Association of sequences in the coat protein/readthrough domain of potato mop-top virus with transmission by Spongospora subterranea

B. Reavy, M. Arif,† G. H. Cowan and L. Torrance

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

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.

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 plasmidiphic fungus Spongospora subterranea (Jones & Harrison, 1969; Arif et al., 1995). The virus particles encasipdate three genomic single-stranded RNA molecules of 6.5, 3 and 2.5 kb (Scott et al., 1994). The smallest genomic RNA (RNA 3) of the T isolate (Harrison & Jones, 1970) of PMTV (PMTV-T) contains a single open reading frame encoding the virus coat protein (CP) of 20 kDa terminated by an amber codon. This is followed by an in-phase coding region for an additional 47 kDa readthrough (RT) domain (Kashikazaki et al., 1995). This RT domain is expressed as a fusion with the CP as a result of suppression of the CP gene termination codon and the resulting 67 kDa RT protein is present near one extremity of PMTV particles (Cowan et al., 1997).

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 (Haebeler 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

---

Author for correspondence: Brian Reavy.
Fax +44 1382 562426. e-mail breavy@scri.sari.ac.uk

† Present address: Dept of Plant Pathology, NWFP Agricultural University, Peshawar, Pakistan.

The nucleotide sequence of the PMTV-S readthrough domain has been submitted to the EMBL database and assigned the accession number AJ224991.
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* leaves was used as a template for first-strand cDNA synthesis using a 22-mer oligonucleotide, TRT-352 (5′ GCCAACGC-TATGTCTGATAGC 3′), complementary to the 3′-terminal sequence 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′ TACGG-TGGGCTGTTGCATAG 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 ~1.4 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′ TATTATTTGAACCGAAGCG 3′) and SRT3 (5′ TATCTGAAAAAAGAGCG 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 Biosystems Sequence Navigator software for sequence editing. Sequence contig assembly and comparisons were performed with the UWCG 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).

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, TRT-352 (5′ GCCAACGC-TATGTCTGATAGC 3′), complementary to the 3′-terminal sequence 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′ TACGG-TGGGCTGTTGCATAG 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 ~1.4 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′ TATTATTTGAACCGAAGCG 3′) and SRT3 (5′ TATCTGAAAAGAGCG 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 Biosystems Sequence Navigator software for sequence editing. Sequence contig assembly and comparisons were performed with the UWCG 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...
Fig. 1. Multiple sequence alignment of the RT domains encoded by PMTV-S, PMTV-T, BSBV and SBWMV performed using the UWGGC programs PILEUP and PRETTYBOX. Amino acids conserved between two or more viruses are shown as white letters on a black background. Regions of conserved sequence (F, G, H, I and J) are indicated by dashed lines under the blocks of sequence.

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...
occurred over this period. However, the region of PMTV-S
years and it is probable that deletions in its RNA 3 have
portion of the RT domain and these deleted isolates were not
during serial passage of virus by manual inoculation and
BNYVV RNA 2 deletion mutants appeared spontaneously
results are similar to the situation with BNYVV in which two
PMTV by its natural fungus vector,
rt domain encoded by PMTV-S RNA 3 contains determinants
hydrophobic regions (Fig. 2D).
BNYVV RT protein is contained within a hydrophilic region
hydrophobic regions (Fig. 2B, C) and the KTER motif of the
SBWMV RT proteins also form hydrophilic regions flanked by
regions (Fig. 2A). The comparable regions of the BSBV and
the RT domain of PMTV-S remained the same over 20
the RT domain of PMTV-S but absent in PMTV-T are indicated by a bar, as are
the corresponding regions of the RT domains of BSBV and SBWMV. The
hydrophilic region of the BNYVV RT domain, which contains the
transmission determinant sequence KTER, is also indicated along with the
two flanking hydrophobic regions.

![Fig. 2. Hydrophobicity plots of the RT domains of PMTV-S (A), BSBV (B), SBWMV (C) and BNYVV (D) derived from the UWGCG program PEPTIDESTRUCTURE using the formula of Kyte and Doolittle. The hydrophilic region and flanking hydrophobic regions present in the sequence of PMTV-S but absent in PMTV-T are indicated by a bar, as are the corresponding regions of the RT domains of BSBV and SBWMV. The hydrophilic region of the BNYVV RT domain, which contains the transmission determinant sequence KTER, is also indicated along with the two flanking hydrophobic regions.](image)

This work was supported by The Scottish Office Agriculture, Environment and Fisheries Department. M. Arif was in receipt of a postgraduate studentship from the Association of Commonwealth Universities. Sequence analysis was performed using the Seqnet facility.

**References**


Received 23 April 1998; Accepted 12 June 1998