For the Record

The Nucleotide Sequence of Satellite St. Augustine Decline Virus

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The complete nucleotide sequence of the satellite virus of St. Augustine decline virus (sSADV) has been determined. It comprises 824 nucleotides and contains a single, large open reading frame that corresponds to the coat protein of the satellite virus, as confirmed by direct amino acid sequencing. The genome contains two other potential open reading frames, similar to the organization of satellite Panicum mosaic virus (sPMV). These two satellite viruses share significant sequence homology at both the nucleotide and the amino acid levels; there are 36 nucleotide differences, resulting in five amino acid changes. Other than the close homology with sPMV, there is no homology with the known satellite viruses at the nucleotide or amino acid level, while there is some similarity in the hydropathy profiles of the coat proteins of these viruses.

A satellite virus requires a helper virus for replication and encapsidates its RNA within its own coat protein. Although the existence of a satellite virus was first reported in 1962 (Kassanis 1962), few satellite viruses are known, particularly in comparison to satellite RNAs. The type A satellites are defined as those which have a genome greater than 0.7 kb and encode a capsid protein (Francki *et al.* 1991). These are satellite tobacco necrosis virus (sTNV) (Ysebaert *et al.* 1980), satellite Panicum mosaic virus (sPMV) (Masuta *et al.* 1987), satellite tobacco mosaic virus (sTMV) (Mirkov *et al.* 1989), and satellite maize white line mosaic virus (sMWLMV) (Zhang *et al.* 1991). St. Augustine decline virus (SADV) is serologically related to Panicum mosaic virus and also possesses a satellite virus (sSADV) (Berger and Toler 1983; Niblett and Toler 1977).

There is relatively little biological information available regarding satellite viruses, and the sequences of helper viruses (other than TNV and TMV) are not known. The nucleotide sequences of all satellite viruses except sSADV have been reported and analyzed to some degree. Here, we report the primary sequence of sSADV and its coat protein and present comparative analyses.

SADV strain N, harboring sSADV, was originally isolated from St. Augustine grass (*Stenotaphrum secundatum*) in Corpus Christi, Texas, and propagated in foxtail millet (*Setaria italica*). The virus was purified from millet by the procedures of Niblett and Paulsen (1975).

Isolated RNA was purified by agarose gel electrophoresis, and the band corresponding to approximately 800 nucleotides was purified. cDNA was produced by three different methods. 1) Random hexanucleotides were used to prime M-MLV reverse transcriptase, and then second-strand cDNA synthesis was conducted. 2) To obtain clones including the 3′ end of the sSADV genome, RNA was poly(A)-tailed using *Escherichia coli* poly(A) polymerase (Bio-Rad Laboratories, Richmond, CA); the newly introduced poly(A) tract was then used for oligo(dT)$_{12-18}$-primed cDNA synthesis, followed by second-strand cDNA synthesis. 3) cDNA was synthesized and directly sequenced using a specific oligonucleotide primer about 100 residues downstream from the 5′ end of the genome. AMV reverse transcriptase was used for the synthesis of chain-terminated reactions (K/RT, Promega, Madison, WI) (Sanger *et al.* 1977). The strategy used to obtain the entire nucleotide sequence of sSADV is described in Figure 1.

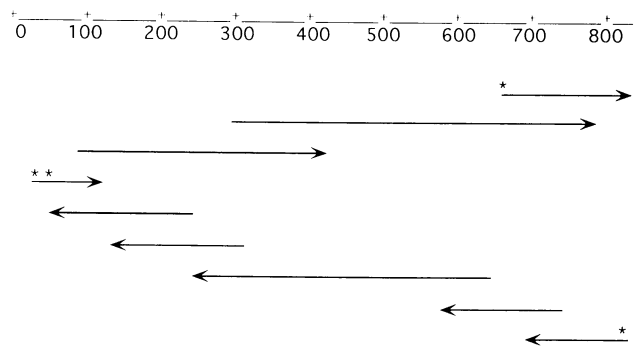

Fig. 1. Strategy used to obtain the entire nucleotide sequence of the satellite St. Augustine decline virus (sSADV) genome. Numbers on the bold line indicate nucleotide positions, and lines with arrows indicate the direction of dideoxynucleotide sequencing. Clones with a single asterisk (*) were derived from cDNA after poly(A) addition. The sequence with two asterisks (**) was generated by reverse transcriptase sequencing using a synthetic oligonucleotide primer.

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The nucleotide sequence reported can be obtained from GenBank as accession no. L10083.

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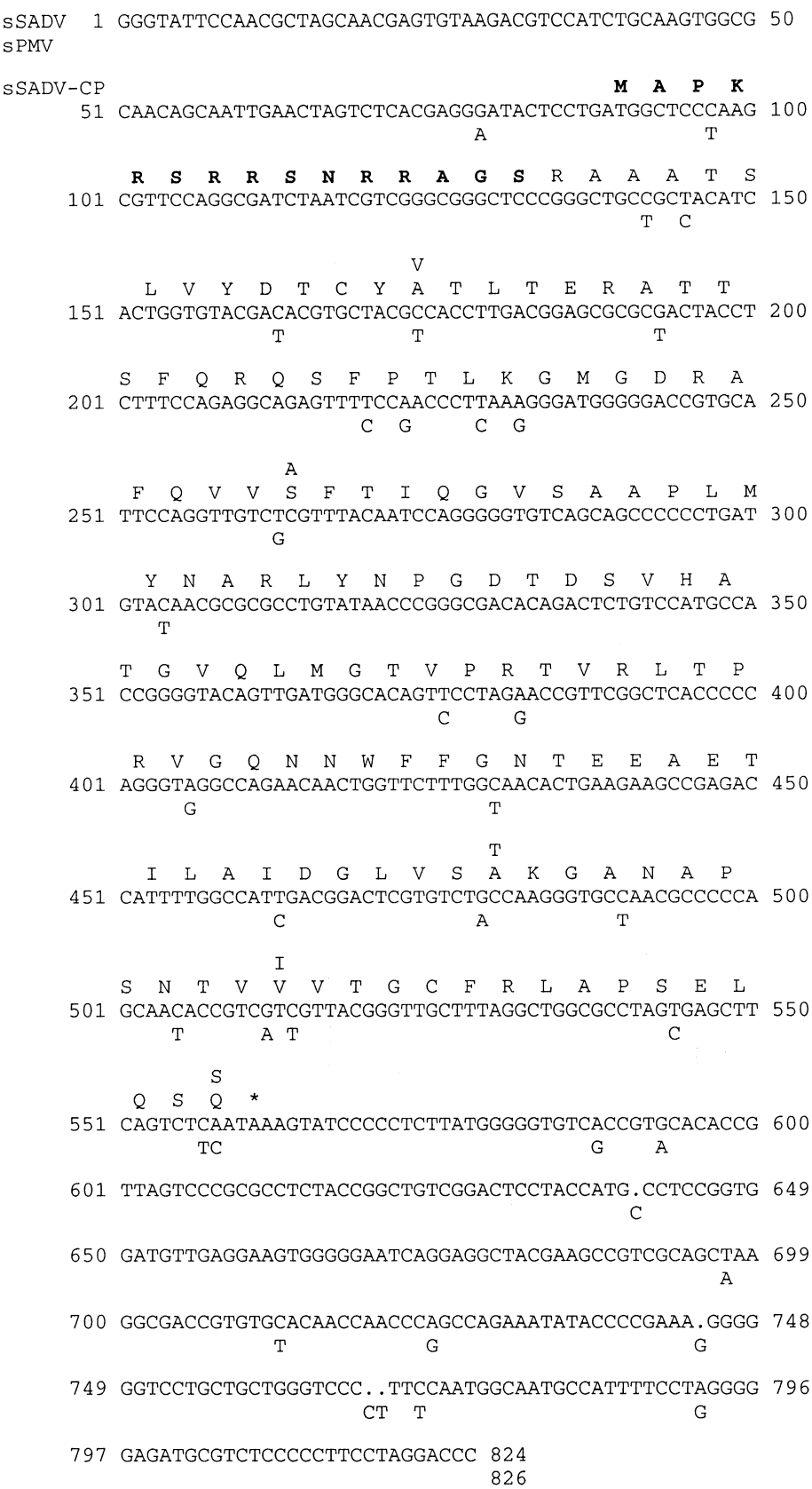

Fig. 2. Nucleotide sequence of the satellite virus of St. Augustine decline virus (sSADV). Nucleotides of satellite Panicum mosaic virus (sPMV) that differ from those of sSADV are shown below the sSADV sequence. The predicted translation of the coat protein open reading frame is shown above the nucleotide sequence. Differences in the sPMV sequence are shown above the sSADV coat protein amino acid sequence. Amino acid residues determined by direct microsequence analysis are represented in boldface type. * = Stop codon.

Like sPMV, sSADV has a large open reading frame (ORF), on the encapsidated (+) strand, that encodes the coat protein. A direct amino acid sequence was obtained by the use of a gas phase sequenator (Applied Bio-Systems Model 475). The sequencer was allowed to perform 15 cycles and confirmed that the ATG beginning at nucleotide 89 was the authentic translation initiation codon in sSADV RNA. Thirteen residues upstream of the initiation codon in sPMV RNA is a putative Shine–Dalgarno sequence (Masuta *et al.* 1987; Shine and Dalgarno 1974). In sSADV, this sequence is AGGGA rather than AGGA. It should be noted that there are additional, smaller ORFs, including one on the (+) strand and one on the (–) strand. These appear in the same relative position as potential ORFs in sPMV RNA, although the second (+) strand ORF is in the same reading frame as the coat protein cistron in sSADV, while it occurs in a different reading frame

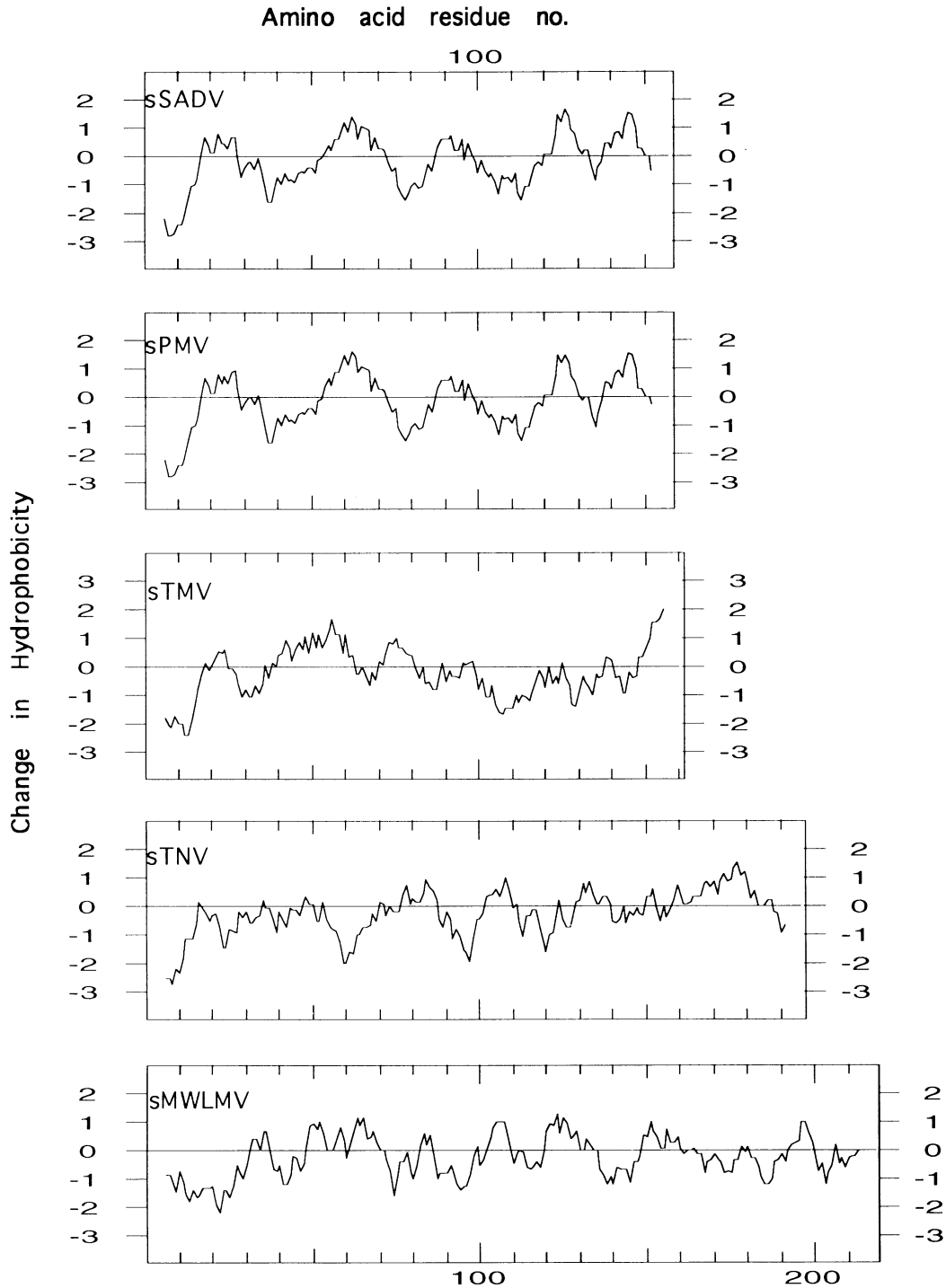

Fig. 3. Hydropathy plots of the coat proteins of the satellite viruses of St. Augustine decline virus (sSADV), Panicum mosaic virus (sPMV), tobacco mosaic virus (sTMV), tobacco necrosis virus (sTNV), and maize white line mosaic virus (sMWLMV).

in sPMV. Whether these ORFs are expressed *in vivo* remains to be determined.

The complete 824-nucleotide sequence of sSADV is presented in Figure 2. sSADV shares significant homology with sPMV, which comprises 826 nucleotides. Differences between the two RNA sequences are shown, together with the resulting amino acid changes (Fig. 2). Interestingly, of the 36 nucleotide changes, 12 are C to T and six are T to C. Only five of them result in changes in the coat protein amino acid sequence. No significant homology was detected when sSADV and sPMV were compared to the other known satellite viruses, at either the nucleotide or the amino acid level. Hydropathy plots of the coat proteins of all known satellite viruses reveals some similarity in patterns of hydrophilic and hydropathic residues (Fig. 3).

When sSADV cDNA was used as a probe for northern hybridization analysis, no signal was detected with helper virus RNA, while a strong signal was observed with sSADV RNA (data not shown). Thus, as in other satellite–helper virus systems, with the notable exception of sTMV (Mirkov *et al.* 1989), no homology is apparent between the satellite and the helper.

ACKNOWLEDGMENTS

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