAGGUACUUGGUCAAUUGAGAUUUCUUGUGAAGGCUACCAGGCAGCGUCUAGCACUUCAGACCCGCAUCGUGGAAAAUGC  4000
          3930                3950                3970                3990

ORF5 D  G  M  I  A  Y  D  D  D  S  S  K  V  W  N  V  G  Q  Q  N  N  V  T  I  T  N  N
4001  GAUGGCAUGAUUGCUUAUGACGACGAUUCAUCCAAGGUGUGGAAUGUUGGCCAGCAGAAUAAUGUAACCAUAACCAACAA  4080
          4010                4030                4050                4070

ORF5  K  A  D  N  D  W  K  Y  G  H  P  D  P  L  D  L  M  I  N  G  D  R  F  D  Q  N  Q
4081  CAAGGCCGAUAAUGAUUGGAAGUAUGGCCACCCAGAUCCUCUAGAUCUGAUGAUCAAUGGUGACAGAUUCGAUCAAAAUC  4160
          4090                4110                4130                4150

ORF5   V  V  E  K  D  G  I  I  S  F  H  L  V  T  T  G  P  N  A  S  F  F  L  V  A  P
4161  AAGUAGUCGAGAAAGAUGGAAUUAUCUCAUUCCAUUUAGUAACUACUGGCCCCAAUGCGAGUUUCUUUCUUGUCGCUCCA  4240
          4170                4190                4210                4230

ORF5 A  V  K  K  T  A  K  Y  N  F  C  V  S  Y  G  D  W  T  D  R  D  M  E  F  G  M  V
4241  GCUGUUAAGAAAACAGCCAAAUACAACUUUUGUGUUUCUUACGGGAUUGGACAGAUAGAGAUAUGGAAUUUGGUAUGGU  4320
          4250                4270                4290                4310

ORF5  S  V  V  L  D  E  H  L  E  G  A  R  S  S  Q  Y  V  R  K  S  P  R  P  G  H  F  G
4321  AUCGGUGGUGCUUGAUGAGCACUUAGAAGGUGCAAGGAGUUCUCAGUAUGUUAGAAAAUCGCCAAGACCAGGUCAUUUUG  4400
          4330                4350                4370                4390

ORF5   V  N  R  S  H  R  L  Q  D  S  F  T  P  V  E  Y  V  S  D  D  D  S  S  S  S  S
4401  GCGUCAAUCGCUCUCACCGAUUGCAAGAUAGUUUCACUCCUGUGGAAUAUGUCAGUGAUGAUGAUAGUAGUAGUAGUAGU  4480
          4410                4430                4450                4470

ORF5 S  I  V  S  N  R  P  S  T  P  D  N  D  S  D  I  Q  F  A  N  S  L  K  G  K  L  P
4481  AGUAUAGUUAGCAAUCGACCAUCAACUCCGGACAAUGAUAGUGAUAUCCAGUUCGCGAAUUCGUUGAAAGGUAAGCUUCC  4560
          4490                4510                4530                4550

ORF5  S  Q  T  K  L  P  P  K  G  F  Q  S  R  L  S  A  R  E  K  E  E  I  S  K  S  K  P
4561  GUCUCAGACGAAACUUCCUCCCAAAGGAUUCCAAUCACGGUUAAGCGCAAGAGAAAAAGAAGAGAUUUCAAAAUCAAAGC  4640
          4570                4590                4610                4630

ORF5   S  N  V  E  R  Q  V  G  P  L  V  D  A  Y  G  Y  P  S  Q  T  G  V  Y  D  A  A
4641  CUUCAAAUGUUGAACGUCAAGUUGGUCCUCUAGUUGAUGCAUAUGGAUACCCUUCCCAGACGGGUGUUUACGAUGCAGCU  4720
          4650                4670                4690                4710

ORF5 R  E  I  L  Q  S  K  E  A  A  E  N  L  A  E  L  E  K  D  L  K  E  I  N  K  L  E
4721  AGGGAGAUUCUGCAGUCUAAAGAGGCUGCAGAAAACUUGGCAGAAUUAGAGAAGGAUCUAAAAGAAAUCAACAAGCUGGA  4800
          4730                4750                4770                4790

ORF5  P  P  D  T  I  E  Q  E  E  I  P  D  F  V  A  P  S  E  R  V  I  A  E  D  D  R  D
4801  ACCGCCGGAUACAAUCGAACAGGAAGAAAUCCCUGAUUUCGUAGCCCCAUCGGAGAGAGUGAUUGCGGAAGAUGAUCGCG  4880
          4810                4830                4850                4870

ORF5   Y  V  P  S  I  W  R  N  A  D  Q  A  V  V  I  S  S  F  E  P  T  D  W  S  R  P
4881  AUUACGUUCCUUCAAUUUGGCGUAACGCCGACCAAGCUGUGGUAAUCUCAUCUUUUGAGCCUACAGAUUGGAGUCGGCCA  4960
          4890                4910                4930                4950

ORF5 A  Y  E  S  G  D  P  P  K  K  A  G  L  L  K  G  T  L  S  K  L  G  G  S  L  R  S
4961  GCUUAUGAGUCAGGUGAUCCUCCUAAGAAGGCAGGUUUGCUCAAAGGCACCCUCUCAAAAUUGGGAGGGUCGCUUAGAAG  5040
          4970                4990                5010                5030

ORF5  G  E  S  S  L  R  G  N  L  R  K  T  Q  D  Q  S  D  L  E  Y  K  L  S  R  L  N  V
5041  UGGCGAGUCGUCUCUUAGAGGUAACCUCCGCAAGACGCAAGAUCAAAGUGAUCUAGAGUACAAGUUGUCUAGACUCAAUG  5120
          5050                5070                5090                5110

ORF5   P  Q  R  S  Q  Y  Q  R  I  L  A  N  L  G  K  V  R  A  R  A  Y  I  D  G  L  D
5121  UUCCGCAGCGAUCCCAAUACCAACGGAUCUUGGCGAACCUUGGGAAAGUGCGUGCGCGGGCAUAUAUCGAUGGUUUAGAU  5200
          5130                5150                5170                5190

ORF5 L  V      gc              g
5201  UUAGUUUAAUAUAAAUAGAAGUAAGUAUGCGCUUUCCAUCACAUUUAGUUGGAGCCAACCUUGGUGAGUUGCUGUCGUGG  5280
          5210                5230                5250                5270
                              Δ                                    u    u
5281  GAUCCUGGGAAACAGGUUCGGUGAACAAAAAGCCGGUAAUCUCGGGCGUUUGUCAGACGCCGUCGCAUCAACACAGUAUG  5360
          5290                5310                5330                5350
                            Δ              guc      +a             g          c
5361  CAUAUCCAUCAAUAACCCAACGUUCUUGUGACUAAAAAUUAAAGUUAGUUUCCGCCUCUCUUAUAAACCACAUAUAAGAU  5440
          5370                5390                5410                5430
                     a                                   c                  g
5441  GCGAGCUUGUGGAGAGUACCGUCCCUUGAAUAAGGAUGGUUGAAAGUUUCUGGUUUCAAUCCAUUGAUCAAAAUUGACAC  5520
          5450                5470                5490                5510
                              a      g          u   uu                       c
5521  UGCUUCUGGUGACUACACUGCCGCUGGCGAUACAGCAUAAACAAUUUCCACUACUUGUUCGAUGAAAUGAGAACAAUUAG  5600
          5530                5550                5570                5590
               u      uc      a         Δ       ΔΔ
5601  UAAAGUCUUGCCGGUCACAUGAUUAUUUGGCGGUGCUACCGUUCGCCCCUUUGAUGGCGUUAAAUGAAAGCGGAGUAAAC  5680
          5610                5630                5650                5670
              u        a  c                       c
5681  CGACAUCACCUCACCAGGUUGGGUUAACCUGGGGAAGCCGGGAAAAUGCCCGGAACACUUGGUUUAGUGGAUUUUAAACU  5760
          5690                5710                5730                5750
                  u                u      .  a  c
5761  CGAGGGCUCUUCCUCGCUAAAAAGGGGGUAGCCAGGUUGAAAACUGGCAUUAGGGAGUGAUCCCUCUUUGUUGUUAAGGU  5840
          5770                5790                5810                5830

5841  GGCUUUGUGUCCACCUGCCCC  5861
          5850
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FIG. 3—*Continued*

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            1                                          42
            |                                          |
SDV         5'-AGUAAAG-UUGAC-ACCUUUACAGAAG-UGGU-CUUACUUGUAAGA
BYDV-PAV    5'-AGUGAAGAUUGACCAUCU-CACAAAAGCUGUUACGUGCUUGUAACA
            |                                          |
            1                                          45
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FIG. 4. Sequence conservation in the 5′ terminal sequences of BYDV-PAV Vic and SDV Tas-1. The sequences were aligned by eye with addition of gaps to maximise the alignment. Sequences immediately downstream of those shown here did not contain any discernable homology.

versus 17K for all other luteoviruses sequenced to date.

Despite an extensive search, we were unable to detect any conserved ORF in the SDV genome corresponding to ORF6 of the BYDV-PAV genome. In all, we sequenced three clones corresponding to the 3′ end of SDV; two of identical sequence from SDV Tas-1, and one from the 3′ end of another SDV isolate, AP-1. Computer analysis of the sequences failed to reveal any homology with the 3′ terminal sequences of BYDV-PAV. Therefore the 654 nt 3′ UTR does not show any obvious similarity with any other luteovirus sequenced to date, because of its length and lack of coding sequence.

DISCUSSION

We present here the full nucleotide sequence of SDV strain Tas-1 comprising 5861 nucleotides. SDV contains some familiar features of luteoviral genome organisation, and is therefore likely to share similar strategies of gene expression. In particular, it is likely that ORF2 is expressed as a frameshift fusion with the product of ORF1, as is the case for BYDV-PAV (Brault and Miller, 1992). This assumption is based on the obvious similarity of gene organisation and sequence shared by the two viruses in this region, as well as the lack of an initiation codon in SDV ORF2. Likewise, ORFs 3, 4, and 5 are probably expressed from the major subgenomic RNA described by Smith *et al.* (1991) and also observed by us (data not shown), as is the case for other luteoviruses. ORF5 is likely expressed as a readthrough product of ORF3, and ORF4 synthesised by internal initiation from the coat protein messenger RNA. These features of expression appear to be common to all luteoviruses described so far (Bahner *et al.*, 1990; Tacke *et al.*, 1990; Dinesh-Kumar *et al.*, 1992).

Despite the conserved features, we argue that SDV represents a variant genome structure within the luteovirus group. The principle argument for this assertion is the divergent homologies of the two blocks of coding sequence: ORFs 1 and 2 resemble those of BYDV-PAV and not PLRV, whereas ORFs 3, 4, and 5 are more closely related to PLRV. Such a chimeric form is likely to have arisen by recombination, possibly between members of the two existing luteoviral subgroups.

Further evidence that SDV is a distinct evolutionary entity from BYDV-PAV, rather than a direct descendent modified by random nucleotide mutation, is the length of the intergenic region. This is similar in SDV and members of the PLRV subgroup (about 200 nucleotides), but substantially smaller in BYDV-PAV (116 nt). Therefore, there is an association between the length of the intergenic region and the subgroup homology of the 3′ coding block. The variance in length between SDV and BYDV-PAV in this region, taken together with the PLRV subgroup homology of ORFs 3, 4, and 5 of SDV, suggests that the 3′ coding block of SDV was obtained independently of that in BYDV-PAV. While RNA recombination is the implicit mechanism involved here, it is impossible to determine whether this occurred between members of the existing luteoviral subgroups, or as a reiteration of the original event leading to the formation of the luteovirus group. The organisation of the luteoviruses into two blocks of coding sequence that presumably reflect the localisation of similar viral activities (replication, transmission) would enhance the probability of productive recombination.

TABLE 1

AMINO ACID SEQUENCE COMPARISONS OF THE PUTATIVE RNA-DEPENDENT RNA POLYMERASES OF SELECTED LUTEOVIRUSES, CARNATION MOTTLE VIRUS, AND SOUTHERN BEAN MOSAIC VIRUS

	CarMV[a]	PLRV[b]	SBMV[c]	SDV
BYDV-PAV[d]	34.0	17.8	20.9	60.8
CarMV	—	16.1	16.9	34.4
PLRV	—	—	31.1	15.6
SBMV	—	—	—	21.4

Note. Numbers are the percentage of amino acids that are identical between the sequences and were derived using the UWGCG program GAP (Devereux *et al.*, 1984).

[a] Guilley *et al.* (1985).
[b] van der Wilk *et al.* (1989).
[c] Wu *et al.* (1987).
[d] Miller *et al.* (1988a).

TABLE 2

AMINO ACID SEQUENCE COMPARISONS OF LUTEOVIRAL COAT PROTEINS

	BYDV-MAV[a]	PLRV[b]	BWYV[c]	BYDV-RPV[d]	SDV
BYDV-PAV[e]	72.7	49.2	51.5	51.0	43.1
BYDV-MAV	—	45.2	47.7	48.0	42.3
PLRV	—	—	64.2	63.2	57.8
BWYV	—	—	—	65.2	57.8
BYDV-RPV	—	—	—	—	58.8

Note. Numbers are percentage of amino acids that are identical between the sequences and were derived using the UWGCG program GAP (Devereux *et al.*, 1984).

[a] Ueng *et al.* (1992).
[b] van der Wilk *et al.* (1989).
[c] Veidt *et al.* (1988).
[d] Vincent *et al.* (1991).
[e] Miller *et al.* (1988a).