Potato mop-top pomovirus (PMTV) is the type member of the recently proposed genus Pomovirus of soil-borne, fungus-transmitted plant viruses (Torrance & Mayo, 1997). PMTV occurs in potato-growing regions of northern and central Europe, the Andean region of South America, China, Japan (Jones, 1988) and Canada, and its fragile, tubular, rod-shaped particles (Harrison & Jones, 1970) are transmitted through soil by the plasmodiophorid fungus Spongospora subterranea. Sequence analysis of the readthrough (RT) protein-coding region of PMTV-S showed the presence of an additional 543 nt in the 3' half of the coding region relative to that of PMTV-T. These additional nucleotides preserved the reading frame of the RT protein and inserted 181 amino acids into the RT protein. This was confirmed by a comparison by immunoblotting of the sizes of the RT protein of PMTV-T and other recent isolates of PMTV.

A monofungal culture of Spongospora subterranea was unable to acquire and transmit the T isolate of potato mop-top pomovirus (PMTV-T), which has been maintained by manual transmission in the laboratory for 30 years. A recently obtained field isolate (PMTV-S) was efficiently acquired and transmitted by the same fungus culture. Sequence analysis of the readthrough (RT) protein-coding region of PMTV-S showed the presence of an additional 543 nt in the 3' half of the coding region relative to that of PMTV-T. These additional nucleotides preserved the reading frame of the RT protein and inserted 181 amino acids into the RT protein. This was confirmed by a comparison by immunoblotting of the sizes of the RT protein of PMTV-T and other recent isolates of PMTV.

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RT proteins are a common feature of soil-borne, fungus-transmitted viruses and are found in beet necrotic yellow vein virus (BNYVV; Bouzoubaa et al., 1986), soil-borne wheat mosaic virus (SBWMV; Shirako & Wilson, 1993) and beet soil-borne virus (BSBV; Koenig et al., 1997). The BNYVV RT protein is also incorporated into virus particles, predominantly at one end (Haederle et al., 1994). The BNYVV RT protein is involved in particle assembly (Schmitt et al., 1992) and transmission of the virus by its fungal vector, Polymyxa betae (Tamada & Kusume, 1991). Repeated manual passage of BNYVV results in spontaneous deletions in the part of RNA 2 which encodes the C-terminal region of the RT protein and this results in a loss of the ability of the virus to be transmitted by P. betae (Tamada & Kusume, 1991; Tamada et al., 1996). Spontaneous deletions have also been observed in the RT protein-coding region of SBWMV RNA 2 (Shirako & Brakke, 1984) and these have been associated with an increase in symptom severity (Chen et al., 1994). Comparison of the RT proteins of BSBV and PMTV-T indicated the presence of two domains (A and B) of conserved sequence near the N termini of the proteins and a third domain (C) at the C-terminal end of the PMTV-T protein or 49 amino acids from the C terminus of the BSBV protein (Koenig et al., 1997). The region between domains B and C of the PMTV-T RT protein was much shorter than that of the BSBV protein and was lacking in two regions of sequence conserved between BSBV and SBWMV. We report here that a field isolate of PMTV (PMTV-S; Arif et al., 1995) has additional amino acids between the B and C domains and that this isolate is transmissible by S. subterranea whereas PMTV-T, which has been maintained by manual inoculation to Nicotiana debneyi or N. benthamiana for more than 30 years, is not.

N. debneyi plants were propagated in tissue culture, then...
transferred into autoclaved quartz sand and watered with nutrient solution (Merz, 1989) in an automatic watering system. The *N. debneyi* plants were inoculated manually with either PMTV-T or PMTV-S (passage 12) and virus-free *S. subterranea* derived from a single cystosorus (isolate N; Arif et al., 1995) was used to infect the roots on the same day. After 4–5 weeks the leaves and roots of the *N. debneyi* plants were tested for PMTV by triple antibody sandwich (TAS)-ELISA (Torrance et al., 1993; Scott et al., 1994) or 5 μg of total cell RNA (Verwoerd et al., 1989) from inoculated *N. benthamiana* leaves was used as a template for first-strand cDNA synthesis using a 22-mer oligonucleotide, TGTCTTGAGGGCTGGTGCATAG (3'), complementary to the 3'-terminal region of RNA 3 (nt 2296–2315; Kashiwazaki et al., 1995) as a primer. The method for cDNA synthesis was as described by Arif et al. (1994). The total cDNA product was amplified by PCR using the primers TRT-352 and TRT-66 (5'-TACGGTGCCCTGATAGACG 3') which corresponds to nt 800–819 of PMTV-T RNA 3 (Kashiwazaki et al., 1995). The predicted size of the PCR product from the published sequence of PMTV-T (Kashiwazaki et al., 1995) is ~ 1 kb. A band of the predicted size was observed for PMTV-T but the PCR product from PMTV-S was ~ 500 nt larger (data not shown).

A reverse transcriptase–PCR approach was devised to amplify the region of RNA 3 encoding the RT domain and the 3' untranslated region of RNA 3. Two μg of viral RNA (Torrance et al., 1993; Scott et al., 1994) or 5 μg of total cell RNA (Verwoerd et al., 1989) from inoculated *N. benthamiana* leaves was used as a template for first-strand cDNA synthesis using a 22-mer oligonucleotide, TGTCTTGAGGGCTGGTGCATAG (3'), complementary to the 3'-terminal region of RNA 3 (nt 2296–2315; Kashiwazaki et al., 1995) as a primer. The method for cDNA synthesis was as described by Arif et al. (1994). The total cDNA product was amplified by PCR using the primers TRT-352 and TRT-66 (5'-TACGGTGCCCTGATAGACG 3') which corresponds to nt 800–819 of PMTV-T RNA 3 (Kashiwazaki et al., 1995). The predicted size of the PCR product from the published sequence of PMTV-T (Kashiwazaki et al., 1995) is ~ 1 kb. A band of the predicted size was observed for PMTV-T but the PCR product from PMTV-S was ~ 500 nt larger (data not shown).

The nucleotide sequence of the PCR product produced from the PMTV-S RNA was determined. PCR products were cloned into the vector pCRII (Invitrogen) or pT7Blue (Novagen). Cloned cDNA was sequenced with M13 forward and reverse sequencing primers using either a Taq Dye Primer or Taq Dye Terminator Cycle Sequencing kit and a model 373 automated DNA sequencer (Perkin Elmer Applied Biosystems). Further PCR products were generated after sequence data had been obtained for the 3' and 5' regions of the PMTV-S RT gene using two primers within the RT gene sequence: SRT2 (5'TATTTATGACGAAAGCGGC 3') and SRT3 (5'TATCTAGCTGCAAAGGACC 3'), corresponding to nt 1590–1609 and the complement of nt 2001–2020 of the PMTV-T RNA 3 sequence, respectively (Kashiwazaki et al., 1995). Sequence analysis was performed using the Applied Bio-systems Sequence Navigator software for sequence editing. Sequence contig assembly and comparisons were performed with the UWCGC software package (Devereux et al., 1984) using the Seqnet computing facility at Daresbury, UK. The region of PMTV-S RNA 3 sequence was 543 nt longer than the corresponding region of PMTV-T. The additional nucleotides in the PMTV-S sequence represent a contiguous sequence inserted between nt 1731 and 1732 of the PMTV-T sequence (Kashiwazaki et al., 1995) and maintain the frame of the protein by inserting 181 amino acids into the RT domain. This would give the RT domain a molecular mass of ~ 67 kDa and the RT protein a molecular mass of 87 kDa. The CP and RT protein of PMTV-S were examined by Western blotting. Leaf tissue from PMTV-infected or uninfected *N. benthamiana* plants was solubilized and proteins were separated by electrophoresis on 12.5% polyacrylamide gels before transfer to nitrocellulose (Towbin et al., 1979). PMTV CP and RT protein were detected using rabbit anti-PMTV polyclonal serum followed by goat anti-rabbit antibody conjugated to alkaline phosphatase using a chemiluminescent substrate (CSPD; Boehringer Mannheim). The CP was of the size predicted from sequence data published.

Table 1. Acquisition and transmission of PMTV isolates by *S. subterranea*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acquisition (A&lt;sub&gt;100&lt;/sub&gt;)&lt;sup&gt;§&lt;/sup&gt;</th>
<th>Transmission by S&lt;sup&gt;†&lt;/sup&gt;</th>
<th>PMTV infection F&lt;sub&gt;t&lt;/sub&gt; A&lt;sub&gt;100&lt;/sub&gt;&lt;sup&gt;§&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMTV-S12 + Ss</td>
<td>1.48 ± 0.070</td>
<td>2.8</td>
<td>20/22 1.257</td>
</tr>
<tr>
<td>PMTV-T + Ss</td>
<td>1.56 ± 0.136</td>
<td>2.8</td>
<td>0/13 0.052</td>
</tr>
<tr>
<td>Ss only</td>
<td>0.075 ± 0.070</td>
<td>2.9</td>
<td>0/9 0.085</td>
</tr>
<tr>
<td>PMTV-S12 only</td>
<td>2.099 ± 0.239</td>
<td>0</td>
<td>0/8 0.0107</td>
</tr>
<tr>
<td>Non-infected</td>
<td>0.061 ± 0.046</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* A<sub>100</sub> values are the mean of four to nine replicate *N. debneyi* plants with each plant tested in triplicate, and indicate the presence of PMTV in plants used for acquisition of virus by *S. subterranea*. A<sub>100</sub> values were obtained after overnight incubation of substrate.

† Mean scores of *S. subterranea* infection on 10 randomly selected lateral root pieces 2–4 mm long from indicator plants exposed to root fragments from plants inoculated as described under the adjacent Acquisition heading.

‡ Frequency of transmission; no. of indicator plants infected/no. of indicator plants tested. Samples were considered to be positive when the absorbance (A<sub>100</sub>) values exceeded the mean of the virus-free sample by at least a factor of two.

§ A<sub>100</sub> values are the means of the indicator plants tested for infection in the adjacent column and were obtained after overnight incubation of substrate.

NA, Not applicable.
previously (Kashiwazaki et al., 1995; Mayo et al., 1996). The molecular mass of the RT protein from leaf tissue infected with isolate S was estimated to be ~87 kDa (data not shown) whereas the predicted mass of the RT protein from the PMTV-T sequence is ~67 kDa (Kashiwazaki et al., 1995).

A multiple sequence alignment of the RT domains of PMTV isolates T and S with those of two other soil-borne, fungus-transmitted viruses, BSBV and SBWMV, indicates the inserted amino acids (305–485) of PMTV-S (Fig. 1). In addition there are single amino acid changes between PMTV-T and PMTV-S.
PMTV-S at positions 98 (V → I), 148 (L → R), 167 (S → L), 196 (F → Y), 200 (G → D), 226 (I → K), 240 (K → N), 244 (I → N), 254 (D → E), 285 (K → E), 287 (R → T) and 519 (L → M). The inserted amino acids in the PMTV-S RT domain have significant similarities with sequences in the RT domain of BSBV, particularly in the blocks of sequence in the conserved regions between BSBV and SBWVMV identified by Koenig et al. (1997). Specifically, the PMTV-S RT domain has amino acids identical with BSBV or SBWVMV in 7/16 amino acids constituting the conserved region F (SBWVMV residues 309–324 in Fig. 1). The similarity between PMTV-S and BSBV extended over the following 10 residues with 5 identical amino acids. The conserved region G (SBWVMV residues 345–384 in Fig. 1) is present in the PMTV-S RT domain at residues 333–372 and the similarity between the PMTV-S and BSBV proteins again extends, this time over the following 7 amino acids with 4 identical. There are two further conserved regions between the RT domains of PMTV-S, BSBV and SBWVMV at PMTV-S residues 384–422 (PMTV-S having 13/40 amino acids identical with either BSBV or SBWVMV), and at PMTV-S residues 447–485 (PMTV-S having 15/39 amino acids identical with either BSBV or SBWVMV). In addition there is a short conserved sequence between the RT domains of PMTV-S and BSBV interrupting these two larger domains at PMTV-S residues 429–439 with 6/11 identical amino acids; this region is absent from the SBWVMV RT protein. The PMTV-S RT domain, like those of BSBV and SBWVMV, does not contain a KTER motif which has been reported to be involved in transmission of BNYVV by *P. betae* (Tamada et al., 1996). However, the additional sequences in the PMTV-S RT protein form a region of hydrophilicity flanked by two hydrophobic regions (Fig. 2A). The comparable regions of the BSBV and SBWVMV RT proteins also form hydrophilic regions flanked by hydrophobic regions (Fig. 2B, C) and the KTER motif of the BNYVV RT protein is contained within a hydrophilic region flanked by hydrophobic regions (Fig. 2D).

The association of lack of transmissibility of PMTV-T with an apparent deletion of sequence in RNA 3, relative to the RNA 3 of the transmissible isolate PMTV-S suggests that the RT domain encoded by PMTV-S RNA 3 contains determinants that play an important role in acquisition and transmission of PMTV by its natural fungus vector, *S. subterranea*. These results are similar to the situation with BNYVV in which two BNYVV RNA 2 deletion mutants appeared spontaneously during serial passage of virus by manual inoculation and contained deletions of ~500 and ~600 nucleotides. The deletions in these mutants are contained within the C-terminal portion of the RT domain and these deleted isolates were not transmissible by *P. betae* (Tamada & Kusume, 1991). PMTV-T has been maintained by manual transmission for more than 30 years and it is probable that deletions in its RNA 3 have occurred over this period. However, the region of PMTV-S RNA 3 encoding the RT domain seems to be relatively stable, at least in the short term, as the size of the sequences encoding the RT domain of PMTV-S remained the same over 20 sequential passages (1 year) (data not shown). In contrast, deletions occur in SBWVMV RNA 2 rapidly and even in a single manual transfer of the virus from naturally infected wheat plants. The SBWVMV particles containing deletions become dominant in the culture only after a few repeated mechanical inoculations and no full-length RNA 2 remains after only five passages (20 weeks) (Chen et al., 1994).

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### References


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