

Tepovirus, a novel genus in the family *Betaflexiviridae*

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Abstract *Tepovirus* is a new monotypic genus of plant viruses typified by potato virus T (PVT), a virus with helically constructed filamentous particles that are 640 nm long, previously classified as unassigned species in the family *Betaflexiviridae*. Virions have a single-stranded positive-sense polyadenylated RNA genome that is 6.5 kb in size, and a single type of coat protein with a size of 24 kDa. The viral genome contains three slightly overlapping ORFs encoding, respectively, the replication-related proteins (ORF1), a putative movement protein of the 30 K type (ORF2) and the coat protein (ORF3). Its structure and organization (number and order of genes) resembles that of trichoviruses and of citrus leaf blotch virus (CLBV, genus *Citrivirus*) but has a smaller size. Besides potato, the primary host, PVT can experimentally infect herbaceous hosts by mechanical inoculation. No vector is known, and transmission is through propagating material (tubers), seeds and pollen. PVT has a number of biological, physical and molecular properties that differentiate it from betaflexiviruses with a 30K-type movement protein. It is phylogenetically distant from all these viruses, but least so from grapevine virus A (GVA), the type member of the genus *Vitivirus*, with which it groups in trees constructed using the sequences of all of the genes.

Introduction

Potato virus T (PVT), a virus with filamentous, slightly flexuous particles that are *c.* 640 x12 nm in size and a positive-sense single-stranded RNA genome, was first isolated in Peru from symptomless potato (*Solanum tuberosum*) plants [14]. The virus has no known vector, is pollen- and seed-borne, and is readily transmitted by inoculation of sap to a restricted range of experimental hosts [15, 16].

PVT had a rather complex taxonomic history. It was first classified as a possible member of the “taxonomic group” Closterovirus [11], but because of its distant serological relationship to apple stem grooving virus (ASGV) [15], the type member of the “taxonomic group” Capillovirus, it was later assigned to this group as a definitive member [4]. Sequencing of the 3' end of the viral genome [12] revealed a structural similarity to that of members of the genus *Trichovirus*, suggesting its re-classification as a member of a definitive trichoviral species [5, 8]. When the genus *Trichovirus* was incorporated into the newly established family *Flexiviridae* [1], the incomplete molecular information prompted a further change in the taxonomic status of this virus, which became a member of an unassigned species in this family [2]. It is currently considered a member of an unassigned species in the family *Betaflexiviridae* [3].

In 2009 the PVT genome was completely sequenced [13] and found to differ sufficiently at the molecular level from representatives of the type species of all genera in the family *Betaflexiviridae* whose members have a 30K-type movement protein (genera *Capillovirus*, *Trichovirus*, *Vitivirus*, *Citrivirus*) to warrant classification in a separate genus. The proposal of the new taxon, denoted genus *Tepovirus*, an abbreviation of the re-arranged name

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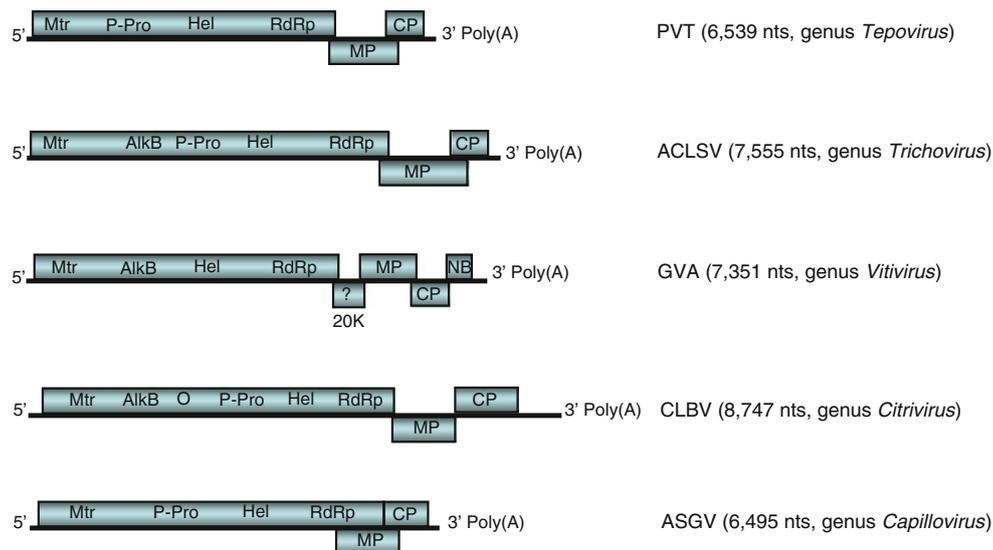


Fig. 1 Schematic representation of the genomes of PVT (EU835937) and of the type members of the genera *Trichovirus*, *Vitivirus*, *Citrivirus* and *Capillovirus*, all of which possess a movement protein of the 30K type. Motifs in the replicase protein (ORF1) are as follows: methyltransferase (Mtr), AlkB domain (AlkB), OTu-like domain (O), papain-like protease (P-Pro), helicase (Hel), RNA-dependent RNA polymerase (RdRp). *MP* movement protein, *CP* coat protein. The 20K protein encoded by GVA ORF2 has an unknown function, whereas the expression product of GVA ORF5 (NB) is the RNA silencing suppressor

Table 1 Properties of PVT compared with those of representatives of the type species of the other genera of the family *Betaflexiviridae* whose members have a 30K-like movement protein

Virus (type species)	PVT	ACLSV	GVA	CLBV	ASGV
Genus	<i>Tepovirus</i>	<i>Trichovirus</i>	<i>Vitivirus</i>	<i>Citrivirus</i>	<i>Capillovirus</i>
Natural hosts	Herbaceous	Woody	Woody	Woody	Woody
Vector	None	None	Mealybugs	Not determined	None
Length of virions (nm)	640	680	800	960	620
Aspect of virions in uranyl acetate mounts	Slightly flexuous cross pattern/faint cross banding	Very flexuous, distinct cross banding	Very flexuous, distinct cross banding	Slightly flexuous, faint cross banding	Very flexuous, distinct cross banding
Serological relationship to PVT		No	No	No	Uncertain (not confirmed)
Genome organization	Three partially overlapping ORFs. No intergenic regions	Three partially overlapping ORFs. No intergenic regions	Five partially overlapping ORFs	Three ORFs. Intergenic region between ORF2 and ORF3	Two nested ORFs
Genome size (nt)	6,539	7,555	7,349	8,747	6,496
Coat protein size (kDa)	24	22	22	44	27
AlkB domain	No	Yes	Yes	Yes	No
OTu-like domain	No	No	No	Yes	No
Papain-like protease	Yes	Yes	No	Yes	Yes
References	[13, 15]	[6, 10, 19]	[9, 10]	[10, 17]	[10, 18, 20]

(*T. potato virus*) of the putative type species, was approved by the Executive Committee of the ICTV in June 2010 and ratified by the ICVT Plenum in February 2012.

Taxonomic structure of the genus

The genus *Tepovirus* has the following taxonomic structure:

Type species: *Potato virus T* (PVT)

Species in the genus: None known

Other related viruses not approved as species: None reported.

Biological properties

The only known natural hosts of PVT are several species of tuber-bearing *Solanum*, which, in most cases, are infected but do not display obvious symptoms. The virus can be transmitted through the tubers to progeny plants and, in *Solanum demissum*, through seed and from pollen to seed, but not to pollinated plants. PVT is readily transmitted by inoculation of sap to a limited range of herbaceous hosts. Seed-transmission has also been observed in *Datura stramonium* and *Nicandra physaloides*. No vectors are known; there is no transmission by aphids [15].

Morphological, physicochemical and serological properties

PVT particles are slightly flexuous filaments, *c.* 640 x 12 nm in size, helically constructed with a pitch of 3.4 nm and 9–10 subunits per turn of the helix. They show a “criss-cross” pattern or a faint cross-banding, depending on whether they are stained with uranyl acetate or sodium phosphotungstate [15]. Virions sediment as a single species with a sedimentation coefficient ($S_{20,w}$) of 99S by analytical or density gradient centrifugation and contain a molecule of single-stranded

positive-sense RNA with a molecular weight of *c.* 2.2×10^6 , constituting about 5 % of the particle weight [15]. Coat protein subunits are of a single type and have a molecular weight of 27 kDa (estimated electrophoretically) or 24 kDa (deduced from sequence data) [13, 15]. PVT has been reported to be serologically related to the capillovirus ASGV [15], but this was not confirmed by Russo et al. [13], who ascertained also that there is no apparent serological relationship with CLBV and representatives of the genera *Vitivirus* and *Trichovirus*.

Molecular properties

The PVT genome is 6,539 nucleotides in size, excluding the poly(A) tail at the 3' end, and it is likely to be capped at the 5' terminus (GenBank accession number EU835937). It contains three slightly overlapping ORFs (Fig. 1) and untranslated regions (UTR) of 74 and 184 nt at the 5' and 3' termini, respectively. ORF 1 codes for a replication-related

Table 2 Pairwise amino acid sequence comparisons of proteins encoded by the PVT genome with corresponding complete gene products of the representatives of the type species of the genera *Trichovirus*, *Vitivirus*, *Citrivirus* and *Capillovirus*

Virus	Amino acid sequence identity (%)		
	Replicase	Movement protein	Coat protein
ACLSV	29	22	28
GVA	30	23	35
CLBV	28	19	22
ASGV	28	24	31

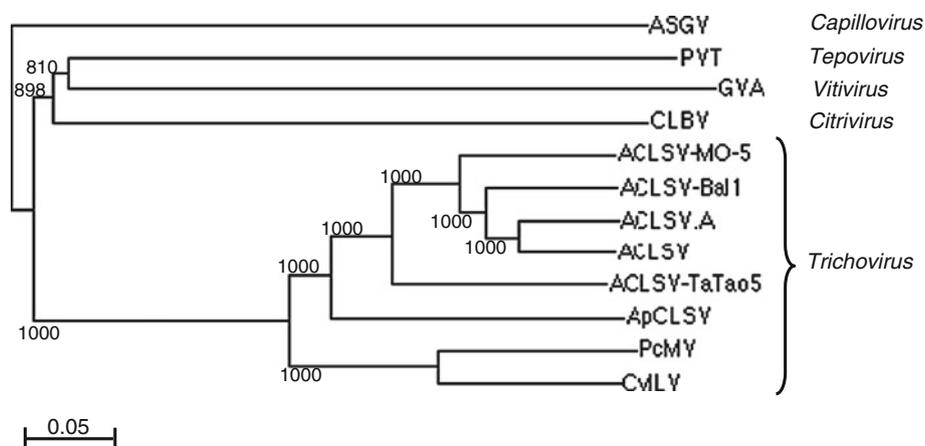


Fig. 2 Unrooted phylogenetic tree constructed using the amino acid sequences of the replicase genes of PVT, members of the genus *Trichovirus*, and one representative member of each genus of the family *Betaflexiviridae* whose members contain a 30K-like movement protein. In trees constructed with the sequences of the movement and coat proteins of the same viruses, clustering was the same. ACLSV (apple chlorotic leaf spot virus, M58152); ACLSV-A (D14996); ACLSV-Bal1 (X99752); ACLSV-MO5 (AB326225); ACLSV-

TaTao5 (EU223295); apricot pseudo-chlorotic leaf spot virus (ApCLSV, AY3379); apple stem grooving virus (ASGV, DI4995); citrus leaf blotch virus (CLBV AJ318061); cherry mottle leaf virus (CMLV, AF170028); grapevine berry inner necrosis virus (GINV, D88448); grapevine virus A (GVA, X75433); peach mosaic virus (PcMV, DQ117579). Alignments, tree construction, and calculation of bootstrap values were done with Clustal W2 [7]

polyprotein that is 185 kDa in size, containing the conserved motifs of the “Alpha-like” supergroup of positive-strand RNA viruses (methyltransferase, helicase and RdRp) and a papain-like cysteine protease signature, but not the AlkB domain found in trichoviruses, vitiviruses and the citrivirus CLB, or the OTu-like domain present in CLB [10] (Table 1). ORF 2 encodes the 40-kDa movement protein (MP), and ORF 3 encodes the 24-kDa coat protein (CP). Intergenic regions are apparently absent [13].

Pairwise amino acid sequence comparisons of each protein encoded by the PVT genome with the comparable gene products of representatives of the type species of the genera *Trichovirus*, *Vitivirus*, *Capillovirus* and *Citrivirus* showed that the relationship to all of these viruses is distant, with sequence identity not exceeding 35% for any of the proteins analysed (Table 2).

Phylogenetic analysis of the proteins encoded by the three PVT ORFs and the corresponding products of all sequenced trichoviruses and of the type members of the other genera of the family *Betaflexiviridae* whose members have 30K-like movement proteins showed that PVT is clearly separated from all trichoviruses, as well as from ASGV (genus *Capillovirus*) and CLB (genus *Citrivirus*), but groups consistently with grapevine virus A (GVA, genus *Vitivirus*), regardless of the viral gene investigated (replicase, MP, and CP) [13] (Fig. 2).

Similarity to other taxa

The common and distinguishing features characterizing members of the genera *Tepovirus*, *Capillovirus*, *Citrivirus*, *Trichovirus* and *Vitivirus* are summarized in Table 1. PVT particles are morphologically similar to those of CLB (genus *Citrivirus*) but are much less flexuous than the virions of representatives of the genera *Trichovirus*, *Capillovirus* and *Vitivirus*. The structure and organization of the PVT genome resemble those of CLB and of members of approved trichovirus species in the number and order of genes and in the absence of intergenic regions. However, ORF1 (replicase) and CP of CLB are larger than those of PVT, whose genome lacks the additional 3'-most ORF that is present in some trichoviruses. The presence of a 30K-like movement protein is common to PVT, CLB, tricho-, capillo-, and vitiviruses. A further point of similarity with capilloviruses, known trichoviruses and CLB is the apparent absence of recognized vectors.

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