A comparative study of the RNA-2 nucleotide sequences of two sweet clover necrotic mosaic virus strains

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The nucleotide sequences of the RNA-2s of two strains of sweet clover necrotic mosaic virus (SCNMV-38 and -59) have been determined. The RNA-2s of SCNMV-38 and -59 consist of 1446 and 1449 nucleotides, respectively, and both contain one major open reading frame (ORF) which potentially can encode polypeptides of 326 amino acid residues (about 36-5K), designated SC38P2 and SC59P2, respectively. The nucleotide sequences of SCNMV-38 and -59 RNA-2s show 93-2% similarity, and the amino acid sequences of SC38P2 and SC59P2 are 91-7% identical, although the identical nucleotides and amino acids are not distributed uniformly in RNA-2 and the encoded proteins. Two highly conserved regions (from positions 23 to 221 and 297 to 326) and a relatively divergent region (from positions 222 to 296) are found in the P2 proteins of these strains. A similar pattern is apparent on comparison of the nucleotide and deduced amino acid sequences of RNA-2 of these SCNMV strains with those of the Australian and Czechoslovakian isolates of red clover necrotic mosaic virus.

Sweet clover necrotic mosaic virus (SCNMV), a member of the dianthovirus group (Francki et al., 1991), is an isometric particle with a diameter of 35 nm. A comparative study has been made on two strains of SCNMV, namely SCNMV-38 and -59, on the basis of physicochemical properties, serology, host range and symptomatology (Pappu et al., 1988). The viral genome consists of two positive-sense RNA segments, RNA-1 and RNA-2, with Mr of 1-35 x 10^6 and 0-55 x 10^6, respectively (Hiruki et al., 1984). Both RNAs are required for whole plant infection (Okuno et al., 1983). RNA-1 can replicate without RNA-2, but RNA-2 is not capable of replicating in cowpea protoplasts in the absence of RNA-1 (Pappu & Hiruki, 1988). Genetic reassortant experiments have shown that there is compatibility of RNA-1 and RNA-2, not only between strains of the same virus, but also between certain members of the dianthovirus group, with an exception (Rao & Hiruki, 1987). It has been demonstrated that physicochemical and serological features of progeny virus are determined by the donor RNA-1 (Pappu & Hiruki, 1989; Okuno et al., 1983). Similar work on red clover necrotic mosaic virus (RCNMV) (Osman & Buck, 1991; Osman et al., 1986, 1991a; Paje-Manalo & Lommel, 1989), another member of the dianthovirus group, has demonstrated that RNA-2 is responsible for cell-to-cell virus movement and that it plays a role in the development of disease symptoms in infected plants. To gain further understanding of the function of SCNMV RNA-2, we have determined the primary structures of RNA-2s of SCNMV-38 and -59.

Both strains were maintained and propagated in Phaseolus vulgaris L. 'Red Kidney'. Virus purification and RNA extraction were carried out as described by Hiruki et al. (1984). Unfractionated viral RNAs were polyadenylated in vitro (Ahlquist et al., 1981) and then purified using an mRNA purification kit (Pharmacia LKB) according to the supplier's instructions. Synthesis of cDNA was performed using a cDNA synthesis kit from Pharmacia LKB following the manufacturer's manual. The double-stranded cDNA, tagged with EcoRI/NotI adapters, was ligated with EcoRI-digested dephosphorylated pBluescript SK+/- . The RNA-2-specific clones were characterized by Northern and Southern blot hybridization (Maniatis et al., 1982) using 32P-labelled viral RNA and nick-translated DNA probes (BRL). The RNA probe was made using a 5' end-labelling method. The gel-purified RNA-2 was partially hydrolysed in 50 μl of 50 mM-carbonate/bicarbonate buffer pH 9-0 at 90 °C for 15 min, followed by neutralization with 0-3 volumes of Tris–HCl pH 7-5. The hydrolysed RNA fragments (0-5 to 1 mg) were then labelled in 10 μl of a reaction mixture containing 1 x kinase buffer (50 mM-Tris–HCl pH 9-0, 10 mM-MgCl2, 1 mM-spermidine and 5 mM-DTT), 10 mCi of [γ-32P]ATP (>4000 Ci/mmol; NEN/Dupont) and 2 units of polynucleotide kinase (Phar-
Fig. 1. Alignment of SCNMV-38 (upper line) and -59 RNA-2 (lower line) sequences. Start codons and stop codons are underlined.
The complete nucleotide sequences of SCNMV-38 RNA-2 and SCNMV-59 RNA-2 were 1446 and 1449 nucleotides in length, respectively (Fig. 1). They contained a 5' non-coding leader sequence (nearly 80 nucleotides), a major open reading frame (ORF) and a 3' non-coding trailing sequence (about 390 nucleotides). The structural features of the 5' non-coding and 3' non-coding sequences, as well as the gene organization of SCNMV RNA-2, were similar to those found for RCNMV RNA-2 (Lommel et al., 1988; Osman et al., 1991b). An alignment analysis revealed that the sequence similarity between the RNA-2s was about 93.2%; however, the distribution of identical nucleotide sequences was not uniform (Fig. 1). For example, the similarity between the regions from position 790 to 850 was only 70%, whereas that between the regions from nucleotide 1293 to 1448 was as high as 98.3%.

The major ORF identified for both strains was capable of directing the synthesis of a protein of 326 amino acids (36.5K), designated SC38P2 and SC59P2 (Fig. 2). The size of this proposed protein is in agreement with that of the *in vitro* translation product of native RNA-2 of both strains (data not shown). The amino acid sequences of SC38P2 and SC59P2 shared 93% amino acid sequence identity, but this was not distributed uniformly along the proteins. Two conserved regions (from positions 23 to 221 and 297 to 326 at the N terminus, designated Cons 1 and Cons 2, respectively) and a variable region (from positions 222 to 296, designated V) were found. The identity of amino acid residues in Cons 1 was nearly 99% and in Cons 2 100%, but in V was only 77.3%.

The RNA-2s of two RCNMV isolates, Aus (Australian) and TpM-34, both 1448 nucleotides in length (Lommel et al., 1988; Osman et al., 1991b), showed great similarity with those of the two SCNMV strains, 84.2% and 94.8% with SCNMV-38 RNA-2, and 82.7% and 94.3% with SCNMV-59 RNA-2. An alignment of the four RNA-2-encoded proteins from these two SCNMV strains and two RCNMV isolates is shown in Fig. 2. The P2 of SCNMV consists of the same number of amino acids as that of RCNMV-TpM-34, but is nine amino acids longer than the predicted P2 of RCNMV-Aus. It is worth noting that the four P2 proteins show a highly conserved region (Cons 1, 92.9% identity) and have a relatively variable region (V, 48% identity) (Fig. 2). In addition, the C-terminal 20 residues of RCNMV-TpM-34 and SCNMV-38 and -59 P2 are 100% identical except for the last amino acid. It is interesting that the similarity between SCNMV RNA-2 and P2 and RCNMV-TpM-34 RNA-2 and P2 is higher than that with RCNMV-Aus, and even higher than that between RCNMV-TpM-34 and -Aus, suggesting that the two strains of SCNMV are more closely related to RCNMV-TpM-34 than to RCNMV-Aus.

### Table 1: Alignment of the amino acid sequences of the P2 proteins of RCNMV-Aus, RCNMV-TpM-34, SCNMV-38 and SCNMV-59

<table>
<thead>
<tr>
<th>P2 Protein</th>
<th>RCNMV-Aus</th>
<th>RCNMV-TpM-34</th>
<th>SCNMV-38</th>
<th>SCNMV-59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conserved 1</td>
<td>100%</td>
<td>100%</td>
<td>99.3%</td>
<td>99.3%</td>
</tr>
<tr>
<td>Conserved 2</td>
<td>100%</td>
<td>97.7%</td>
<td>98.3%</td>
<td>98.3%</td>
</tr>
<tr>
<td>Variable</td>
<td>93.2%</td>
<td>97.7%</td>
<td>97.7%</td>
<td>97.7%</td>
</tr>
</tbody>
</table>

Some potentially functional modules of RNA-2 have been highly conserved during their evolution. The stable secondary structures generated by RNA-2 seem to be a common feature for SCNMV and RCNMV, and may be involved in viral replicase recognition during replication, as suggested by Dreher et al. (1984) and Bujarski et al. (1986) for brome mosaic virus. The existence of the Cons 1 region may enable these viruses to complete their life cycle. On the other hand, alteration of the amino acid sequence in the C terminus of P2 may promote better survival of these viruses under different environmental pressures, probably contributing to the creation of a new strain of the same virus or a new virus. A recent example is a mutant of RCNMV-TpM-34, which was produced spontaneously by deleting an A residue in a sequence of four A residues (positions 790 to 793) located in the supposed variable region of RCNMV RNA-2 (Osman et al., 1991b). This mutant, which is capable of directing synthesis of a polypeptide truncated in the C terminus of the protein encoded by the P2 ORF of RCNMV-TpM-34 RNA-2, causes symptoms in cowpea plants different from those produced by the parent virus.
More recently, an extensive degree of sequence identity between the N-terminal 230 amino acids of P2 of RCNMV and those of carnation ringspot virus, a type member of the dianthoviruses (64.3% and 62.6% with RCNMV-Aus and -TpM-34, compared with 59.6% and 55.7% sequence identity of the whole P2), has been found (Kendall & Lommel, 1992). The current evidence suggests that the gene organization of RNA-2 and the functional domains of P2 may be similar among the dianthoviruses.

Combined with previous work on pseudorecombination of RCNMV and SCNMV (Okuno et al., 1983; Rao & Hiruki, 1987), the high degree of similarity of the RNA-2 nucleotide sequences and P2 amino acid sequences, not only between SCNMV-38 and -59, but also between these SCNMV strains, RCNMV-Aus and -TpM-34, suggests closely related evolution of the RNA-2s of these viruses. However, full consideration of the classification of dianthoviruses must wait until the complete nucleotide sequences of the member viruses are known.

We thank Steven A. Lommel for critically reviewing the manuscript, and Thomas Tribe and Gina Figueiredo for technical assistance. This study was supported by a Strategic Grant (G1450) and an Operating Grant (A3843) from the Natural Sciences and Engineering Research Council of Canada.

References


(Received 19 March 1992; Accepted 3 June 1992)